CD117 Expression Is a Sensitive but Nonspecific Predictor of FLT3 Mutation in T Acute Lymphoblastic Leukemia and T/Myeloid Acute Leukemia

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Key Words: CD117; FLT3; T acute lymphoblastic leukemia; T/myeloid acute leukemia

Abstract

Others have suggested that CD117, or an immunophenotypic profile including CD117, can serve as surrogate for FLT3 mutation in T acute lymphoblastic leukemia (ALL), thereby guiding targeted therapy. We report the results of flow cytometry immunophenotypic analysis in 42 cases of T-ALL and T/myeloid acute leukemia also assessed for FLT3 mutation. CD117 was expressed in 21 (50%), and FLT3 was mutated in 8 cases (19%; 1 T-ALL and 7 T/myeloid). FLT3-mutated cases were terminal deoxynucleotidyl transferase (TdT)+/CD2+ (7/8), cytoplasmic CD3+/CD5+ (5/8), CD7+/CD13+/CD15+ (4/6), CD33+ (4/8), CD34+, and CD117+ (bright). Cytochemistry showed myeloperoxidase-positive cells in all T/myeloid acute leukemias (3%-50%). We conclude that FLT3 mutation is rare in T-ALL, and its presence supports T/myeloid lineage. CD117 expression alone is sensitive but not specific for FLT3 mutation. The immunophenotypic profile of TdT, CD7, CD13, CD34, and CD117 (bright) is helpful for predicting FLT3 mutation, with a sensitivity of 100% and specificity of 94%.

The FMS-like tyrosine kinase 3 gene (FLT3) is a class III receptor tyrosine kinase, and its product is expressed by early hematopoietic progenitors. Mutations of FLT3 cause constitutive activation of this signaling pathway. FLT3 mutations are known to occur mainly in cases of acute myeloid leukemia or myelodysplastic syndrome in leukemic transformation and are associated with a high risk for relapse and an overall poor prognosis.1 There are 2 general means of FLT3 mutation. The most common mechanism is internal tandem duplication (ITD), but missense point mutations of codon 835 of the kinase region of FLT3 also occur.

Acute lymphoblastic leukemia (ALL) cases uncommonly carry FLT3 mutations, and mutated cases are usually of B-cell lineage and associated with mixed lineage leukemia (MLL) gene alterations.2 Data regarding FLT3 mutation in cases of T-ALL are limited, but FLT3 mutations have been reported to be uncommon, in fewer than 5%. Therefore, testing for FLT3 mutations in T-ALL is low yield, and methods to identify a more likely population to be positive would be helpful for increasing yield and avoiding unnecessary testing. In 2004, Paietta and colleagues3 reported 3 cases of T-ALL that were positive for FLT3 mutation. In each case, CD117 was expressed. They concluded that CD117 expression could be used as a surrogate for FLT3 mutation and, therefore, could direct therapy with FLT3 inhibitors. Subsequently, Paietta4 suggested that an immunophenotypic profile was even more helpful for predicting the presence of FLT3 mutation in T-ALL; this profile was CD2+/CD7+/CD13+/CD34+/CD62L+/CD117+/surface CD3–/CD4–/CD5–/CD8–. Paietta emphasized that deviations from this profile, especially expression of CD5 and CD33 (instead of CD13), obviated the predictive power of this immunophenotype.4

To address the potential usefulness of CD117 expression and immunophenotype in general to predict FLT3 mutations.
in T-ALL, we reviewed our experience with cases assessed for CD117 expression and FLT3 mutation at our institution. Because the criteria for acute leukemias changed in the 2008 World Health Organization (WHO) classification, we also included cases of T/myeloid acute leukemia in this study.

### Materials and Methods

#### Study Group

We retrospectively searched the files of the Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston, for cases of acute leukemia with T-cell differentiation that were assessed for FLT3 mutations as part of the clinical workup from January 2002 to August 2009. In this search, we included T-ALL and T/myeloid acute leukemia. Clinical data were obtained by review of medical records.

#### Morphologic Analysis

For each case in this study, Wright-Giemsa–stained peripheral blood and bone marrow aspirate smears were reviewed. Aspirate clot and biopsy specimens were fixed in formalin and routinely processed, and histologic sections stained with H&E were reviewed. Bone marrow aspirate smears in all 42 cases were assessed by a standard cytochemical method for myeloperoxidase, using a 3% cutoff for positive, as suggested by the French-American-British classification. Each case was classified according to the criteria of the 2008 WHO classification after the integration of morphologic findings and ancillary data.

