Use of sorafenib for post-transplant relapse in *FLT3/ITD*-positive acute myelogenous leukemia: maturation induction and cytotoxic effect

Internal tandem duplication (ITD) mutation of the *FLT3* receptor tyrosine kinase (*FLT3/ITD*) is one of the most common gene mutations in patients with acute myeloid leukemia (AML) and is a notoriously poor prognostic factor. The ligand-independent constitutive activation of the FLT3 kinase and its downstream signaling pathway stimulates AML cell proliferation.[1] It has been previously hypothesized that *FLT3/ITD* alone leads to myeloproliferation but does not lead to impaired differentiation of hematopoietic progenitors unless it occurs alongside other gene rearrangements, such as t(8;21) and t(15;17).[2] However, *in vitro* studies have shown that *FLT3/ITD* mutation can induce a differentiation block, and that targeted FLT3 tyrosine kinase inhibitors (TKIs) can overcome it. FLT3/ITD expression blocks G-CSF-mediated myeloid differentiation in murine myeloid cell progenitor lines and also represses transcription factors involved in myeloid maturation. But when those FLT3/ITD-expressing cell lines are treated with a FLT3 tyrosine kinase inhibitor (TKI), both phenomenona are reversed.[3]

Lestaurtinib and quizartinib are TKIs with activity against FLT3 whose *in vivo* use has been associated with myeloid maturation. Two out of 3 AML patients treated with lestuartinib experienced minimally reduced bone marrow blasts but increased markers of myeloid maturity (CD15 and CD11b).[4] A multicenter study of quizartinib treatment in 13 patients with relapsed/refractory FLT3/ITD-positive AML showed clearance of myeloid

