FLT3 inhibitor-induced neutrophilic dermatosis

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**Key Points**

1. Infiltrating FLT3-ITD neutrophils identified in skin confirms terminal differentiation of FLT3-ITD blasts after FLT3 inhibitor therapy.

2. Neutrophilic dermatosis after FLT3 inhibition may be a manifestation of a differentiation syndrome associated with this treatment.

**Abstract**

The *FLT3-ITD* mutation is associated with poor outcomes in acute myeloid leukemia (AML). Multiple FLT3-inhibitors have been studied in clinical trials. Recently, potent FLT3 inhibition was shown to induce terminal differentiation of FLT3-mutant myeloblasts. In three patients who developed characteristic skin nodules upon initiation of FLT3-inhibition, we conducted dermatopathologic evaluation of skin samples, as well as FLT3 and NPM1 mutational analysis and fluorescence in situ hybridization (FISH). All three patients demonstrated characteristically deep dermal and subcutaneous neutrophilic infiltrates, without evidence of myeloblasts. Discovery of *FLT3-ITD* and *NPM1* mutations in two of the samples, as well as presence of *FLT3-ITD* and deletion of 7q in the other, confirmed the ancestry of the differentiated neutrophils as that of the original *FLT3*-mutant myeloblasts. FLT3 inhibition can lead to a clinically distinct dermatoses, which suggest the impact of FLT3 inhibition on myeloid differentiation and a manifestation of a broader “syndrome” associated with this therapy.
Introduction:

The FMS-like tyrosine kinase 3 (FLT3) gene is mutated in approximately 30% of patients with acute myeloid leukemia (AML), with the majority being internal tandem duplication (ITD) mutations, an alteration associated with higher relapse rates, and worse outcomes. The FLT3-ITD protein product is a ligand-independent, constitutively active receptor tyrosine kinase, causing activation of multiple signaling cascades, suppression of apoptosis, and proliferation of myeloblasts. Several inhibitors of the FLT3-ITD protein are under clinical investigation.

FLT3 inhibition causes direct cytotoxicity to myeloblasts. However, intriguingly, terminal differentiation of FLT3-ITD myeloblasts was recently reported in patients treated with quizartinib, a potent FLT3 inhibitor. We now report the incidence of neutrophilic dermatoses in three FLT3-ITD patients treated with FLT3 inhibitors, and demonstrate that these infiltrating neutrophils retain FLT3-ITD expression, confirming the process of terminal differentiation induced by this therapy.

Methods:

Skin biopsy samples from three patients seen at the Massachusetts General Hospital and Johns Hopkins Sidney Kimmel Comprehensive Cancer Centers were evaluated. All patients were diagnosed with FLT3-ITD AML, were treated with FLT3 inhibitors, and subsequently developed skin nodules. Samples were obtained through protocols approved by each institution’s IRB. The study was conducted in accordance with the Declaration of Helsinki.
FLT3-ITD mutation status was assessed using previously reported primers. The NPM1 exon 12 insertion mutation status was assessed using custom designed primers (Forward 5'-AGTTAACTCTCTGTGCTATAGATGTAATG-3'[labeled w/NED] and Reverse 5'-GGACAGCCAGATATCAACTGTAC-3'[labeled w/HEX]). Primer sets were combined to perform multiplex polymerase chain reaction (PCR: 94°C 2 minutes, 36 cycles x 94°C 15 seconds, 60°C 30 seconds, 72°C 30 seconds) for samples, with subsequent analysis by Applied Biosystems Genetic Analyzer capillary electrophoresis.

EGFR and MET gene copy number was evaluated by fluorescence in situ hybridization (FISH) on formalin-fixed paraffin-embedded tissue sections, as described previously.

