

## Brief report

## Compassionate use of sorafenib in FLT3-ITD–positive acute myeloid leukemia: sustained regression before and after allogeneic stem cell transplantation

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**Acute myeloid leukemia (AML) patients with internal tandem duplication (ITD) mutations in the Fms-like tyrosine-3 (FLT3) gene have a dismal prognosis. Here we report compassionate-use results with the multikinase and FLT3-ITD inhibitor sorafenib for the treatment of relapsed or refractory FLT3-ITD–positive AML. Sorafenib induced clinically meaningful and**

**very rapid responses in all 6 patients treated either before (n = 2), after (n = 3), or both before and after (n = 1) allogeneic stem cell transplantation (allo-SCT). Sorafenib-induced remissions facilitated allo-SCT in 2 of the 3 refractory patients. Two of the 4 patients who were treated after allo-SCT survived 216 and 221 days, respectively, whereas the other 2 remain**

**in ongoing complete molecular remission. Sorafenib response was associated with an inhibition of the antiapoptotic FLT3-ITD target Stat-5 in vivo. Together, sorafenib monotherapy before or after allo-SCT has remarkable clinical activity in poor risk FLT3-ITD–positive AML and deserves further evaluation in prospective clinical trials. (Blood. 2009;113:6567-6571)**

## Introduction

Acute myeloid leukemia (AML) is the most frequent acute leukemia of adults and has an unfavorable prognosis.<sup>1</sup> In 20% to 30% of the AML patients, mutations in the Flt3-receptor tyrosine kinase (Flt3-RTK) occur, leading to internal tandem duplications in the juxtamembrane domain of the receptor (FLT3-ITD).<sup>2,3</sup> FLT3-ITD dictates a particularly poor clinical outcome.<sup>4-8</sup> Therefore, several specific FLT3 inhibitors have been developed and evaluated in clinical trials.<sup>9</sup> However, their overall clinical efficacy in AML must so far be considered as minor.<sup>9</sup>

Sorafenib (Nexavar, formerly BAY 43-9006; Bayer HealthCare) has been approved for the treatment of metastatic renal cancer and advanced hepatocellular carcinoma.<sup>10</sup> It inhibits the serine threonine kinase RAF-1, but also the FLT3-RTK and FLT3-ITD,<sup>11</sup> suggesting that it may have a role also in AML.<sup>12</sup> Sorafenib was recently tested in a phase I clinical trial on 16 patients with AML and was found to be particularly active in 6 of the 7 Flt3-ITD–positive patients.<sup>11</sup> However, treatment duration was short (21-70 days), and no durable responses were reported. Notably, a complete molecular remission has recently been reported in a patient relapsing after stem cell transplantation (SCT).<sup>13</sup> We here show, in a cohort of 6 FLT3-ITD–positive AML patients, that single-agent sorafenib given on a compassionate-use basis led to notable long-term responses in refractory and relapsed disease before and after allogeneic SCT (allo-SCT).

## Methods

## Patients, treatment, and objective

From November 2007 to November 2008, refractory or relapsed Flt3-ITD–positive AML patients (n = 6) before or after allo-SCT were treated after

informed consent at the University of Marburg with sorafenib on a compassionate-use basis. Treatment was performed in the absence of alternative therapeutic options outside a clinical trial. The initial dose was 2 × 400 mg orally daily and was adjusted in case of cytopenia, suspected toxicity, or resistance (dose range, 200-800 mg daily).

## Response

Treatment response was monitored and remission was defined according to standard criteria<sup>14</sup>: (1) complete remission (CR): marrow blasts less than 5%, neutrophils more than 10<sup>9</sup>/L, platelets more than 100 × 10<sup>9</sup>/L for at least 4 weeks; (2) bone marrow response (BMR): marrow blasts reduction by more than 50% from start of sorafenib without hematologic recovery; (3) hematologic response (HR): disappearance of blasts from the peripheral blood; and (4) complete molecular response (CMR): CR plus molecular negativity for FLT3-ITD by polymerase chain reaction.

## Intracellular p-STAT-5 staining

Intracellular staining with phycoerythrin-conjugated antiphospho-STAT-5 (Y694) was performed as previously described<sup>15</sup> after informed consent was obtained in accordance with the Declaration of Helsinki, according to a vote of the local ethics committee of the University of Marburg.

