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Use of Sorafenib for relapse posttransplant in FLT3/ITD+ acute myelogenous leukemia: maturation induction and cytotoxic effect

Running Title: Sorafenib-induced in vivo myeloid maturation

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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 notorious 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 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 phenomenons 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 three AML patients treated with lestaurtinib 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 AML showed clearance of myeloid blasts from the marrow with little or no change in cellularity and a concomitant increase in mature myelocytes, suggesting terminal differentiation [5]. Sorafenib 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 AML and relapsed disease after allogeneic hematopoietic cell transplant (alloHCT) treated with sorafenib. Both patients responded to the drug and one patient displayed evidence of in vivo maturation which 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 after induction chemotherapy, the patient was enrolled in an institutional trial of quizartinib for relapsed/refractory AML and achieved morphologic and molecular remission after 28 days. He proceeded to myeloablative alloHCT from his sibling using cyclophosphamide 60mg/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 which were predominantly CD34 negative. In addition there was a new complex cytogenetic abnormality involving two unrelated clones accounting for 65% of metaphases (13/20) on G-banding. Immune-suppression was rapidly tapered; he entered 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 he was instead started on sorafenib as an off-label use. Peripheral blasts cleared within 15 days with gradual increase in monocyte and neutrophil count (Figure 1.1). Bone marrow biopsy done after 40 days of treatment remained 65% cellular but with reduction of blasts to 9%. Moreover, there was an evidence of monocytic maturation on morphology and flow cytometry with brighter CD45 and slightly increased side-scatter (Figure 1.2). 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 (14/20). 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 morphologic and molecular remission with resolution of prior cytogenetic
abnormalities and 100% donor engraftment by one month and remains in remission now three months post-DLI and now 5 months post-sorafenib.

Case 2

A 56-year-old female with 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 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, using cyclophosphamide 50 mg/kg x 1 dose + fludarabine 30 mg/m² 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+. Immune-suppression was rapidly tapered and sorafenib was started. Peripheral blasts cleared in 9 days (Figure 2.1). At day 13 there was evidence of granulocytic maturation on marrow morphology and flow cytometry with increased side scatter (Figure 2.2). At day 50 post sorafenib, cellularity was similar but blasts were <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 morphologic and molecular remission while continued on the drug now more than 6 months.

FLT3/ITD mutation confers an increased risk for relapse even after early bone marrow transplantation [6]; however sustained relapse-free remissions with use of sorafenib can still be attained up to two years [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 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 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 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 in AML [8]. NPM1 encodes nucleophosmin, which is involved in diverse cellular processes through its interactions with ribosomes and nucleic acids and 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 relapse setting given 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 approximately half of the patients with FLT3/ITD mutated AML patients have NPM1 mutation and respond to a TKI alone [14], it suggests that an agent possible effect on NPM1 like ATRA may not be critical to add a TKI to obtain clinical response in these patients.
References


Figure Legends

**Figure 1.1** 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.

**Figure 1.2** The pre-sorafenib bone marrow aspirate (A, 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 (B, 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 (C) identified a blast population with dim CD45 and low side-scatter. Post-sorafenib flow cytometric evaluation of the aspirate (D) showed a smaller blast population with more events within the monocyte and lymphocyte gates.

**Figure 2.1** Complete blood counts and differentiation show that peripheral blasts cleared in 9 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.

**Figure 2.2**. The pre-sorafenib bone marrow sample (A, 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 (B, 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 (C) identified a large blast population with dim CD45 and low side-scatter. Post-sorafenib flow cytometric evaluation of the aspirate (D) showed a smaller blast population with more events within the granulocyte, lymphocyte, and monocyte gates.
Figure 2.2

Pre-sorafenib

Day 13 Post-sorafenib

C

D

- blasts
- granulocytes
- lymphocytes
- monocytes