Aberrant Expression of CD7 in Myeloblasts Is Highly Associated With De Novo Acute Myeloid Leukemias With FLT3/ITD Mutation

Veronica Rausei-Mills, MD, Karen L. Chang, MD, Karl K. Gaal, MD, Lawrence M. Weiss, MD, and Qin Huang, MD, PhD

Key Words: CD7; Acute myeloid leukemia; FLT3/ITD mutation

DOI: 10.1309/NRTX9AKXHR5JB793

Abstract

Acute myeloid leukemia (AML) with normal cytogenetics represents approximately 40% to 50% of de novo AML. This heterogeneous AML subgroup constitutes the single largest cytogenetic group with an intermediate prognosis. Previous studies have suggested that the Fms-like tyrosine kinase-3 internal tandem duplication (FLT3/ITD) mutation–positive de novo AML may represent a distinctive subgroup of AML. We analyzed the clinical and pathologic features of 15 cases of de novo AML with normal cytogenetics and with the FLT3/ITD mutation. In comparison with patients with AML without the FLT3/ITD mutation, patients with FLT3/ITD+ AML are relatively younger, more often have marked peripheral leukocytosis with a higher number of circulating blasts at initial examination, more often have minimal differentiation morphologic features, more frequently have abnormal CD7 coexpression, and have poorer outcome. Close association of aberrant CD7 expression and FLT3/ITD mutation in the myeloblasts of FLT3/ITD+ AML suggests that FLT3/ITD-mediated leukemic transformation occurs in the more early stage of myeloid progenitor cells.

The 2001 World Health Organization classification of leukemia and lymphomas clearly defines an unequivocal role of certain recurrent cytogenetic abnormalities in de novo acute myeloid leukemia (AML) for identifying the disease entity and predicting patient prognosis and outcomes. However, AML with normal cytogenetics (normal chromosomal karyotype) represents approximately 40% to 50% of de novo acute leukemia cases and constitutes the single largest cytogenetic group of AML. The cases are composed of a heterogeneous group of de novo acute leukemias considered more or less in the intermediate prognostic category. In this heterogeneous category, stratified prognostic determinants are required to predict increased risk of relapse, resistance to therapy, and long-term disease outcomes.

Fms-like tyrosine kinase-3 (FLT3) is a member of the class III type of tyrosine kinase receptor superfamily expressed widely in hematopoietic cells, placenta, gonads, and neural tissue. The FLT3 gene encodes a 993-amino-acid protein tyrosine kinase receptor that regulates growth, proliferation, and differentiation of hematopoietic progenitors. The FLT3 receptor consists of an extracellular structure with 5 immunoglobulin-like domains, a simple transmembrane region, a juxtamembrane domain, and a cytoplasmic kinase domain. The binding of FLT3 ligand to the extracellular domain of the receptor induces receptor dimerization and autophosphorylation. Its phosphorylation subsequently activates PI-3 kinase/akt, Ras/MAPK, and STAT downstream signal transduction pathways and has an important role in cellular growth, proliferation, and differentiation of hematopoietic progenitors.

Several acquired FLT3 gene mutations have been identified in patients with AML, and these mutations may contribute to leukemic pathogenesis and may have clinically
prognostic significance. Recently, a unique mutation has been described in the FLT3 gene, whereby a fragment of the juxtamembrane domain coding sequence is duplicated in direct head-to-tail orientation, a so-called internal tandem duplication (ITD) mutation. The length of the ITD varies from approximately 20 to 200 base pairs, and the duplicated sequence is always in-frame. Early investigations suggest that the FLT3/ITD + de novo AML may represent or define a distinctive subgroup of AML with normal cytogenetics. However, the clinical, pathologic, and immunophenotypic features of de novo AML with normal cytogenetics and with the FLT3/ITD mutation have not been described previously. In the present study, we analyzed the clinical and pathologic features of de novo AML with normal cytogenetics with or without FLT3/ITD mutations.

**Materials and Methods**

**Clinical Information**

Patient information was obtained from a search of case files from 2002 to 2006 at the City of Hope National Medical Center, Duarte, CA. In initial diagnostic AML cases submitted to the molecular diagnostic laboratory, 15 cases of de novo AML with the FLT3/ITD mutation and normal cytogenetics were identified. Clinical, pathologic, and immunophenotypic data were analyzed and compared with those for 16 cases of de novo AML with normal cytogenetics but without the FLT3/ITD mutation from the same period.

**Histologic and Cytologic Examination**

Peripheral blood and bone marrow aspirate smears were prepared with Wright-Giemsa staining for morphologic evaluation. The bone marrow trephine biopsy specimen was fixed in 10% neutral buffered formalin. Paraffin sections were stained with H&E for routine histologic examination.