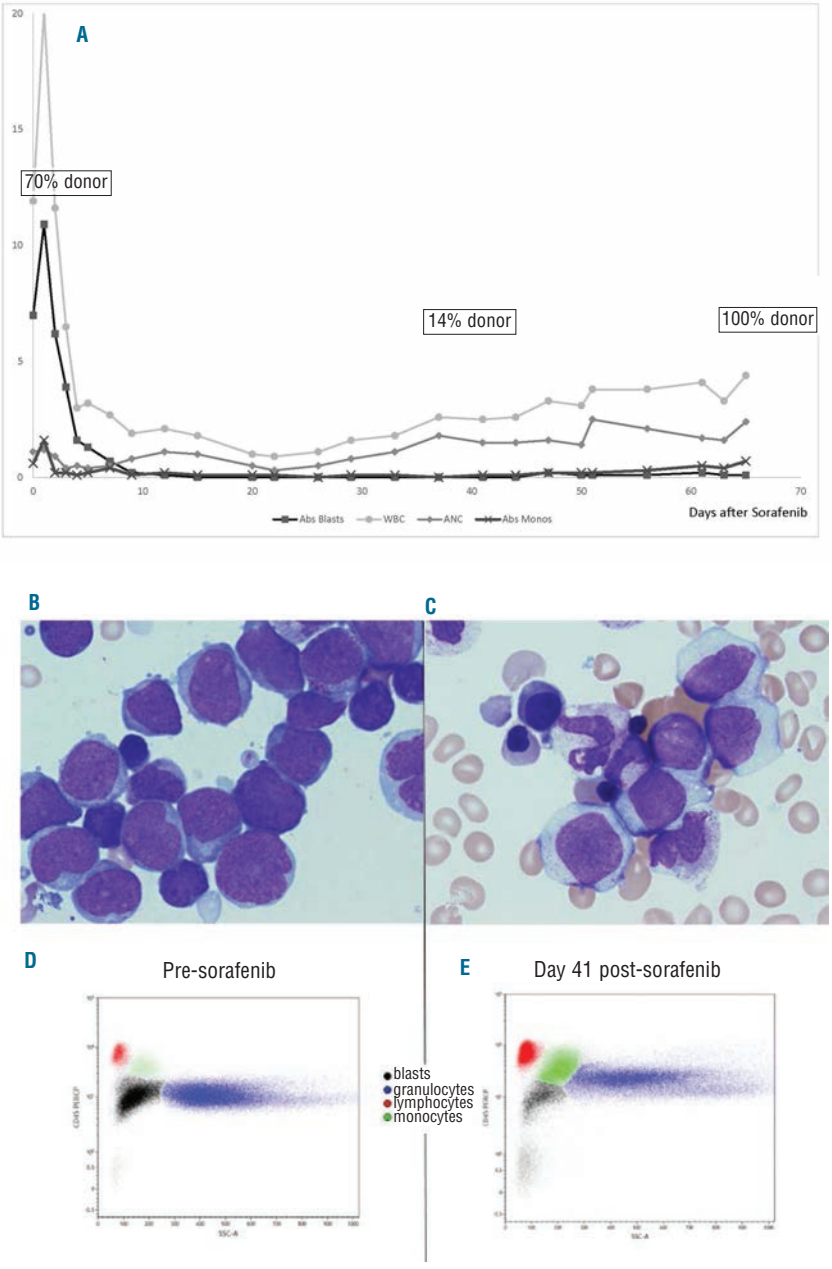

Figure 1. (A) Complete blood counts and differentiation indicates disappearance of blasts within 15 days with gradual increase in monocyte and neutrophil count on sorafenib. Boxes refer to results (as percentage) of chimerism analysis at various time points performed on bone marrow cells without selection for cell surface markers. Genomic DNA is extracted and amplified by PCR using a series of fluorescently labeled oligonucleotide primers specific for highly polymorphic genetic markers from which the pre and posttransplant specimens are compared. (B-E) The pre-sorafenib bone marrow aspirate (B) (Wright-Giemsa stain, 100X) showed a monotonous population of blasts (36% on aspirate differential) with scant basophilic cytoplasm, slightly irregular nuclear contours, fine chromatin and prominent nucleoli. In contrast, the Day 41 post-sorafenib biopsy (C) (Wright-Giemsa stain, 100X) had a lower blast percentage (9% on aspirate count), with the cellularity composed predominantly of erythroid precursors, maturing neutrophils, and monocytes. Pre-sorafenib flow cytometric evaluation of the aspirate (D) identified a blast population with dim CD45 and low side-scatter. Post-sorafenib flow cytometric evaluation of the aspirate (E) showed a smaller blast population with more events within the monocyte and lymphocyte gates.