## Results and discussion

## Sorafenib monotherapy in relapsed FLT3-ITD–positive AML after allo-SCT

Four patients (median age, 50 years; range, 42-62 years) with relapsed FLT3-ITD–positive AML after allo-SCT were treated with

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Table 1. Patient response details

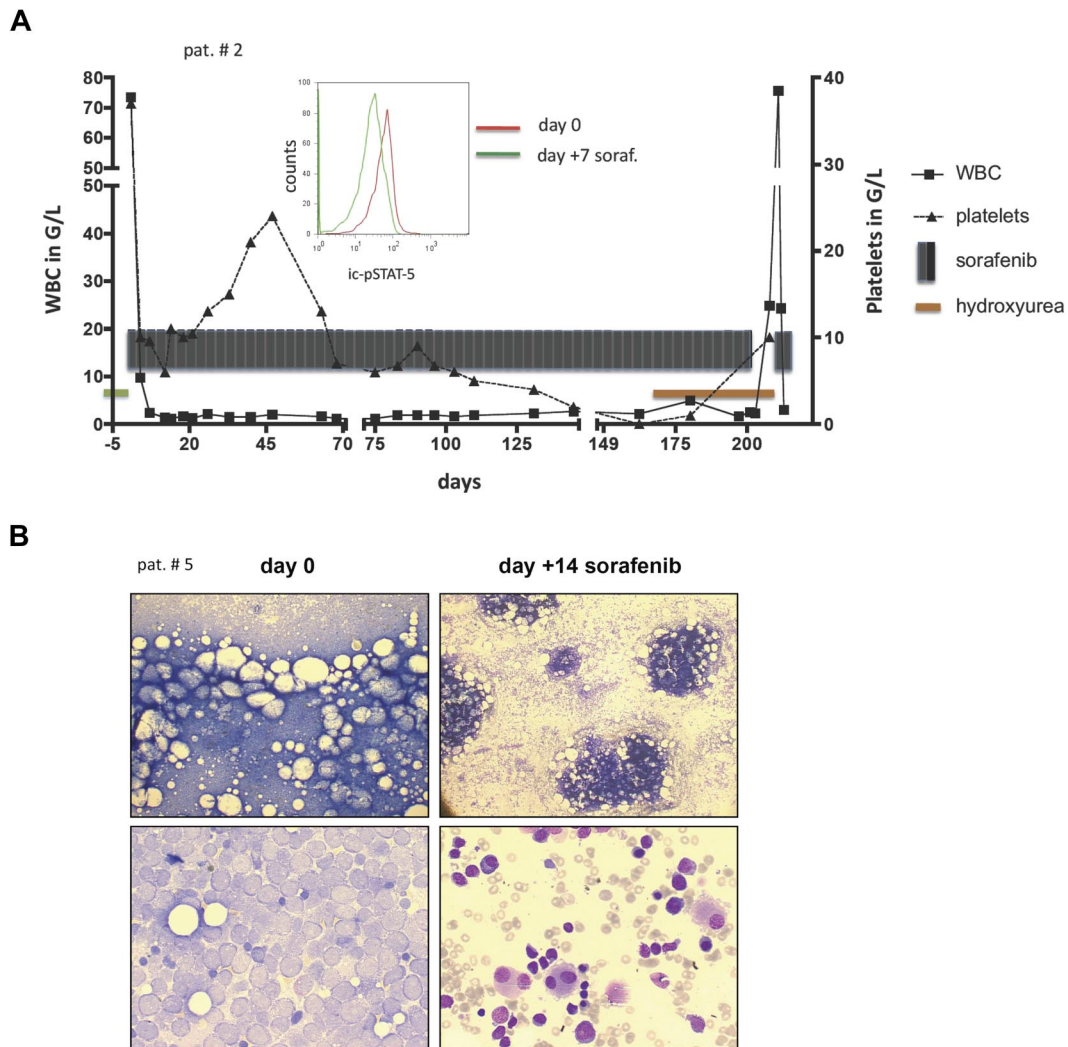
Patient no.	Clinical status before sorafenib	Dose, mg	Treatment duration, days	WBC, × 10 <sup>9</sup> /L (blasts %)		Best response	Side effects (grade*)	Emergence of sorafenib resistance	Outcome at last visit on sorafenib (grade*)
				Before sorafenib	After 7 days of sorafenib				
<b>Sorafenib after allo-SCT</b>									
1	Relapse day 87 after allo-SCT	200-800	211	39.6 (78)	1.5 (14)	HR, BMR	Neutropenia (IV), thrombopenia (IV), pneumonia hemolysis	Yes	Exitus letalis on day 216 in relapse
2	Relapse day 168 after allo-SCT	200-800	221	73.5 (84)	2.45 (0)	HR, BMR	Neutropenia (IV), thrombopenia (IV)	No	Exitus letalis on day 221 with cerebral mass, neutropenia (IV), thrombopenia (IV)
3	Relapse day 322 after allo-SCT	800	81	18.6(80.inBM)	5.94(NA)	CMR, CR	Maculopapulous exanthema of the skin (GVHD)	No	CR and ongoing CMR with sorafenib
<b>Sorafenib before allo-SCT</b>									
4	Relapse day 30 after first consolidation cycle	400-800	50	44 (91)	1.54 (6)	HR, BMR	Neutropenia (IV), thrombopenia (III), sepsis	No	Exitus letalis on day 58 in sepsis, neutropenia (IV), thrombopenia (IV)
5	Primary refractory	800	13†	36.2 (94)	0.6 (40)	HR, BMR,‡ CMR	No	No	Relapse on day 111 after allo-SCT, sorafenib monotherapy 400 mg twice a day → ongoing CMR
6	Primary refractory	800	82	8 (26)	8 (1)	HR	Hand-foot syndrome, hyperkeratosis	Yes	Relapse on day 71 RIC-allo-SCT → CR