**Immunophenotypic Analysis**

Immunophenotyping was performed by 4-color flow cytometric analysis of bone marrow aspirates with a Coulter Epics XL cytometer (Beckman Coulter, Miami, FL) using whole blood lysis. The bone marrow was studied for surface antigen expression by using a panel of monoclonal antibodies (all Coulter), including CD34, CD33, CD45, CD7, CD38, CD56, CD117, CD41, GlyA, CD15, CD14, CD13, HLA-DR, CD19, CD10, and CD20. Cytoplasmic terminal deoxynucleotidyl transferase expression was also tested. For all of the markers, expression was considered positive if 20% or more of the population expressed the antigen. The “blasts” were gated for analysis by using CD45 expression and lower right-angle light scatter, as previously described.

**FLT3/ITD Mutational Analysis**

Genomic DNA from fresh bone marrow aspirate samples was isolated by using routine laboratory procedures. The FLT3/ITD mutation was detected by using FLT3/ITD-specific polymerase chain reaction (PCR) followed by separation and detection with capillary electrophoresis. The FLT3/ITD mutation specific primers included the following: forward, 5'-FAM/GCAATTTAGGTATGAAAGCCAGC-3'; and reverse, 5'-CTTCACGATTTTGAACGGCAACC-3'. A specific set of β-globin primers was also used simultaneously with FLT3 primers to ensure DNA integrity and PCR amplification. The amplified PCR products were separated and detected by capillary electrophoresis on an ABI 310 or ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

**Statistical Analysis**

Statistical analysis was performed between various subgroups by using the Student t test and the χ² test by using a software program (SAS software, version 9.1, SAS Institute, Cary, NC). A P value of .05 or less was considered to indicate a statistically significant difference.

**Results**

All 31 studied de novo AML cases demonstrated to have normal chromosomal karyotypes by conventional cytogenetic analysis were submitted to the molecular laboratory for FLT3/ITD mutational analysis. During the 2002-2006 period at City of Hope National Medical Center, there were 15 cases of de novo AML with the FLT3/ITD mutation identified. An additional 16 cases of de novo AML with normal cytogenetics but without the FLT3/ITD mutation in the same period were also analyzed for comparison. All 31 cases studied were reviewed by hematopathologists (K.L.C., K.K.G., and Q.H.) at City of Hope National Medical Center and classified as the “acute myeloid leukemia not otherwise categorized” subgroup according to the current World Health Organization classification.

For histologic reasons, the French-American-British (FAB) classification for these AML cases was also included in Table 1. The FLT3/ITD mutation–positive cases included 2 FAB-M0 cases, 6 FAB-M1 cases, 3 FAB-M2 cases, 3 FAB-M4 cases, and 1 FAB-M5 case. The patients were 5 men and 10 women with a median age of 49 years (range, 30-81 years). The FLT3/ITD mutation was identified from the initial diagnostic bone marrow materials, before treatment, by specific PCR amplification followed by separation and detection with capillary electrophoresis. Clinically, all patients with FLT3/ITD+ AML presented with variable leukocytosis with an average WBC count of...
86,900/µL (86.9 × 10⁹/L; range 10,700-325,000/µL [10.7-325.0 × 10⁹/L]) and having a higher percentage of circulating blasts (average, 50%) in comparison with the cases of AML without FLT3/ITD mutation, who presented with an average WBC count of 11,300/µL (11.3 × 10⁹/L; \( P < .05 \)) and average circulating blasts of 26% (\( P < .05 \)) (Table 1).

Histomorphologically, the myeloblasts of FLT3/ITD+ AML more often showed minimally differentiated morphologic features. The blasts were usually medium to large with round or slightly indented nuclei with 1 or 2 nucleoli and agranular, basophilic cytoplasm. The blasts in some cases displayed monocytoid differentiation. Immunophenotypic analysis by flow cytometry demonstrated that the myeloblasts in FLT3/ITD+ cases were usually positive for CD33 and CD13 with variable expression of CD34 or CD117 (Table 1). Notably, the myeloblasts in 11 (73%) of 15 FLT3/ITD+ cases displayed aberrant coexpression of CD7, a T cell–associated antigen, whereas only 1 (6%) of 16 cases of AML without the FLT3/ITD mutation expressed CD7 (\( P < .001 \)) 11 Image 2. Of 4 FLT3/ITD+ AML cases without CD7 expression, 2 showed aberrant coexpression of CD4.

