Erythroid differentiation of myeloblast induced by gilteritinib in relapsed *FLT3-ITD*–positive acute myeloid leukemia

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The article by McMahon et al described 2 patterns of responses to gilteritinib treatment in acute myeloid leukemia (AML) patients with FLT3-internal tandem duplication (*ITD*) mutation: responses with and without hematopoietic differentiation.[1] In this study, gilteritinib treatment resulted in myeloid differentiation in almost one-half of AML patients (10/21, 47.6%) with granulocytic hyperplasia without dysplastic changes. Interestingly, the proportion of erythroid lineage was unaffected or hypoplastic.

In contrast to those findings in the article, here we describe a unique differentiation pattern of myeloblasts into erythroid lineage induced by gilteritinib highlighting biological impacts of pharmacological blockade in *FLT3* signaling.

A 63-year-old female with no significant medical history presented with fever, shortness of breath, and generalized weakness. Physical examination at presentation was unremarkable. A complete blood count showed a white blood cell count of 137.6×10^9/L with 38% blasts and 42% monocytes on differential, hemoglobin 8.0 g/dL, and platelet 72×10^9/L. A peripheral blood smear showed numerous monocytes with blasts (Figure 1A). A bone marrow biopsy revealed 95% hypercellular marrow and 86% myeloblasts with monocytic differentiation (Figure 1B). *NPM* immunostain of the biopsy demonstrated both nuclear and cytoplasmic *NPM* immunopositivity, indicating the presence of an *NPM1* mutation (Figure 1C). Flow cytometry showed bright CD45; bright cytoplasmic myeloperoxidase; dim CD117, CD13, CD33; and dim CD4, HLA-DR, CD38. A small subset expressed CD34 (13%) and CD7 (12%). Cytogenetics showed a normal karyotype, and a molecular study demonstrated *NPM1* and *FLT3-ITD* mutations. The mutated-to-unmutated peak height ratio for *FLT3-ITD* (*FLT3-ITD* allelic ratio) was 0.8. Unfortunately, midostaurin was not available to the patient during hospitalization for induction chemotherapy. Hence, the patient underwent induction chemotherapy with high-dose cytarabine and mitoxantrone. A bone marrow biopsy at day 14 postinduction showed no evidence of leukemia. The patient initially refused allogeneic hematopoietic stem cell transplantation (HCT) and elected consolidation chemotherapy alone with 4 cycles of high-dose cytarabine and midostaurin. At the end of consolidation therapy, her complete blood count showed white blood cells 3.12×10^9/L with hemoglobin 10.1 and platelet 56×10^9/L (Figure 1D); a bone marrow biopsy revealed hypercellular marrow (80%) and 47% pleiomorphic blasts in marrow aspirates (Figure 1E). Flow cytometry identified 30% of aberrant myeloblasts expressing CD117, CD13, CD33, and CD7 with a subset expressing CD34. Myeloid:erythroid ratio (ME ratio) was 0.4:1, with erythroid precursors 35%. Immunostain for *NPM1* showed an abnormal pattern of nuclear and cytoplasmic staining, indicating presence of the mutation (Figure 1F). A variable allelic frequency of *FLT3-ITD* from peripheral blood at relapse was 21.5%, equivalent to allelic ratio 0.28, and the length of *ITD* insertion was 120 bp [*FLT3* c.1837+12_1837+13ins(120)]. She was started on gilteritinib 120 mg daily and she tolerated the treatment well. A bone marrow biopsy after a 2-month gilteritinib treatment demonstrated a remarkable improvement; a peripheral blood smear failed to detect circulating blasts (Figure 1G) and the cellularity of bone marrow decreased to 30% with 1% blasts in the aspirates and abundance of dysplastic erythroid precursors (Figure 1H). Flow cytometry showed 2% of myeloblasts with aberrant CD7 expression. *NPM* immunostain of the biopsy again demonstrated the mutant staining pattern with nuclear and cytoplasmic reactivity in almost all of the marrow cells (Figure 1I), the majority of which were normoblasts with marked dysplasia in the aspirate smear. Interestingly, the distribution of erythroid precursors identified by E-cadherin[2] and CD71[3] in the bone marrow before and after initiation of gilteritinib treatment were within *NPM*-stained areas, indicating that erythroid precursors were differentiated from mutant myeloblasts (Figure 1J-M). Furthermore, *FLT3-ITD* allelic ratio increased to 2.8 and the karyotype remained normal after initiation of gilteritinib. Notably, the ME ratio strikingly decreased to 0.2:1, with 80% of normoblasts indicating skewed differentiation of
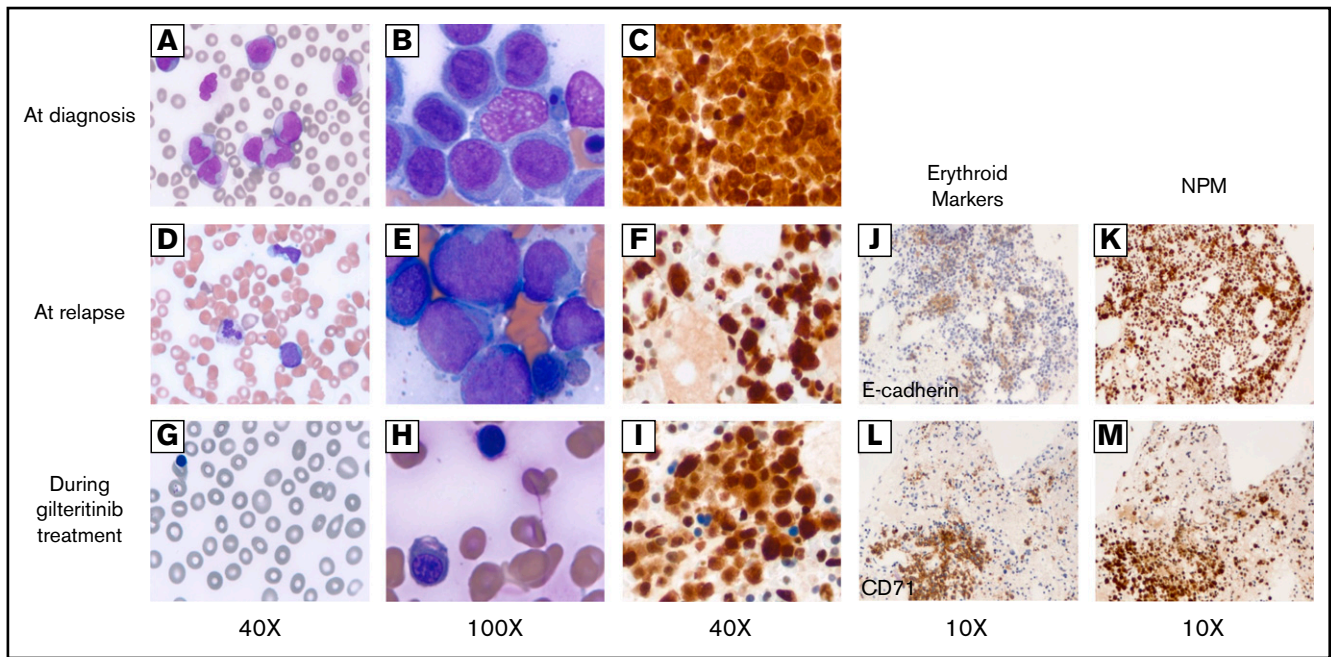
Figure 1. Peripheral blood smear and bone marrow biopsy before and after gilteritinib treatment. (A-C) Before gilteritinib treatment. (D-F) At relapse. (G-I) Two months after gilteritinib treatment. (A) Multiple monocytes and myeloblasts identified in peripheral blood smear (×40). (B) Clusters comprising blasts, monocytoid cells, and dysplastic normoblasts in bone marrow smear (×100). (C) *NPM* immunostain demonstrating both nuclear and cytoplasmic stains (×40). (D) A few monocytes and blasts in peripheral blood (×40). (E) Multiple monocytoid cells and blasts in clusters in bone marrow smear (×100). (F) Both nuclear and cytoplasmic stains by *NPM* immunostaining (×40). (G) Leukopenia without circulating blasts, polychromasia, and nucleated red blood cells in peripheral blood smear (×40). (H) Numerous erythroid precursors, dysplastic normoblasts identified in bone marrow smear (×100). (I) *NPM* immunostain redemonstrating abnormal staining pattern in both cytoplasms and nuclei (×40). (J) Erythroid precursors indicated by E-cadherin (×10) were within the distribution of *NPM* immunostain (×10) (K) at relapse. (L) Erythroid precursors indicated by CD71 (×10) that markedly increased after gilteritinib treatment were (M) a large proportion of *NPM1*-mutated cells in bone marrow.

