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Compassionate-use of sorafenib in Flt3-ITD positive acute myeloid leukemia: sustained regression prior and post allogenic stem cell transplantation

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Running title: Sorafenib for relapsed Flt3-ITD+AML prior and post stem cell transplantation

Abstract

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 multi-kinase-, 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 six patients treated either prior (n=2), post (n=3), or both, prior and post (n=1) allogenic stem cell transplantation (allo-SCT). Sorafenib-induced remissions facilitated allo-SCT in two of the three refractory patients. Two of the four patients that were treated after allo-SCT survived 216 and 221 days, respectively, while the other two remain in ongoing complete molecular remission. Sorafenib-response was associated with an inhibition of the anti-apoptotic Flt3-ITD target Stat-5 in vivo.
Together, sorafenib-monotherapy prior or post allo-SCT has remarkable clinical activity in poor risk Flt3-ITD-positive AML and deserves further evaluation in prospective clinical trials.

Introduction

Acute myeloid leukemia (AML) is the most frequent acute leukemia of adults and has an unfavorable prognosis [1]. In 20-30% 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) [2,3]. Flt3-ITD dictates a particularly poor clinical outcome [4-8]. Therefore, several specific Flt3-inhibitors have been developed and evaluated in clinical trials (for review see ref. [9]). However, their overall clinical efficacy in AML must so far be considered as minor [9].

Sorafenib (Nexavar ™, formerly BAY 43-9006) has been approved for the treatment of metastatic renal cancer and advanced hepatocellular carcinoma [11]. It inhibits the serine threonine kinase Raf-1, but also the Flt3-RTK, and Flt3-ITD [10], suggesting that may have a role also in AML [12]. Sorafenib was recently tested in a phase I clinical trial on 16 patients with AML, and was found to be particularly active in six of the seven Flt3-ITD positive patients [10]. 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 SCT [13]. We here show, in a cohort of six 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 prior and post allogenic stem cell transplantation (allo-SCT).

Material and Methods

Patients, treatment and objective

From November 2007 to November 2008 refractory or relapsed Flt3-ITD positive AML patients (n=6) prior or post 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 x 400mg orally (p.o.) daily, and was adjusted in case of cytopenia, suspected toxicity or resistance (dose range: 200-800mg daily).

Response

Treatment response was monitored and remission was defined according to standard criteria [14]: i) complete remission (CR): marrow blasts < 5%, neutrophils > 1 $x10^9$/L, platelets > 100 $x10^9$/L for at least 4 weeks; ii) bone marrow response (BMR): marrow blasts reduction by > 50% from start of sorafenib without hematologic recovery; iii) hematologic response (HR): disappearance of blasts from the peripheral blood; iv) complete molecular response (CMR): CR plus molecular negativity for Flt3-ITD by polymerase chain reaction.

Intracellular p-Stat-5 staining

Intracellular staining with phycoerythrin (PE)-conjugated anti-phospho-Stat-5 (Y694) was performed as previously described [15] after informed consent was given, in accordance with the Declaration of Helsinki, according to a votum 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) with relapsed Flt3-ITD positive AML after allo-SCT were treated with sorafenib on a compassionate-use basis. The median time from allo-SCT to relapse was 192 days (range, 87 to 322 days). All patients had a normal karyotype. For treatment and response details see Table 1.

Patient #1 was a 62-year-old male 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. Due to leucopenia sorafenib was repeatedly paused and the sorafenib dose level was reduced. The patient died from AML-progression that was resistant to sorafenib on day +216 (Table 1).

Patient # 2 was a 42-year-old female with normal karyotype AML-M4 (Flt3-ITD-ratio: 1.15). After two DA-induction cycles she underwent allo-SCT in first CR and relapsed despite experiencing a graft versus host disease (GvH) (intestine and skin). With sorafenib at 400mg p.o. bid, she obtained a rapid and sustained HR and BMR (Figure 1A, Table 1). Sorafenib inhibited the anti-apoptotic Flt3-ITD-target Stat-5 in vivo (Figure 1A). Concomitantly to an increasing donor chimerism (from 47% to 91%) GvH re-occurred, indicating that sorafenib did not compromise a GvL response. The patient died on day+221 of sorafenib, but remained essentially sorafenib-sensitive (Figure 1A).