Clinically, more than half of FLT3/ITD+ AML cases showed persistent disease or relapsed shortly after the first cycle of chemotherapy. In 12 patients with FLT3/ITD+ AML with available follow-up data, 8 (67%) died within 1 year after the initial diagnosis (Table 1).

### Discussion

AML is a clinically, biologically, and genetically heterogeneous disease characterized by various genetic defects. 1 These defects include chromosomal translocations involving many oncogenes and transcription factors and/or various mutations leading to activation of signal transduction pathways and alterations of growth factor receptors. 1-5

The FLT3/ITD mutation has been identified in 20% to 30% of de novo adult AML cases and 10% to 15% of pediatric AML cases. 8-10 Enforced expression of the FLT3/ITD mutation in human CD34+ cells confers properties of...
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self-renewal and enhanced erythropoiesis.\textsuperscript{12} Studies on a transgenic mouse model demonstrated that the \textit{FLT3/ITD} mutation alone or in collaboration with the \textit{AML1-ETO} fusion gene induces myeloproliferative disorders, lymphoid disease, or acute leukemia in experimental animals,\textsuperscript{13,14} indicating that the \textit{FLT3/ITD} mutation is indeed involved in leukemogenesis.

Clinically, the \textit{FLT3/ITD} mutation has been demonstrated in a higher frequency of patients with de novo AML with normal cytogenetics and may be associated with lower rates of complete remission, progression-free survival, and overall survival.\textsuperscript{2-7} Furthermore, the length of the mutation varies from patient to patient and from initial presentation to relapse in a single patient. Longer mutations of \textit{FLT3/ITD} have been linked to higher relapse rates and poor outcome.\textsuperscript{8-10} The studies suggest that de novo AML with the \textit{FLT3/ITD} mutation may represent a distinctive subgroup of AML with an unfavorable clinical outcome.

In the present study, we analyzed the clinical, hematologic, and immunophenotypic features of \textit{FLT3/ITD}+ AML cases by comparing the features of \textit{FLT3/ITD}– AML cases occurring during the same period. The present study confirms the findings of previous studies that the \textit{FLT3/ITD} mutation is an adverse prognostic factor for de novo AML with normal cytogenetics. Of 12 patients with \textit{FLT3/ITD}+ AML, 8 (67%) died within 1 year after initial diagnosis despite extensive treatments with chemotherapy and/or hematopoietic stem cell transplantation. In comparison, only 5 (36%) of 14 patients with AML without the \textit{FLT3/ITD} mutation died during the same period, although no statistically significant difference was found owing to the relatively small sample (\textit{P} = .11). Our study also demonstrates that patients with de novo AML with the \textit{FLT3/ITD} mutation and normal cytogenetics, when compared with similar patients without the \textit{FLT3/ITD} mutation, are
relatively younger and more often present with marked leukocytosis with higher numbers of circulating blasts, more often have minimal differentiation morphologic features, more frequently have aberrant CD7 expression, and have a poorer prognosis (Table 1).

The higher frequency of aberrant CD7 coexpression in FLT3/ITD+ AML with normal cytogenetics is striking, and the findings have not been described previously. CD7 is generally considered a T cell–associated antigen expressed on some myeloid progenitors, and then its expression is down-regulated as myeloid progenitors undergo differentiation and maturation. Early studies of clinical and pathologic features of so-called CD7+ AML revealed that this subtype of AML usually has minimally differentiated FAB-M1 morphologic features, is often hypersensitive to stem cell factor and interleukin-3 in vitro, and completely lacks B- and T-cell receptor gene rearrangements, suggesting that these AML tumor cells are derived from early committed myeloid progenitor cells. The CD7+ AMLs described in previous reports displayed very similar clinical and pathologic features to those of FLT3/ITD+ de novo AML cases found in the present study, suggesting they might represent the same category of AML. In fact, evidence that FLT3/ITD mutations are frequently identified in leukemic stem cells and in immature leukemic CD34+/CD33– cells further supports the hypothesis. 

Close association of aberrant CD7 expression and the FLT3/ITD mutation in the myeloblasts of FLT3/ITD+ AML suggests that FLT3/ITD-mediated leukemic transformation occurs in the earlier stage of myeloid progenitor cells, and aberrant CD7 expression may serve as a surrogate marker for predicting FLT3/ITD mutation. However, further studies with a larger sample are warranted to verify these results.

From the Division of Pathology, City of Hope National Medical Center, Duarte, CA.

Presented in part at the 96th Annual Meeting of the United States and Canadian Academy of Pathology; March 2007; San Diego, CA.

Address reprint requests to Dr Huang: Division of Pathology, City of Hope National Medical Center, Duarte, CA 91010.

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