myeloblasts into normoblasts following gilteritinib treatment (Figure 2). The patient underwent matched unrelated peripheral blood HCT under a reduced intensity conditioning with fludarabine, melphalan, and total body irradiation following a 3-month course of gilteritinib treatment. A bone marrow biopsy 1 month after HCT revealed no morphologic or flow cytometric evidence of residual AML. Cellularity was 50% with trilineage maturation, and ME ratio was 1:1. Notably, *NPM* immunostain did
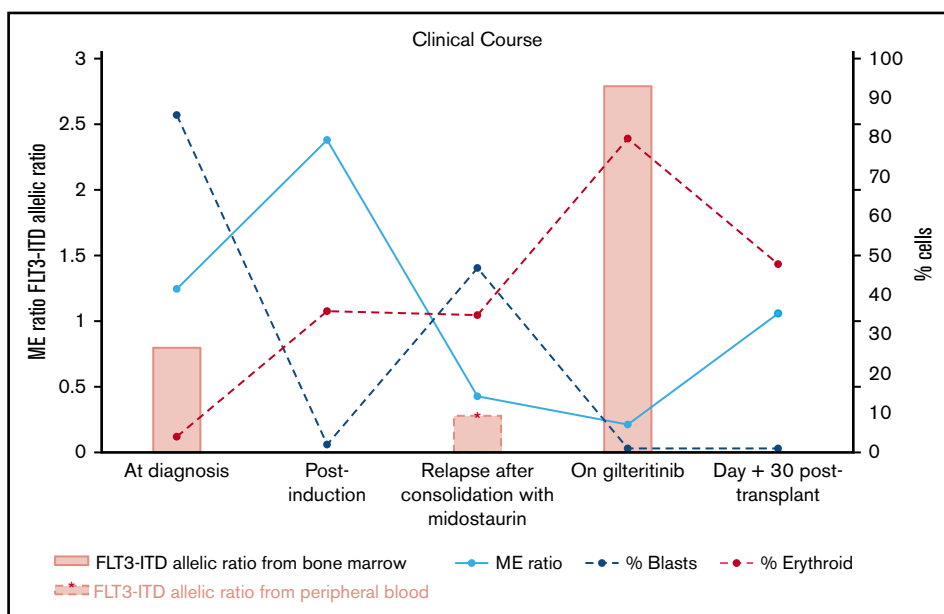
Figure 2. Summary of clinical courses with *FLT3-ITD* allelic ratio, ME ratio, and % blasts of bone marrow. ME ratio strikingly decreased by numerous erythroid precursors identified after initiation of gilteritinib treatment, whereas blasts significantly decreased. *FLT3-ITD* allelic ratio increased following gilteritinib treatment. Both *FLT3-ITD* and blasts became undetectable at day 30 after HCT.