#### Flow Cytometric Immunophenotypic Methods

All cases were bone marrow aspirate samples that were assessed by multicolor flow cytometry (at least 3-color; more recent cases, 6-color) using a large panel of antibodies, including CD2, cytoplasmic and surface CD3, CD5, CD7, CD13, CD15, CD33, CD34, CD117, cytoplasmic terminal deoxynucleotidyl transferase (TdT), and myeloperoxidase. Antigens were scored as positive by using an arbitrary cutoff of 20% or more leukemic blasts staining brighter than an isotype-matched negative control. A cutoff of 10% was used for cytoplasmic CD3 and TdT, as proposed by others. Data were reviewed in the light of the 2008 WHO classification criteria for mixed acute T/myeloid leukemia. Expression of CD117 was further specified as bright and uniform if greater than 70% of analyzed events were positive or as partial if only a subset of events were positive, often with variable intensity. This cutoff was derived by determining the distribution of CD117 expression after reviewing histograms of CD117 for all cases in this study.

#### Molecular Methods

Bone marrow aspirate samples were assessed for FLT3 mutation using a multiplex, fluorescent-based polymerase chain reaction (PCR) method to detect internal tandem duplication (ITD) and D835 point mutations, as described previously. Cases were scored positive for FLT3 ITD if amplimers were larger than 330 base pairs (bp). For the analysis of FLT3 D835 point mutations, we amplified a 112-bp fragment of FLT3 and digested with the EcoRV restriction enzyme. Digestion normally results in 2 fragments of 64 and 48 bp, but digestion is precluded by the presence of a D835 point mutation. The sensitivity of the FLT3 assay is approximately 2%.

T-cell clonality was assessed in 33 cases by analysis of the T-cell receptor (TCR)-γ chain gene as a part of the diagnostic workup. The TCR-γ chain gene was assessed by a PCR method using a mixture of 4 family-specific, multicolor, fluorescently labeled variable region primers and 4 unlabeled joining region primers, as described previously. The sensitivity of this assay for the detection of a monoclonal T-cell population is 1:100 to 1:10,000 lymphocytes, depending on the number of polyclonal T cells present.

#### Statistical Analysis

Statistical analysis was performed using the Fisher exact test (GraphPad, version 5.0a, GraphPad Software, La Jolla, CA) to evaluate differences in immunophenotype, T-cell clonality, and FLT3 mutation status. A P value of less than .05 was considered statistically significant.

### Results

The study group included 27 cases of T-ALL and 15 cases of acute T/myeloid leukemia. CD117 was expressed in 21 cases (50%); it was bright and uniform in 14 and partial in 7. A total of 8 cases had an FLT3 mutation. The immunophenotype and genotype are summarized in Table 1 and Table 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Immunophenotype and Genotype of 27 T-ALL and 15 T/Myeloid Acute Leukemia Cases*</th>
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<tbody>
<tr>
<td></td>
<td>FLT3</td>
</tr>
<tr>
<td>T-ALL (n = 27)</td>
<td>1/27 (4)</td>
</tr>
<tr>
<td>T/myeloid (n = 15)</td>
<td>7/15 (47)</td>
</tr>
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</table>

ALL, acute lymphoblastic leukemia; c, cytoplasmic; MPO, myeloperoxidase; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.
* Data are given as number positive/total tested (percentage).
Hematopathology / Original Article

T Acute Lymphoblastic Leukemia

In this subgroup (n = 27), CD117 was positive in 11 (41%) cases—bright in 7 (64%), and partial in 4 (36%). The immunophenotype of these cases was as follows: cytoplasmic CD3 (100%), CD7 (26/27 [96%]), TdT (24/27 [89%]), CD5 (22/27 [81%]), CD34 (19/27 [70%]), CD33 (17/27 [63%]), CD2 (16/27 [59%]), CD13 (14/27 [52%]), CD10 (11/27 [41%]), and CD15 (5/20 [25%]). By using flow cytometry and/or cytochemistry, we found that all 27 cases were negative for myeloperoxidase. Molecular analysis using PCR showed monoclonal TCR-γ chain gene rearrangements in 17 (85%) of 20 cases. An FLT3 ITD mutation was identified in 1 case.

