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Case Report

Emergence of Natural Killer Cell Large Granular Lymphocytes during Gilteritinib Treatment in Acute Myeloid Leukemia with FLT3-ITD Mutation

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Abstract: As the potent, selective Fms-Like Tyrosine Kinase 3 (FLT3) inhibitor gilteritinib has only been approved for use for a few years, its efficacy and complications remain incompletely understood. We herein report an elderly patient with FLT3 internal tandem duplications (FLT3-ITD) mutated acute myeloid leukemia (AML) who developed natural killer cell large granular lymphocytes (NK-LGL) in the bone marrow and peripheral blood during gilteritinib treatment. Case: A 79-year-old Japanese female had been diagnosed with FLT3-ITD-mutated AML. The patient received hydroxycarbamide 2000 mg daily for induction chemotherapy but did not achieve remission at day 28 postinduction. The treatment was then changed to gilteritinib 120 mg daily. Although the reduction of blasts in peripheral blood occurred immediately, it was revealed abnormal lymphocytes with large granules developed in bone marrow and peripheral blood. These lymphocytes were analyzed by flow cytometry, which revealed that these cells were NK-LGL because they expressed CD2, CD7, CD16, and CD56 and did not express CD3, CD19, and CD20. The patient achieved partial remission (PR) in a month with gilteritinib treatment. Leukemia eventually could not be controlled, but PR persisted for about 4 months and leukemia was controlled for 4 months after progression disease (PD) with gilteritinib treatment alone. Conclusion: Gilteritinib may induce the NK-LGL. The exact mechanism and effect of LGL in patients with FLT3 mutated AML treated with gilteritinib warrants further investigation.

Keywords: acute myeloid leukemia (AML); Fms-Like Tyrosine Kinase 3 internal tandem duplications (FLT3-ITD) mutation; Gilteritinib; natural killer cell large granular lymphocytes (NK-LGL)

Fms-Like Tyrosine Kinase 3 (FLT3), a cytokine receptor tyrosine kinase that is expressed on the surface of hematopoietic progenitor cells, regulates proliferation, survival, and differentiation of multipotent stem cells [1]. Mutations in FLT3 internal tandem duplications (FLT3-ITD) and FLT3 tyrosine kinase domain (FLT3-TKD) are involved in approximately 20% and 7% of acute myeloid leukemia (AML), respectively, and are reported to be associated with a poor prognosis [2]. FLT3-ITD is a strong risk factor for relapse, particularly in the absence of NPM1 mutation or when a high ITD allele ratio is present at diagnosis, and shows inferior overall survival (OS) compared to FLT3-TKD or wild-type FLT3 because of the high rate of relapse. Use of the potent, selective FLT3 inhibitor gilteritinib has resulted in significantly longer survival and higher percentages of patients with remission than salvage chemotherapy for relapsed and/or refractory FLT3-mutated AML [3]. As gilteritinib has only

been approved for use for the past year, its efficacy and complications remain incompletely understood. In this report, we describe an elderly patient with FLT3-ITD mutated AML who developed abnormal lymphocytes with large granules in the bone marrow and peripheral blood during gilteritinib treatment. We identified these lymphocytes as natural killer cell large granular lymphocytes (NK-LGL) ($CD16^+$, $CD56^+$). As far as we know, this is the first report describing the induction of NK-LGL by gilteritinib.