Results and Discussion:

Case histories are summarized below, and in Table 1:

Case 1:
Patient was a 29-year old female, presenting with a white blood cell count (WBC) of 30,000/μL, and diagnosed with cytogenetically-normal AML, with FLT3-ITD and nucleophosmin (NPM1) mutations. After induction chemotherapy, she achieved complete remission (CR). She received one cycle of high-dose cytarabine and underwent myeloablative haplo-identical stem cell transplantation (SCT). She experienced relapse four months later, and quizartinib was initiated. Of note, prior to initiation of quizartinib, the FLT3-ITD to WT ratio was 0.2 (ITD allelic burden of 20%). Two months later, she
developed tender, erythematous nodules on her lower extremities, which resolved after therapy with oral dexamethasone. Quizartinib was ultimately discontinued due to worsening performance status, and she died soon thereafter.

Skin biopsy revealed subcutaneous, lobular mixed inflammatory infiltrates, including numerous neutrophils, consistent with lobular panniculitis. The infiltrates lacked morphologically identifiable blasts, and lacked CD34 or CD117 expression (markers expressed in her leukemic blasts) by immunohistochemistry. Sizing analysis by PCR detected both FLT3-ITD and NPM1 insertional mutations in the skin sample.

Case 2:
Patient was a 60-year old female, presenting with WBC of 40,000/µL. She was diagnosed with FLT3-ITD and NPM1-mutant AML, characterized also by translocation t(10;17) (q24;p13), and partial deletion of chromosome 15 (q15q24). She received induction chemotherapy, and achieved CR. After two cycles of decitabine, she relapsed, and received MEC (mitoxantrone, etoposide, and cytarabine) re-induction. She achieved remission with incomplete peripheral hematopoietic recovery. Disease relapse followed within weeks, and quizartinib was started. Prior to initiation of quizartinib, the FLT3-ITD allelic burden was 95%. Six weeks later, pulmonary nodules were noted on imaging, as was a tender, erythematous skin nodule on her right thigh (Figure 1A), which was biopsied. Treatment with oral dexamethasone led to resolution of the skin nodule within days. She subsequently underwent allogeneic transplant, but died in the peri-transplant period from complications of sepsis.
Dermatopathologic evaluation revealed deep dermal and subcutaneous inflammatory infiltrates, consisting of abundant neutrophils, but no blasts. Sizing analysis by PCR detected both FLT3-ITD and NPM1 insertional mutations (Figure 1B) in the skin sample.

Case 3:
Patient was a 56-year old male with history of osteosarcoma, for which he underwent chemotherapy and resection. Two years later, he developed therapy-related AML, with deletion of 7q, and no FLT3 or NPM1 mutations. He was treated with induction chemotherapy, achieving CR, followed by a myeloablative matched related donor SCT. Four months later, he suffered relapse of his disease, which was refractory to MEC and then to decitabine. Bone marrow biopsy, 11 months after diagnosis, revealed persistent AML, but now with a FLT3-ITD mutation, with ITD allelic burden being 15%. He was started on lenalidomide and sorafenib. Two weeks later, he developed tender subcutaneous nodules on his left thigh, which were biopsied. Imaging revealed concurrent pulmonary nodules. He ultimately succumbed to progressive disease.

Dermatopathologic evaluation revealed dermal and subcutaneous lobular neutrophilic infiltration (Figure 1C), with no CD34 or CD117-positive blasts, consistent with lobular neutrophilic dermatosis and panniculitis. Flow cytometry on this specimen revealed no evidence of myeloblasts. PCR sizing analysis on the skin sample detected a FLT3-ITD mutation. FISH copy analysis for EGFR (located on 7p11.2) and MET (located on 7q21.11) revealed aggregates of cells within the neutrophilic infiltrates, with each
nucleus showing two red \textit{EGFR} signals and one green \textit{MET} signal located on 7p and 7q, respectively, confirming clonal derivation from the original leukemic myeloblasts (Figure 1D).