WBC indicates peripheral white blood cell count; allo-SCT, allogeneic stem cell transplantation; HR, hematologic remission; BMR, bone marrow remission; CMR, complete molecular remission; CR, complete remission; and RIC, reduced-intensity conditioning therapy.

\*According to the National Cancer Institute common toxicity criteria, version 2 (<http://www.fda.gov/cder/cancer/toxicityframe.htm>).

†This patient was treated for only 14 days before SCT, when a donor became available and conditioning could be started. A hematologic relapse occurred 111 days after SCT, and the patient responded again to sorafenib (400 mg twice a day), achieving a CMR.

‡BMR is not according to Cheson et al.<sup>14</sup> Bone marrow was hypocellular (Figure 1B); but after only 14 days, nucleated cells consisted of still more than 50% blasts.



**Figure 1. Sorafenib treatment response.** (A) Patient 2: A fast response to sorafenib was associated with a dephosphorylation of the antiapoptotic FLT3-ITD target STAT-5, measured intracellularly in the blastic population by fluorescence-activated cell sorter (middle panel histogram): the left shift of the green curve documents a decrease in intracellular STAT-5 phosphorylation 7 days after commencing sorafenib compared with baseline (day 0, red line). Sorafenib was withdrawn on day 204 of sorafenib and hydroxyurea commenced because of a newly diagnosed cerebral mass. AML was resistant to hydroxyurea (peripheral white blood cell count increase to  $75.6 \times 10^9/L$ ), but reexposure to sorafenib again led to an instant response (white blood cell count decline to  $1.3 \times 10^9/L$ ). (B) Patient 5: Bone marrow light microscopy to evaluate bone marrow response (patient 5) before allo-SCT. Giemsa staining shows blast infiltration before (left panels) and extensive blast clearance 14 days after commencing sorafenib (right panels). Low and high magnification of bone marrow smear (top and bottom panels, respectively). Images were viewed with an Olympus BH-2 microscope using Giemsa stain; left panel,  $10\times/0.3$  numeric aperture (NA); right panel,  $60\times/1.4$  NA oil objective. Images were taken with a Sony Exwave HAD camera using DHS Bilddatenbank software (Dietermann & Heuser Solution GmbH, Greifenstein-Beilstein, Germany).

sorafenib on a compassionate-use basis. The median time from allo-SCT to relapse was 192 days (range, 87-322 days). All patients had a normal karyotype (treatment and response details, Table 1).

Patient 1 was a 62-year-old man with AML-M4 (*FLT3*-ITD ratio, 11.4). After daunorubicin/cytarabine (DA) induction therapy and one consolidation cycle, he underwent allo-SCT in first CR but relapsed. He obtained a rapid HR and BMR under sorafenib. Because of leucopenia, sorafenib was repeatedly paused and the sorafenib dose level was reduced. The patient died of AML progression that was resistant to sorafenib on day 216 (Table 1).

Patient 2 was a 42-year-old woman with normal karyotype AML-M4 (*FLT3*-ITD ratio, 1.15). After 2 DA-induction cycles, she underwent allo-SCT in first CR and relapsed despite experiencing graft-versus-host disease (GVHD; intestine and skin). With sorafenib at 400 mg orally twice a day, she obtained a rapid and sustained HR and BMR (Figure 1A; Table 1). Sorafenib inhibited the antiapoptotic FLT3-ITD target STAT-5 in vivo (Figure 1A). Concomitantly to an increasing donor chimerism (from 47% to

91%), GVHD reoccurred, indicating that sorafenib did not compromise a graft-versus-leukemia response. The patient died on day 221 of sorafenib but remained essentially sorafenib-sensitive (Figure 1A).