A 79-year-old Japanese female with no significant medical history presented with fever and generalized weakness that had lasted for a month. Physical examination at presentation was unremarkable, and the performance status (PS) was 3. A complete blood count revealed a white blood cell count of 252.7×10^9/L with 62% blasts, hemoglobin 8.6 g/dL, and platelet 149×10^9/L. In addition, the presence of hypercellular marrow with abnormal myeloblasts (42.2% of bone marrow cells) was revealed by a bone marrow smear. Furthermore, flow cytometry analyses showed bright CD45, bright CD13, CD33, CD117, and HLA-DR. Cytogenetics showed a normal karyotype, and molecular study revealed FLT3-ITD mutation. The patient had been diagnosed with AML with myelodysplasia related changes according to the World Health Organization criteria and was identified to have the FLT3-ITD mutation. The patient received hydroxycarbamide 2000 mg daily for induction chemotherapy, as she had a risk of severe adverse events associated with intensive chemotherapy. She did not achieve remission at day 28 postinduction; her complete blood count showed a white blood cell count of 4.1×10^9/L with 40% blasts, hemoglobin 6.8 g/dL, and platelet 346×10^9/L. The treatment was then changed to gilteritinib 120 mg daily. As the reduction of blasts in peripheral blood occurred immediately and the patient tolerated the treatment well, she was discharged from the hospital after the 2-week gilteritinib treatment. However, 2 weeks later, she visited the emergency room of our hospital for slight fever and lassitude. Physical examination, CT scan, and blood and urine culture showed no evidence of infection and immunoreaction such as pleural effusion and rash. A complete blood count revealed a white blood cell count of 1.8×10^9/L with no blast and 29% neutrophils, hemoglobin 7.1 g/dL, and platelet 29.0×10^9/L. At first, the patient was treated as having febrile neutropenia (FN) with antibiotics and gilteritinib was stopped, but a few hours later, it was found that abnormal lymphocytes with large granules developed in peripheral blood (18.0% of peripheral white blood cell). Bone marrow aspiration was performed, revealing the presence of normocellular marrow with 1% myeloblast and 15.2% abnormal lymphocytes, as observed in peripheral blood (Figure 1a). For further analysis of these abnormal lymphocytes, bone marrow and peripheral blood cells were analyzed by flow cytometry, which revealed that these lymphocytes were NK-LGL because they expressed CD2, CD7, CD16, and CD56 and did not express CD3, CD19, and CD20 (Figure 1b). The number of NK-LGL gradually decreased in two weeks, and they disappeared thereafter. Her clinical symptoms also improved over several days. After a 2-week drug withdrawal owing to the requirement for frequent blood transfusion, gilteritinib was administered again at a reduced dose of 80 mg, but NK-LGL did not recur (Figure 2). As there was no evidence of malignant Granular lymphocyte-proliferative disorders (GLPD) and NK-LGL was decreased immediately after gilteritinib withdrawal, we determined that NK-LGL in this case was induced by gilteritinib. The patient achieved partial remission (PR) in a month with gilteritinib treatment. Leukemia eventually could not be controlled, but PR persisted for about 4 months and leukemia was controlled for 4 months after progression disease (PD) with gilteritinib treatment alone. Patient consent was obtained for this case report.

LGL is characterized by a high cytoplasmic–nuclear ratio and abundant azurophilic granules [4]. LGL is shown to be composed of both cytotoxic T lymphocytes ($CD3^+$) and NK cells ($CD3^-$), both of which belong to the lymphoid lineage and serve as the main executors of cell-mediated cytotoxicity [5]. GLPD are characterized by lymphocytic proliferation with malignant LGL lymphocytosis; these disorders include T-cell large granular lymphocytic leukemia (T-LGL), chronic lymphoproliferative disorders of NK cells (CLPD-NK), and aggressive NK cell leukemia (ANKL), or reactive LGL lymphocytosis induced by viral infection and dasatinib [6,7]. In the case described here, there was no evidence of malignant GLPD. GLPD caused by dasatinib in Philadelphia chromosome-positive (Ph^+) chronic myeloid leukemia (CML) and acute lymphoid leukemia (ALL) was reported in some of the

literature. The dasatinib patients with LGL had a significantly lower percentage of regulatory T cells in peripheral blood and achieved a high rate of complete molecular remission (CMR) in CML and OS in Ph^+ALL than did patients without LGL or healthy controls [6,7].