Discussion:

Potent FLT3 inhibition appears to cause terminal differentiation of arrested myeloid precursors in AML\textsuperscript{13}. The impact of \textit{FLT3-ITD} mutation on differentiation is previously described, and release of differentiation block appears to be a feature of FLT3 inhibition\textsuperscript{16,17}. Differentiation and cell cycling are coordinated phenomena, and the transcription factor C/EBP\textalpha{} has been identified as an important mediator\textsuperscript{17}. Dysfunction of C/EBP\textalpha{} activity is well-described in AML, and \textit{FLT3-ITD} mutations inhibit C/EBP\textalpha{}\textsuperscript{17-19}. FLT3 inhibition leads to cytotoxicity within days, with clearance of peripheral blasts\textsuperscript{13,20,21}, but its impact on terminal differentiation usually follows over a period of weeks\textsuperscript{13}. A similar timeframe was noted in our patients, with characteristic skin findings occurring weeks after therapy initiation.

We here describe a dermatologic manifestation of a “differentiation syndrome” that appears to follow initiation of FLT3 inhibition. These patients all developed painful subcutaneous nodules within weeks of starting therapy, with pathological evaluation revealing deep neutrophilic infiltration and panniculitis. Skin biopsy samples contained no myeloblasts but did express \textit{FLT3-ITD}, as well as other cytogenetic or molecular aberrations that had characterized the patients’ leukemic clone, suggesting that the infiltrating neutrophils were terminally differentiated from \textit{FLT3}-mutant precursors. In
addition, two of these patients developed concurrent pulmonary nodules, without clear evidence of infection, suggesting this too as a manifestation of differentiation. Intriguingly, in two patients, steroid therapy led to resolution of skin nodules. To date, in 22 patients treated with quizartinib at Johns Hopkins, three have developed biopsy-confirmed neutrophilic dermatoses, an occurrence rate of 13.6%. At MGH, in ten \(FLT3-ITD\) patients treated with single agent sorafenib since 2007, one developed confirmed neutrophilic dermatosis, a rate of 10%. Based on this limited data, we therefore estimate a likelihood of occurrence for this phenomenon in relapsed/refractory AML patients treated with a FLT3 inhibitor of approximately 10% during the treatment period. Our patients were treated with single agent quizartinib and sorafenib, with subsequent development of characteristic skin nodules, suggesting that the affect is class-specific and a feature of potent FLT3 inhibition. We chose to include our experience with single-agent FLT3 inhibitors, and not their combination with cytotoxic chemotherapy, such as that of the FLT3 inhibitor midostaurin and induction chemotherapy, for which we have not observed similar dermatologic phenomena. This is likely due to the fact that concurrent cyto reduction with traditional chemotherapy suppresses differentiation and its clinical manifestations.

Our patients did have some unique clinical features, in that cases 2 and 3 displayed karyotypic abnormalities, and in case 3, \(FLT3-ITD\) was only detected at relapse. Although the majority of patients with \(FLT3-ITD\) AML have a normal karyotype and exhibit the mutation on presentation, studies have demonstrated that \(FLT3-ITD\) can be acquired on relapse and can accompany a range of karyotypic abnormalities.\(^3\,22,23\)
addition, in our patients, the extent of *FLT3*-mutant allelic burden did not appear to correspond to the development of neutrophilic dermatosis, although it is difficult to draw conclusions from this limited number of cases.

This syndrome appears to share features with the well-described phenomena associated with use of all-trans retinoic acid in acute promyelocytic leukemia, characterized by neutrophilic differentiation, with possible pulmonary and skin manifestations.\textsuperscript{24} ATRA-differentiated cells are known to over-express CXCR4, which interacts with the stromal factor, SDF-1, residing in affected tissues.\textsuperscript{25} CXCR-4 expression is elevated in *FLT3-ITD* AML,\textsuperscript{26,27} potentially priming these cells upon differentiation for CXCR-4-mediated migration and SDF-1 interaction in tissues.