Patient #3 was a 46-year-old female with Flt3-ITD positive AML-M2 (Flt3-ITD-ratio: 4.2). After two DA-induction cycles she underwent allo-SCT in first CR, but relapsed (Table 1). She also had experienced chronic GvH of the skin, joints, and poly-serositis

requiring immune suppressive therapy with steroids. The relapse was treated with sorafenib 400mg p.o. bid and within two month a still ongoing CMR was achieved.

Sorafenib facilitates allogenic stem cell transplantation 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 male 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 400mg sorafenib, bid, he instantly achieved a HR (Table 1) and thrombopoiesis recovered in part (Supplemental Figure 1). Sorafenib inhibited Stat-5 activation in AML blasts in vivo, confirming in vivo the presumed mode of action of sorafenib via inhibiting anti-apoptotic targets of Flt3-ITD (Supplemental Figure 1).

Patient #5 was a 40-year-old female 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 was seen (Figure 1B). The patient underwent allo-SCT using a dose-reduced conditioning regimen [16]. She achieved a CR, but relapsed again on d+111 post allo-SCT. Sorafenib was re-started at 400mg bid, and a still ongoing CMR was obtained (Table 1).

Patient #6 was a 38-year-old male 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 400mg bid on an outpatient basis. A 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 [10 13]. Apparently, sorafenib has a consistent activity in relapsed and refractory Flt3-ITD-positive AML. With an median treatment duration of 158 days post allo-SCT (range, 90-221), only two patients developed sorafenib drug resistance, whereas two 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 GvL response (pat #2, #3, and #5). This reminds on the successful scenario seen with pre-emptive imatinib treatment after allo-SCT in BCR/ABL+ acute lymphatic leukemia (Ph+ALL) [17].

However, sorafenib could also be a valuable compound to bridge the time to allo-SCT. It spared chemotherapy-toxicity, while still exerting anti-leukemic activity (pat. #5, #6). Equally important in this regard, circumventing ineffective cycles of chemotherapy prior to allo-SCT was shown to translate into better overall survival in high-risk AML [16, 18]. 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 [11], but may be explained by the compromised normal bone marrow reservoir in relapsed AML post 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 CMR's 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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Authors contribution

A.B., A.N. and S.M. designed the research, 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. M.W., E.E., and M.E. contributed vital reagents and analyzed the data. None of the authors have declared any conflict of interest.

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Legend Table 1)

Abbreviations: SCT, allogenic stem cell transplantation; WBC, peripheral white blood cell count, HR, hematological remission, BMR, bone marrow remission; CMR, complete molecular remission, RIC, reduced intensity conditioning therapy.

[#] Patient was treated for only 14 days prior to SCT, when a donor became available and conditioning could be started. A hematological relapse occurred 111 days after SCT and the patient responded again to sorafenib (400mg bid), achieving a CMR.

[†] BMR is not according to ref. [14]. Bone marrow was hypocellular (Figure 1b) but after only 14 days, nucleated cells consisted of still more than 50% blasts.

[*] according to the National Cancer Institute common toxicity criteria, version 2 (http://www.fda.gov/cder/cancer/toxicityframe.htm)

Table 1. Individual patient response details; light grey: sorafenib post allo-SCT, grey: sorafenib prior to allo-SCT

Patient no.	Clinical status before sorafenib	Dose, mg	Treatment duration, days	WBC, x 10^9 /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				
1	relapse day +87 post allo-SCT	200mg to 800mg	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 post allo-SCT	200mg to 800mg	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 post allo-SCT	800mg	81	18.6 (80, in BM)	5.94 (n.a)	CMR, CR	maculo-papulous exanthema of the skin (GvH)	no	CR and ongoing CMR with sorafenib
4	relapse d+30 after first cons.	400mg to 800mg	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	800mg	13#	36.2 (94)	0.6 (40)	HR BMR† CMR	no	no	relapse on d+114 after allo-SCT, sorafenib monotherapy 400mg bid => ongoing CMR
6	primary refractory	800mg	82	8 (26)	8(1)	HR	hand-foot-syndrome hyperkeratosis	yes	relapse on d+71 RIC-allo-SCT => CR