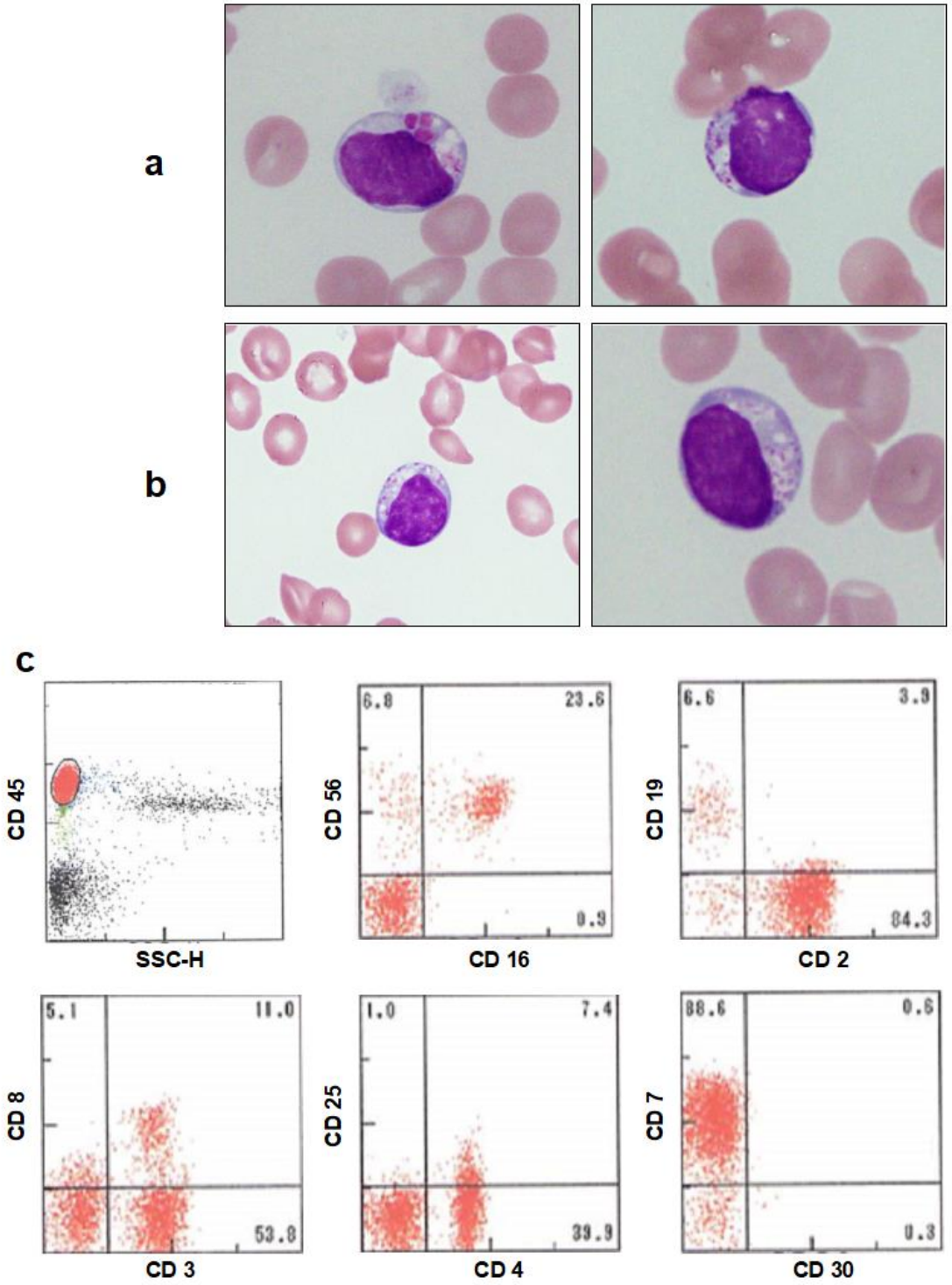

Figure 1. Natural killer cell large granular lymphocyte (NK-LGL) lymphocytosis induced by gilteritinib. (**a**,**b**) May Giemsa stained (**a**) peripheral blood and (**b**) bone marrow smear; original magnification × 400. (**c**) Flow cytometry analysis of lymphocytes in peripheral blood.