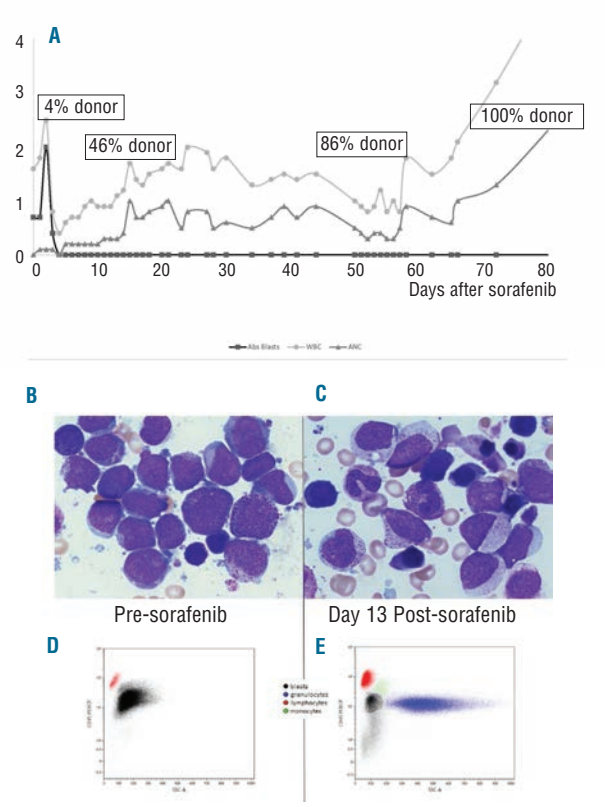

Figure 2. (A) Complete blood counts and differentiation show that peripheral blasts cleared in nine days on sorafenib treatment. Boxes refer to results (as percentage) of chimerism analysis at various time points performed on bone marrow cells without selection for cell surface markers. (B) The pre-sorafenib bone marrow sample, (Wright-Giemsa stain, 100X) showed numerous blasts (73% on aspirate count) with an increased nuclear to cytoplasmic ratio, fine chromatin, variably prominent nucleoli, and occasional azurophilic cytoplasmic granules. In contrast, the Day 13 post-sorafenib biopsy (C) (Wright-Giemsa stain, 100X) showed fewer blasts (6% on aspirate count) and greater numbers of neutrophil precursors, erythroid precursors, and monocytes. Pre-sorafenib flow cytometric evaluation of the aspirate (D) identified a large blast population with dim CD45 and low side-scatter. Post-sorafenib flow cytometric evaluation of the aspirate (E) showed a smaller blast population with more events within the granulocyte, lymphocyte, and monocyte gates.

blasts from the marrow with little or no change in cellularity and a concomitant increase in mature myelocytes, suggesting terminal differentiation.[5] Sorafanib is another TKI with activity against FLT3 that is increasingly employed in the treatment of AML, but has not been previously associated with myeloid maturation. In this report, we present 2 patients with *FLT3/ITD*-positive AML and relapsed disease after allogeneic hematopoietic cell transplant (alloHCT) treated with sorafenib. Both patients responded to the drug and one patient showed evidence of *in vivo* maturation that has not previously been reported.

Case 1: A 50-year old male presented with hyperleukocytosis and was diagnosed with AML. Bone marrow showed 87% myeloid blasts that were predominately negative for CD34. *FLT3/ITD* mutation and NPM1 exon 12 frameshift mutation were both present with a normal karyotype. Due to persistent (<1%) leukemic cells and *FLT3/ITD* positivity after induction chemotherapy, the patient was enrolled in an institutional trial of quizartinib for relapsed/refractory AML and achieved morphological and molecular remission after 28 days. He proceeded to myeloablative alloHCT from his sibling using cyclophosphamide 60 mg/kg x 2 doses with 8 fractions of total body irradiation (165 cGy). At Day 100 post-transplant evaluation, he remained in complete remission, but at Day 180 he was found to have relapsed disease on surveillance bone marrow biopsy with recurrence of *FLT3/ITD* and *NPM1* mutations. Bone marrow was 95% cellular with 36% blasts that were predominantly CD34 negative. In addition, there was a new complex cytogenetic abnormality involving two unrelated clones accounting for 65% of metaphases (13 of 20) on G-banding. Immunosuppression was rapidly tapered; he entered a clinical trial with IL-15 superagonist therapy but had no response. Although his initial disease responded to quizartinib, the trial was no longer available, so instead he was started on sorafenib in off-label use. Peripheral blasts cleared within 15 days with gradual increase in monocyte and neutrophil count (Figure 1A). Bone marrow biopsy performed after 40 days of treatment remained 65% cellular but with a reduction of blasts to 9%. Moreover, there was evidence of monocytic maturation on morphology and flow cytometry with brighter CD45 and slightly increased side-scatter (Figure 1B). Despite these improvements, donor chimerism of bone marrow cells decreased from 70% to 14% and *FLT3/ITD* and *NPM1* mutations persisted as well as the two unrelated clones, now 70% of metaphases (14 of 20). With improved control of disease but waning graft function he was treated with donor lymphocyte infusion (DLI) after 65 days of sorafenib and has been continued on the drug. He achieved complete morphological and molecular remission with resolution of prior cytogenetic abnormalities and 100% donor engraftment by one month; he currently remains in remission at three months post DLI and five months post sorafenib.