In light of these and similar findings,\textsuperscript{13} we suggest that remissions resulting from FLT3 inhibition, as recently reported,\textsuperscript{7,21} may differ in quality from those achieved following conventional chemotherapy. The latter are “kinetic” remissions due to repopulation, by normal precursors, of a cytocoreduced marrow milieu, whereas remissions resulting from FLT3-inhibitors may be driven by terminal differentiation and gradual replacement of *FLT3-ITD* precursors. This distinction may have broader clinical implications, and responses to FLT3-targeted therapies require further study to determine their clinical trajectory versus more traditional states of remission.

In summary, FLT3 inhibition can lead to clinically and pathologically distinct dermatoses, consisting of deeply infiltrative, terminally differentiated *FLT3*-mutant
neutrophils. As use of FLT3 inhibitors expands, physicians will increasingly detect these manifestations, which may be part of a broader differentiation syndrome associated with this therapy.
Authors’ Contributions:

A.T.F. developed the study design, performed data collection, and wrote the manuscript; L.L. performed molecular and FISH assays on tissue; R.P.H. performed histopathological evaluation, provided images, and helped edit the manuscript; H.S. helped with sample collection and editing of manuscript; M.L. and Y.C. helped with study design and development, and edited the manuscript.

Conflicts of Interest:

M.L. is on the clinical advisory board for Ambit Biosciences. Y.C. receives grant support from Bayer/Onyx to conduct clinical trials. All other authors have no relevant conflicts of interest to declare.
References:


Table 1: Clinical characteristics of three patients with FLT3-ITD mutations, treated with FLT3 inhibitors, and who subsequently developed tender skin nodular, consisting of FLT3-mutant infiltrating neutrophilic infiltrates.

WBC: White blood cell count, ITD: Internal tandem duplication, WT: wildtype

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age/Gender</th>
<th>WBC (/µL) at presentation</th>
<th>WBC (/µL) (Neutrophil, Blast %) at time of skin lesion</th>
<th>Karyotype</th>
<th>FLT3 status</th>
<th>NPM1 status</th>
<th>FLT3 Inhibitor</th>
<th>Time to onset of skin lesion</th>
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<tr>
<td>#1</td>
<td>29 / Female</td>
<td>30,000</td>
<td>550 (4%, 0%)</td>
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<td>Mutant</td>
<td>Quizartinib</td>
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<tr>
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<td>60 / Female</td>
<td>40,000</td>
<td>570 (76%, 0%)</td>
<td>t (10;17), del (15)</td>
<td>ITD</td>
<td>Mutant</td>
<td>Quizartinib</td>
<td>6 weeks</td>
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<tr>
<td>#3</td>
<td>56 / Male</td>
<td>6,600</td>
<td>1,100 (56%, 8%)</td>
<td>del (7q)</td>
<td>ITD</td>
<td>WT</td>
<td>Sorafenib</td>
<td>2 weeks</td>
</tr>
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Figure Legends:

Figure 1A-D:

(A) Photograph of the erythematous, tender nodule on the outer right thigh of patient #2, arising six weeks after initiation of the FLT3 inhibitor quizartinib.

(B) Fragment Size Analysis for FLT3 ITD and NPM1 Insertion Mutations: A representative analysis for FLT3 ITD and NPM1 insertion mutations using a PCR fragment size assay targeting FLT3 exon 15 and NPM1 exon 12 in genomic DNA extracted from the skin biopsy sample from patient #2. The arrows indicate the respective insertion mutations found in the patient but not in the normal Control.

(C) Histology of skin biopsy from Patient #3. Clusters of neutrophils are present within subcutaneous fat, admixed with histiocytes. No blasts are identified (original magnification 1000x, images taken on Olympus BX41 microscope).

(D) EGFR (7p) and MET (7q) FISH: Copy number analysis by FISH was performed on a skin biopsy formalin-fixed paraffin-embedded tissue section from Patient #3 using probes specific for EGFR (green) which corresponds to chromosome 7p and MET (red) which corresponds to chromosome 7q. The arrows indicate nuclei showing one copy loss of MET or 7q, identified within the subcutaneous neutrophilic infiltrates.
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