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

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Supplementary Appendix

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Supplementary Methods

Platelet aggregation assay

The platelet aggregation assay was performed at Charles River Laboratories Montreal ULC (Senneville, QC, Canada) and is based on previously published methods.[1] The principle of this in vitro assay is that human platelets in platelet rich plasma (PRP) samples will aggregate in the presence of ADP or collagen agonists. Eptifibatide was used to the prevent aggregation caused by ADP or collagen. A vehicle control and three concentrations of mivavotinib were evaluated for their effects on ADP- or collagen-mediated platelet aggregation. Citrated whole blood samples were collected from six donors (3 males and 3 females). Once the citrated whole blood samples were collected, an adequate number of tubes from each donor were centrifuged at 200 RCF for 10 minutes at 21°C (break set to off). The plasma fraction was then isolated, pooled, capped and kept at room temperature. This sample was identified as the Platelet Rich Plasma (PRP) pool. In parallel, all the remaining citrated whole blood tubes from each donor were centrifuged at 2400 RCF for 10 minutes at 21°C. The plasma fraction was then isolated, pooled,

capped and kept at room temperature. This sample was identified as the Platelet Poor Plasma (PPP) pool. No sample was lipemic, icteric, or hemolyzed. PRP platelet counts were established using an ADVIA 120 Hematology Instrument, then standardized to obtain final platelet concentrations of 200 to 300 x 10_3 cells/µL, using autologous PPP diluent, when necessary. Platelet counts were confirmed using an ADVIA 120 Hematology Instrument. PPP was also used as blank for this analysis. PRP and platelet poor plasma (PPP) were prepared for each donor. PRP samples were standardized by dilution with homologous PPP sample to have a platelet count between 200 and 300 x 10^3 cells/µL. Prior to incubation at 37°C for 15 minutes, standardized PRP samples were spiked with 0.5% Methylcellulose and mivavotinib at 0.107, 1.07 or 10.7 µM. PPP samples served in the reference sample cuvette, standardized PRP samples served as positive control and PRP spiked with 0.5% Methylcellulose served as the Vehicle Control. To determine the effect of mivavotinib on ADP-induced platelet aggregation, the platelet aggregation was evaluated at the end of a 15-minute incubation period, in the presence of ADP at 10 µM. To determine the effect of mivavotinib on collagen-induced platelet aggregation, the platelet aggregation was evaluated at the end of a 15-minute incubation period, in the presence of collagen at 2 µg/mL. Following the incubation period of 15 minutes at 37°C, the samples were loaded in the instrument prior to measurement of platelet aggregation. Each of the groups was tested in duplicate with the individual samples from 3 male and 3 female donors. The samples were analyzed using a Chrono-Log aggregometer 700.

1. Methods Mol Biol. 2004; 272:13-28

Dose-limiting toxicities

Any of the following adverse events (AEs) occurring in cycle 1 that were considered by the investigator to be at least possibly related to mivavotinib:

- Prolonged myelosuppression with the persistence of grade ≥4 neutropenia or grade ≥4 thrombocytopenia in the absence of leukemia (blast count <5% in bone marrow) at least 42 days after the initiation of cycle 1 therapy;
- Any grade ≥3 nonhematologic toxicity with the following exceptions: 1) grade ≥3 nausea and/or vomiting or diarrhea that resolved to grade ≤1 or baseline in a week after standard supportive treatment; 2) Brief (<1 week) grade 3 fatigue; 3) Asymptomatic grade 3 laboratory abnormalities that were not considered to be clinically significant;
- Failure to administer ≥75% of planned doses of the study drug due to mivavotinib-related or possibly related toxicities; or
- Other mivavotinib–related grade ≥2 nonhematologic toxicities that required a dose reduction or discontinuation of treatment with mivavotinib

Inclusion and exclusion criteria

Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–1, and adequate organ function. Patients were excluded if they had clinically significant toxicity from prior chemotherapy, had received hematopoietic stem cell transplantation (HSCT) within 60 days of the first dose of mivavotinib, had clinically significant graft-*versus*-host disease requiring ongoing immunosuppressive therapy, or had a current history of cardiovascular conditions. Patients with a white blood count >50,000 u/L were also excluded; however, hydroxyurea was permitted at study entry and during the first 28 days up to a

maximum dose of 5g/day if needed to control circulating blasts. Patients were excluded if they had prior exposure to FMS-like tyrosine kinase 3 (FLT3) inhibitors.

Assessments

Progressive disease defined as one of the following:

- >50% increase in bone marrow blasts from baseline value;
- >50% increase in circulating blasts from baseline value with absolute blast count >1000/mm^3;
- Development of biopsy-proven extramedullary disease; or
- New sites of extramedullary leukemia

If the initial marrow blast percentage was too high to base progression on a >50% increase in bone marrow blasts, then peripheral blood criteria were to be used.

Blood samples for PK assessment were collected within 1 hour pre-dose on days 1, 2, 15, and 16, and 0.5-, 1-, 2-, 3-, 4-, and 8-hours post-dose on days 2 and 16 of cycle 1. Mivavotinib plasma concentration was measured using high performance liquid chromatography with tandem mass spectrometry, which was validated over the concentration range of 0.5 to 2000 ng/mL.