T/Myeloid Acute Leukemia

In this subgroup (n = 15), CD117 was positive in 10 (67%) cases—bright in 7 (70%) and partial in 3 (30%). The immunophenotype of these cases was as follows: CD7 (100%), CD13 (100%), CD34 (100%), cytoplasmic CD3 (100%), TdT (100%), CD2 (10/15 [67%]), CD5 (10/15 [67%]), CD33 (7/15 [47%]), CD15 (5/14 [36%]), and CD10 (4 [27%]). By using cytochemistry, we found that all 15 cases were positive for myeloperoxidase (cutoff, >3%), also shown by flow cytometry in 10 of 15 cases (cutoff, 10%). Molecular analysis using PCR showed monoclonal TCR-γ chain gene rearrangements in 5 (33%) of 15 cases. An FLT3 mutation was identified in 7 (47%) of 15 cases.

FLT3-Mutated Subgroup

An FLT3 mutation was identified in a total of 8 cases, 6 ITD, 1 D835, and 1 with both ITD and D835 point mutation. Seven cases were T/myeloid acute leukemia, and 1 case was T-ALL. The point mutation occurred in a case of T/myeloid acute leukemia. The combination of both ITD and D835 point mutation also occurred in a case of T/myeloid acute leukemia. CD117 expression was bright in 7 cases Image 1 and partial in 1 case. Of interest, a low level of FLT3 mutation was detected in the case with partial CD117 expression Image 2. The pattern of CD117 expression in ITD+ cases was similar to that of the D835-mutated case Image 3.

All 8 cases with FLT3 mutation expressed cytoplasmic CD3, CD7, CD13, CD34, and TdT. Additional immunophenotypic markers expressed by a subset of cases within this subgroup included CD15 (4/6 [67%]), CD33 (4/8 [50%]), and CD5 (5/8 [63%]). Cytochemistry for myeloperoxidase showed 3% to 50% positive cells in the 7 cases of T/myeloid acute leukemia and was negative in 1 FLT3-mutated T-ALL. Conventional cytogenetic analysis showed that 1 case of T-ALL and 3 cases of T/myeloid acute leukemia had a normal karyotype. Four cases of T/myeloid acute leukemia were associated with an abnormal karyotype, but there were no recurrent abnormalities, and no case had an abnormality of chromosome 11q23 (MLL locus) Table 3. TCR gene rearrangement was detected in the 1 case of T-ALL and was not detected in all 7 cases of T/myeloid acute leukemia.

Correlation of Immunophenotype With FLT3 Mutation

CD117 as a sole marker was sensitive but not specific for FLT3 mutation. For CD117 expression alone, sensitivity was 100% and specificity was 48%. By contrast, the immunophenotypic profile of TdT+/CD7+/CD13+/CD34+/CD117+ (bright) was more helpful for predicting the presence of FLT3 mutation, with a sensitivity of 100% and a specificity of 94%.

Table 2* Phenotype and Genotype of Eight Cases of T-ALL or T/Myeloid Leukemia With FLT3 Mutation

<table>
<thead>
<tr>
<th>Type/Case No.</th>
<th>FLT3</th>
<th>TCR</th>
<th>CD2</th>
<th>cCD3</th>
<th>CD5</th>
<th>CD7</th>
<th>CD10</th>
<th>TdT</th>
<th>CD13</th>
<th>CD15</th>
<th>CD33</th>
<th>CD34</th>
<th>CD117</th>
<th>MPO</th>
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<tbody>
<tr>
<td>T/Myeloid</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>ITD</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>–</td>
<td>+</td>
<td>10</td>
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<td>2</td>
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<td>+</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>3</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
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*By cytochemistry.
CD5 expression also may be helpful for predicting FLT3 mutation. CD5 was expressed in 5 of 8 cases with FLT3 mutation—4 T/myeloid acute leukemias and 1 T-ALL. Two cases of T-ALL with an immunophenotypic profile of TdT+/CD7+/CD34+/CD117+ (bright) did not have the FLT3 mutation; both were CD5−.

**Discussion**

FLT3 is known to have important roles in hematopoietic stem/progenitor cell survival and proliferation. FLT3 is constitutively activated by mutation, and FLT3 mutations occur in an appreciable subset of cases of acute myeloid leukemia (AML) and myelodysplastic syndrome in transformation to AML. Inhibitors of FLT3 have been developed and used in clinical trials. Although there have been setbacks in drug potency and other factors related to FLT3 ligand in serum, it seems likely that these problems will be overcome. As a result, virtually all cases of AML are tested for FLT3 mutation as a part of the diagnostic workup at our institution.