not show aberrant cytoplasmic staining. A molecular study revealed no *FLT3-ITD* and tyrosin kinase domain mutations. The patient has currently been in complete remission at 80 days after HCT.

FLT3-ITD is a common molecular aberration identified in AML[4] and is associated with arrest of myeloid differentiation via suppression of *C/EPBα* expression.[5,6] The negative effect of *FLT3-ITD* on myeloid differentiation is further supported by the fact that *FLT3* inhibitors, including quizartinib[7] and sorafenib[8] as well as gilteritinib,[1] induced in vivo myeloid maturation in patients with *FLT3-ITD* mutation. In fact, no prior studies demonstrated that pharmacological blockade of *FLT3* signaling induced skewed erythroid differentiation from the blasts. In our case, a striking decrease in ME ratio by increased normoblasts with *NPM1* mutation following gilteritinib treatment and persistence of *FLT3-ITD* indicates that erythroid differentiation was originated from leukemic myeloblasts. Interestingly, our patient's *FLT3-ITD* allelic ratio increased, whereas the proportion of blasts was markedly decreased following gilteritinib treatment. This is consistent with the study by McMahon et al, in which FLT3 mutation frequencies increased in 2 patients with the differentiation responses. The prior midostaurin treatment in our case did not induce myeloid nor erythroid differentiation despite midostaurin treatment targeting *FLT3-ITD*.[9] This might be explained by myelosuppression resulted from potent blockade of *KIT* signaling by midostaurin but not with gilteritinib.

Why was the erythroid differentiation prominently observed in our case following *FLT3* inhibition? What are the biological underpinnings? Injections with human *FLT3* ligand to mice resulted in decreased erythropoiesis with loss of erythroid island macrophages in murine bone marrow.[10] In a transgenic mouse model, BM erythroid progenitor numbers were 5.4-fold decreased along with a significant reduction in hematocrit in mice expressing the human *FLT3* ligand (*FLT3*L-Tg mice) compared with wild-type mice.[11] Moreover, the expression of *Mpl* and *Klf1* (megakaryocyte/erythroid lineage genes) was increased with decreased expression of *Mpo*, *Csf3r*, and *Cebpa* (myeloid-specific genes) in murine hematopoietic stem cells (Lineage⁻SCA1⁺CD117⁺) of *FLT3*L-Tg mice, suggesting that overactivation of *FLT3* signaling by increased access to *FLT3*L skewed hematopoiesis toward myelopoiesis at the expense of erythropoiesis and thrombopoiesis. Furthermore, mean serum *FLT3L* concentrations in patients with Fanconi or aplastic anemia were much higher than in healthy individuals.[12] Taken together, it is plausible that selective blockade of *FLT3* signaling by gilteritinib may induce rebound of erythroid differentiation previously suppressed by excessive *FLT3* signaling by *FLT3-ITD* before gilteritinib treatment. The mechanistic underpinnings of erythroid differentiation induced by selective *FLT3* signaling blockade need to be further elucidated. In AML patients with *FLT3-ITD*, higher allelic burden of *FLT3-ITD* and failure to achieve complete molecular remission before transplantation were associated with poorer clinical outcomes.[13] Despite the persistence of *FLT3-ITD* following gilteritinib treatment without achieving complete remission before transplantation, the patient has remained in complete molecular remission.

As in our case, novel targeted therapeutic agents (eg, *FLT3* inhibitors, *IDH1/2* inhibitors[14-16]) have been reported to induce maturation from leukemic blasts. Therefore, this phenomenon may complicate the definition of complete remission and its clinical significance. The effect of differentiation induced by gilteritinib and other targeted agents on clinical outcomes warrants further investigations.

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