Blood samples for assessment of phosphorylated FLT3 (pFLT3) by PIA were collected pre-dose on days 1 and 15 of cycles 1 and 2. Plasma inhibitory activity for FLT3and pFLT3 in circulating blasts from study participants was determined as previously described.[4, 5]

Statistical analysis

The safety population included all enrolled patients who received ≥1 dose of mivavotinib. The dose-limiting toxicity (DLT) evaluable population included all patients who either experienced a DLT during cycle 1 or completed ≥75% of the planned doses of mivavotinib and had sufficient follow-up data to determine whether a DLT had occurred. The response-evaluable population included all patients who received ≥1 dose of study drug, had baseline bone marrow blast counts, and one post-baseline disease assessment. Statistical analyses were primarily descriptive and graphical in nature. No formal statistical hypothesis testing was performed. Pharmacokinetic parameters were estimated for the acute myeloid leukemia (AML) dose-escalation cohorts (cycle 1, day 1 and cycle 1, day 15) using noncompartmental methods with WinNonlin Professional version 7.0 or higher (Certara USA).

Supplementary results

Disease response

One patient diagnosed with *de novo* high-risk AML and a World Health Organization (WHO) classification of acute erythroleukemia, achieved a complete response (160 mg daily cohort). The FLT3 status by local and central testing were unknown and quantity not sufficient, respectively. The patient had received 1 prior therapy of decitabine with a best response of progressive disease. The patient entered the study with a bone marrow biopsy and aspirate blast count of 18% and a peripheral blast count of 0%. The patient achieved a complete response on cycle 4 day 15 with a reduction in bone marrow biopsy and aspirate blasts to 2%. A subsequent bone marrow biopsy and aspirate at cycle 9 revealed a largely unchanged blast count of 3% but no objective assessment was collected. The peripheral blast count remained unchanged until cycle 11, when it increased to 4%. Study drug was permanently discontinued after cycle 11 due

to a related, recurrent grade 3 gastrointestinal hemorrhage. According to the data, the patient was transfusion independent until just before discontinuation.

A second responding patient with incomplete count recovery was assigned to the 60 mg twice daily cohort and diagnosed with *de novo* AML, FLT3- internal tandem duplication by central and local testing, and a poor risk classification. The patient had been treated previously with intensive chemotherapy, sorafenib, and midostaurin. The patient entered the study with a bone marrow biopsy and aspirate blast count of 11% and 72%, respectively, and a peripheral blast count of 3% on cycle 1 day 1 as a result of ongoing hydroxyurea use. The patient achieved an incomplete count recovery by the end of cycle 1 with a reduced bone marrow biopsy and aspirate blast count of 1% and a peripheral blast count of 0%. In cycle 2, despite ongoing disease control in the marrow with a bone marrow biopsy and aspirate blast count of 0% and 11%, respectively, the patient's response changed to clinical benefit despite progressive disease on account of study drug being held for the majority of the cycle for grade 3 parainfluenza virus infection and grade 2 cerebral hemorrhage. The patient was maintained on study treatment for 4 additional cycles with intermittent dose modification due to grade 3 infection and grade 2 pneumonitis, before stopping due to altered mental status.

Supplementary Tables

Supplementary Table 1. Hemorrhagic events on study. Values are number (%).

Hemorrhagic event	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 any bleed	3 (75)	5 (71)	2 (50)	3 (60)	6 (67)	5 (63)	6 (100)	30 (70)
Epistaxis	1 (25)	3 (43)	-	1 (20)	1 (11)	3 (38)	2 (33)	11 (26)
Gastrointestinal hemorrhage	-	1 (14)	-	1 (20)	1 (11)	1 (13)	1 (17)	5 (12)
Gastric hemorrhage	-	-	-	-	-	-	4 (67)	4 (9)
Gingival bleeding	1 (25)	1 (14)	1 (25)	-	-	-	1 (17)	4 (9)
Rectal hemorrhage	-	1 (14)	-	1 (20)	1 (11)	-	1 (17)	4 (9)
Hematochezia	-	-	1 (25)	-	1 (11)	-	1 (17)	3 (7)
Hemoptysis	-	1 (14)	-	-	-	1 (13)	1 (17)	3 (7)
Melaena	1 (25)	-	-	-	-	1 (13)	1 (17)	3 (7)
Retinal hemorrhage	-	-	-	-	-	1 (13)	2 (33)	3 (7)
Subdural hematoma	1 (25)	-	-	-	1 (11)	-	1 (17)	3 (7)
Hematuria	-	-	-	-	-	1 (13)	2 (33)	3 (7)
Mouth hemorrhage	1 (25)	1 (14)	-	-	-	-	1 (17)	3 (7)

Pharyngeal hemorrhage	-	-	-	1 (20)	-	-	1 (17)	2 (5)
Subarachnoid hemorrhage	-	-	-	1 (20)	1 (11)	-	-	2 (5)
Hematemesis	-	-	-	-	-	-	2 (33)	2 (5)
Cerebral hemorrhage	-	-	-	-	-	1 (13)	-	1 (2)
Conjunctival hemorrhage	-	-	-	-	1 (11)	-	-	1 (2)
Diarrhea hemorrhagic	-	1 (14)	-	-	-	-	-	1 (2)
Gastritis hemorrhagic	-	1 (14)	-	-	-	-	-	1 (2)
Hemarthrosis	-	1 (14)	-	-	-	-	-	1 (2)
Hemorrhage intracranial	-	-	-	-	-	-	1 (17)	1 (2)
Lower gastrointestinal hemorrhage	-	1 (14)	-	-	-	-	-	1 (2)
Esophageal hemorrhage	-	-	-	-	-	-	1 (17)	1 (2)
Oral mucosa hematoma	-	1 (14)	-	-	-	-	-	1 (2)
Upper gastrointestinal hemorrhage	-	-	-	-	1 (11)	-	-	1 (2)

BID: twice daily; QD: once daily