Unlike AML, FLT3 mutations are uncommon in cases of ALL and are found most frequently in cases of B-ALL with MLL gene alterations. Fewer than 5% of cases of T-ALL are reported to carry FLT3 gene mutations. With such a low frequency, it would be helpful to avoid routine testing for FLT3 mutation on all cases of T-ALL if a reliable surrogate marker is available that can reduce unnecessary testing. In 2004, Paietta and colleagues suggested that CD117 expression could be used as a surrogate for FLT3 mutation. They also
suggested that patients with CD117 T-ALL could potentially benefit from FLT3 inhibitors. Since their initial study, Paietta has slightly modified that view, suggesting that an immunophenotype profile may be more helpful for predicting the presence of FLT3 mutation in T-ALL. The profile suggested was CD2+/CD7+/CD13+/CD34+/CD62L+/CD117+/CD3–/CD4–/CD5–/CD8–.

These prior studies and the need to streamline molecular testing as much as possible at our institution led us to review our experience with CD117 expression in T-ALL. Based on earlier studies by others that have shown that CD117 is generally considered a marker of leukemic cells committed to the myeloid lineage, as well as changes in the criteria for mixed lineage specified in the 2008 WHO classification, we reviewed T-ALL and T/myeloid acute leukemia cases in this study.

Our results show that CD117 expression is sensitive, but not specific, for the presence of FLT3 mutation. More than 50% of cases expressed CD117, including approximately 40% of T-ALL and 70% of T/myeloid acute leukemia. By contrast, FLT3 mutation was relatively uncommon, in only 1 case of T-ALL and 7 cases of T/myeloid acute leukemia. These results are in accord with a letter by Scharnhorst and colleagues, who described a patient with CD117+ T-cell ALL without evidence of FLT3 mutation. In our study, the negative predictive value of CD117 is 100% because no CD117– cases had an FLT3 mutation, but the positive predictive value of CD117 is only 35%. We conclude that knowledge of CD117 expression is helpful in identifying a subset of cases, approximately 60% of T-ALL and 25% of T/myeloid acute leukemia, that do not need FLT3 mutational analysis. However, it would be helpful to have a surrogate with a better specificity and positive predictive value.

In this study, we found an immunophenotypic profile that is far more helpful than CD117 alone for predicting the presence of FLT3 mutation. All 8 FLT3-mutated cases in this study had the following immunophenotype: TdT+/CD7+/CD13+/CD34+/CD117+ (bright). In contrast, only 2 cases in...
this study without an FLT3 mutation had this immunophenotype. Therefore, the presence of this immunophenotype has a sensitivity of 100%, specificity of 94%, and positive predictive value of 80%. Expression of CD117 was bright in all FLT3-mutated cases except 1; this case with partial CD117 expression had a low level of FLT3 mutation. It is also important to note that the immunophenotype we report does not exclude the expression of other markers. All FLT3-mutated cases in this study expressed cytoplasmic CD3, 7 (88%) of 8 expressed CD2, 5 (63%) of 8 expressed CD5, and 4 (50%) of 8 expressed CD33.

Seven cases of FLT3-mutated acute leukemia in this study had a T/myeloid lineage. In these cases, none of the cases had monoclonal TCR-γ gene rearrangements. These findings are in accord with the findings in a recent study by Zaremba and colleagues who, in an abstract, reported 3 FLT3-mutated cases thought to arise from early thymic T-cell progenitors that have T-cell and myeloid potential. These cases are also consistent with newly proposed models of lineage commitment. Bell and Bhandoola proposed a model of lineage commitment in which early thymic progenitors have lymphoid and myeloid lineage potential, challenging the previous concept that T cells arise from progenitors committed to lymphoid differentiation only. Kawamoto and Katsura also have proposed a
“myeloid-based” model of hematopoiesis, suggesting myeloid potential is retained by cells in the process of committing to lymphoid lineage. Of interest, 2 of 3 FLT3-mutated cases of T-ALL initially reported by Paietta and colleagues would be considered T/myeloid acute leukemia according to the current WHO classification as myeloperoxidase was expressed in 3% to 5% of cytoplasmic CD3+ blasts.

In summary, CD117 is expressed in a large subset of cases of T-ALL and T/myeloid acute leukemia. Solo CD117 expression, although present in FLT3-mutated cases, is not specific because CD117 is expressed in many other cases without FLT3 mutation. However, the immunophenotypic profile of TdT+/CD7+/CD13+/CD34+/CD117+ (bright) is highly suggestive of FLT3 mutation, with 100% sensitivity and 94% specificity in this study. FLT3 mutation is rare in T-ALL, and its presence in blastic leukemias usually indicates a mixed T/myeloid phenotype. Our data also support testing for FLT3 mutation in all cases of T/myeloid acute leukemia and T-ALL with the immunophenotypic profile TdT+/CD7+/CD13+/CD34+/CD117+ (bright).

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References