Figure Legends

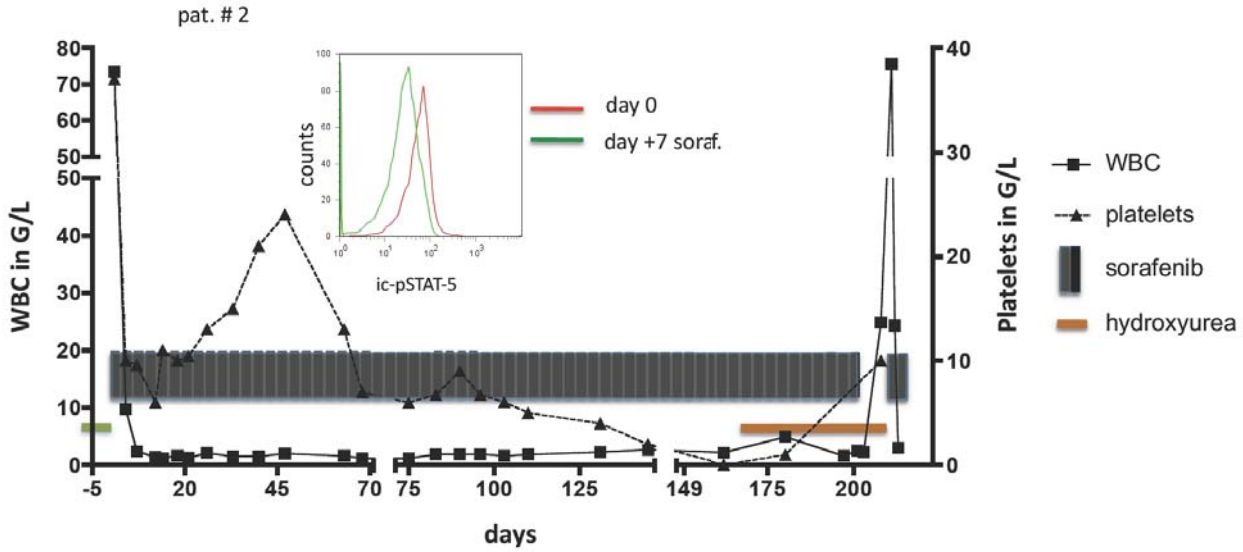
Figure 1)

Sorafenib treatment response

A) Patient #2: A fast response to sorafenib was associated with a dephosphorylation of the anti-apoptotic Flt3-ITD target Stat-5, measured intracellularly in the blastic population by FACS (middle panel histogram): the left shift of the green curve documents a decrease in intracellular Stat-5 phosphorylation 7 days after commencing sorafenib compared to 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 (WBC count increase to 75.6 x 10^9/ L), but re-exposure to sorafenib again led to an instant response (WBC decline to 1.3 x 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 prior (left panels) and extensive blast clearance 14 days after commencing sorafenib. Right panels: low and high magnification of bone marrow smear (upper and lower panels, respectively).

Figure 1

A.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WBC in G/L
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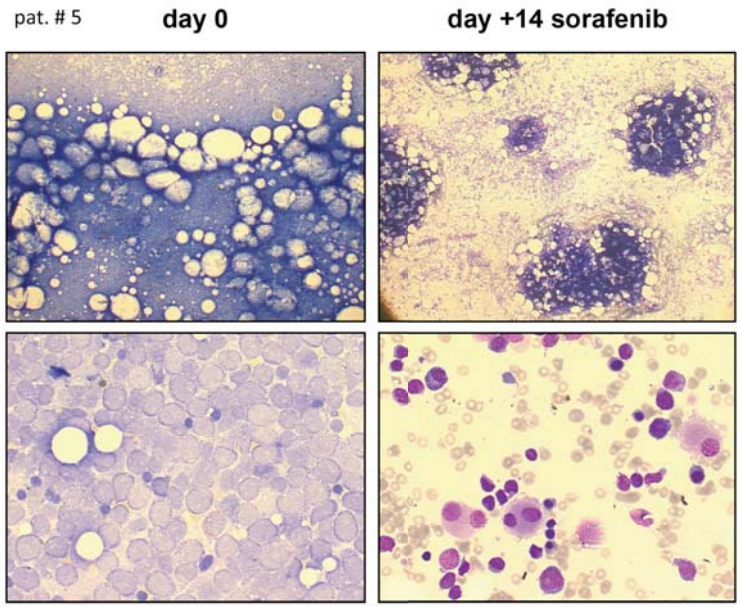
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