Patient 3 was a 46-year-old woman with FLT3-ITD-positive AML-M2 (*FLT3*-ITD ratio, 4.2). After 2 DA-induction cycles, she underwent allo-SCT in first CR but relapsed (Table 1). She also had experienced chronic GVHD of the skin, joints, and polyserositis requiring immune suppressive therapy with steroids. The relapse was treated with sorafenib 400 mg orally twice a day; and within 2 months, a still ongoing CMR was achieved.

#### Sorafenib facilitates allo-SCT in chemotherapy-refractory FLT3-ITD-positive AML

Three patients were rescued with sorafenib before allo-SCT. The median age of these patients was 40 years (range, 38-57 years).

Patient 4 was a 56-year-old man with FLT3-ITD–positive (*FLT3*-ITD ratio, 1.16) AML, who relapsed after one cytarabine consolidation cycle. The patient's clinical status was poor. Under 400 mg sorafenib, twice a day, he instantly achieved a HR (Table 1) and thrombopoiesis recovered in part (Figure S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Sorafenib inhibited STAT-5 activation in AML blasts in vivo, confirming in vivo the presumed mode of action of sorafenib via inhibiting antiapoptotic targets of FLT3-ITD (Figure S1).

Patient 5 was a 40-year-old woman with AML-M5a (*FLT3*-ITD ratio, 0.8) experiencing primary induction failure. Sorafenib was commenced; and within 14 days of treatment, a fast HR and BMR were seen (Figure 1B). The patient underwent allo-SCT using a dose-reduced conditioning regimen.<sup>16</sup> She achieved a CR but relapsed again on day 111 after allo-SCT. Sorafenib was restarted at 400 mg twice a day, and a still ongoing CMR was obtained (Table 1).

Patient 6 was a 38-year-old man with primary chemotherapy–refractory AML-M2. He had the lowest *FLT3*-ITD ratio in this cohort (0.39). A donor was not available, and sorafenib was commenced at 400 mg twice a day on an outpatient basis. An HR was achieved (Table 1). The patient relapsed on day 71 of sorafenib; but at this time, allo-SCT could be performed. The patient remained in CR at last visit.

Here we support and significantly extend initial findings on the efficacy of sorafenib monotherapy in AML.<sup>11,13</sup> Apparently, sorafenib has a consistent activity in relapsed and refractory FLT3-ITD–positive AML. With a median treatment duration of 158 days after allo-SCT (range, 90–221 days), only 2 patients developed sorafenib drug resistance, whereas 2 patients obtained an ongoing CMR. Based on these observations and the limited literature on sorafenib monotherapy in AML, it is tempting to speculate that sorafenib may be most effective when applied in the context of an allo-SCT because it has the potential to reduce leukemic burden, without compromising the restoration of a graft-versus-leukemia response (patients 2, 3, and 5). This is similar to the successful scenario seen with preemptive imatinib treatment after allo-SCT in BCR/ABL<sup>+</sup> acute lymphatic leukemia (Ph<sup>+</sup> ALL).<sup>17</sup>

However, sorafenib could also be a valuable compound to bridge the time to allo-SCT. It spared chemotherapy toxicity while still exerting antileukemic activity (patients 5, 6). Equally important in this regard, circumventing ineffective cycles of chemotherapy before allo-SCT was shown to translate into better overall

survival in high-risk AML.<sup>16,18</sup> Notably, sorafenib did not adversely affect engraftment in patients 5 and 6. However, long-term sorafenib treatment was associated with neutropenia and thrombopenia in many patients. This is not typically seen during sorafenib treatment of renal or hepatic cancer<sup>10</sup> but may be explained by the compromised normal bone marrow reservoir in relapsed AML after allo-SCT.

Taken together, the role of sorafenib monotherapy in FLT3-ITD–positive AML deserves evaluation in clinical trials. Based on its ability to induce CMRs in relapsed patients after allo-SCT, a prophylactic treatment strategy after allo-SCT may hold the promise to markedly improve the poor outcome of FLT3-ITD–positive AML.

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## Authorship

Contribution: A.B., A.N., and S.M. designed the research and analyzed the data; A.B. wrote the paper; S.M., Y.W., M.W., S.T., and A.C. performed the in vitro research; A.B., E.W., S.M., and A.N. treated the patients; and M.W., E.E., and M.E. contributed vital reagents and analyzed the data.

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