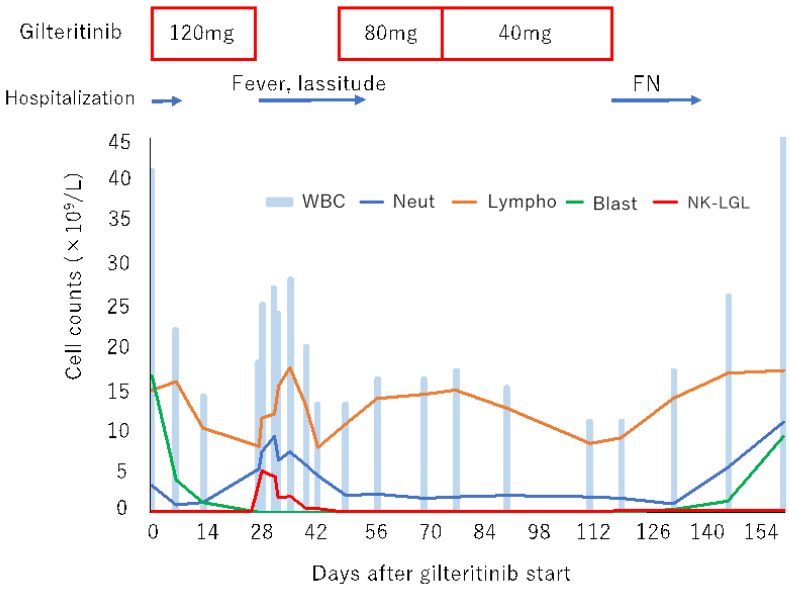

Figure 2. Clinical course from the start of gilteritinib therapy to progression disease. The patient had achieved partial response (PR) immediately because of gilteritinib treatment. Natural killer cell large granular lymphocytes (NK-LGL) was detected at the early stage of gilteritinib treatment and gradually decreased in about 2 weeks. The patient was hospitalized owing to fever, lassitude, and febrile neutropenia (FN), but long-term outpatient management was possible. Acute myeloid leukemia (AML) eventually could not be controlled but PR persisted for approximately 4 months.

Some studies have reported a relationship between dasatinib-related LGL and cytomegalovirus (CMV) [8,9], but the factors underlying LGL lymphocytosis with dasatinib treatment remain uncertain. Recently, work by a Japanese group indicated that how CMV plays a role in dasatinib-related LGL lymphocytosis. They applied principal component analysis (PCA) to an extensive panel of NK cell markers to explore underlying factors in NK cell activation. PCA provided phenotypic divergence of NK cells that reflects CMV-associated differentiation and genetic differences, and the divergence was markedly augmented in CMV-seropositive dasatinib-treated patients. Therefore, they presume that the onset of leukemia elicits subclinical CMV reactivation and consequent NK cell activation, and that subsequent dasatinib treatment induces further subclinical CMV reactivation, leading to CMV-associated strong NK cell differentiation and expansion in CMV+ dasatinib-treated patients [10].

Sorafenib, a multikinase inhibitor active against FLT3-ITD, caused an increase in $CD8^+CD107a^+IFN\text{-}\gamma^+$ T-cells through IL-15 production in leukemia cells with FLT3-ITD mutation. The synergism of T-cells and sorafenib is mediated via reduced ATF4 expression, causing activation of the IRF7-IL-15 axis in leukemia cells and thereby leading to metabolic reprogramming of leukemia-reactive T cells in humans [11]. In fact, sorafenib maintenance after allogeneic hematopoietic stem cell transplantation (allo-HSCT) was associated with a significantly improved OS and progression-free survival (PFS) by promoting graft-versus-leukemia (GVL) activity [12,13]. IL-15 is also required for survival and activation of NK cells as well as expansion of NK cell populations [14]. IL-15 production from leukemia cells was dependent on sorafenib-sensitivity and sorafenib-induced serum IL-15 subsided when leukemia cells were reduced [11]. Therefore, in this case, we assume that the transient development of NK-LGL was due to reduction of leukemia cells and, in the PD period, leukemia cell lost the sensitivity to gilteritinib enough to produce IL-15. Recently, the possibility of GVL effects and developing NK cell induced by gilteritinib after an allo-HSCT was reported [15].

Herein, we describe an unusual case of a patient with FLT3-ITD mutated AML who developed NK-LGL induced by gilteritinib. The exact mechanism and effect of LGL in patients with FLT3 mutated AML treated with gilteritinib warrants further investigation. Hence, this patient experience could be used to inform the relationship between gilteritinib and NK-LGL.

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