Case 2: A 56-year old female with a history of breast cancer in remission presented with pancytopenia and was diagnosed with treatment-related AML. Bone marrow biopsy identified 68% myeloid blasts, with partial CD34 positivity. *FLT3/ITD* mutation and *NPM1* exon 12 frameshift mutation were both present with a normal karyotype. Six weeks later, after induction chemotherapy, the patient was in first complete remission (CR1) with absence of *FLT3/ITD* and *NPM1* mutations. She underwent non-myeloablative matched sibling alloHCT as our institutional age limit for myeloablative conditioning is 55 years, using cyclophosphamide 50 mg/kg x 1 dose + fludarabine 30 mg/m^2 x 5 daily doses and one fraction of total body irradiation (200 cGy). She relapsed on Day +8 with circulating blasts and recurrence of *FLT3/ITD* and *NPM1* mutations. At the time of relapse, bone marrow cellularity was 40% with 70% blasts, of which 24% were CD34+. Immunosuppression was rapidly tapered and sorafenib was started. Peripheral blasts cleared in nine days (Figure 2A). At Day 13, there was evidence of granulocytic maturation on marrow morphology and flow cytometry with increased side-scatter (Figure 2B). At Day 50 post sorafenib, cellularity was similar but blasts were less than 1%. The patient has now been continued on sorafenib for 80 days and remains in remission. During this time period, donor chimerism of bone marrow cells increased from 4% to 86%, and is now 100%. *FLT3/ITD* and *NPM1* mutations initially persisted but became undetectable at Day 80. She remains in complete morphological and molecular remission while having been on the drug now for more than six months.

FLT3/ITD mutation confers an increased risk for relapse even after early bone marrow transplantation;[6] however,

use of sorafenib can still achieve sustained relapse-free remissions for up to two years duration.[7] Drug resistance occurs later than in patients who have not undergone transplant and there may be synergism with graft-*versus*-leukemia effects to induce durable remissions.[7] Case 1 demonstrates this pattern with the cytotoxic effect of sorafenib on leukemia blasts allowing for an eventual increase in donor engraftment and attainment of remission due to allogeneic-immune effects. In case 2, however, the response to relapsed leukemia occurred despite waning graft function and thus cannot be attributed to graft-*versus*-leukemia effect. Rather, the predominant mechanism is likely an induction of myeloid maturation. Initiation of sorafenib led to rapid blast clearance but the marrow remained hypercellular with persistence of *FLT3/ITD* mutation and the abnormal cytogenetic clones. The concurrent increased side scatter and rise in neutrophil count is suggestive of differentiation stemming from the leukemic precursors similar to that noted with prior reports of TKI-induced maturation.[5] Lastly, both patients harbored *NPM1* mutations, as is frequently seen in the setting of *FLT3/ITD* mutation in AML.[9] *NPM1* encodes nucleophosmin, which is involved in diverse cellular processes through its interactions with ribosomes and nucleic acids and but has no known tyrosine kinase activity.[9,10] Sorafenib, as a non-specific FLT3 inhibitor, targets other similar families of kinases[11] but does not directly affect the histone chaperone families like nucleophosmin protein (NPM). Therefore, sorafenib might have effects in downstream pathways of *NPM1* mutations or most likely the response seen with sorafenib in the above cases is solely dependent on *FLT3/ITD* mutation. Sorafenib can be more effective in a relapse setting given that leukemic cells have a higher mutant/wild-type FLT3 ratio at relapse (more addicted to *FLT3/ITD* to survive).[12] In a recent case series, 3 relapsed AML patients with *FLT3/ITD*+ and *NPM1*+ responded to a combination of sorafenib and all-trans-retinoic acid (ATRA).[13] The authors concluded that the addition of ATRA was important to evoke response due to the possible effects of ATRA on NPM1. However, given that approximately half of the patients with *FLT3/ITD* mutated AML patients have *NPM1* mutation and respond to a TKI alone,[14] it suggest that a possible agent that has effects on *NPM1* like ATRA may not be critical to the addition of a TKI to obtain clinical response in these patients.

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