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Distinct immunophenotype of early T-cell progenitors in T lymphoblastic leukemia/lymphoma may predict FMS-like tyrosine kinase 3 mutations

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Abstract FMS-like tyrosine kinase 3 (*FLT3*) mutation in T lymphoblastic leukemia/lymphoma (T-LL) is rare (~4%) and reported only in cases with CD117 expression. This study aimed to identify the immunophenotypic features that may predict *FLT3* mutations. We report 3 (43%) of 7 $CD117^+$ T-LL cases harboring *FLT3*-internal tandem duplication mutation. Compared with 4 *FLT3*-unmutated cases, all 3 *FLT3*-mutated cases had a distinct immunophenotype ($CD1a^-/CD2^+/CD7^+/CD34^+/CD117^{uniform+}/Tdt^+$) corresponding to the stage of earliest thymic T-cell progenitors possessing myeloid lineage potential. Indeed, all *FLT3*-mutated T-LL cases expressed myeloperoxidase on a very small subset of blasts and, thus, may be further considered a mixed phenotype acute leukemia, T/myeloid, by the 2008 World Health Organization classification scheme. We conclude that this unique immunophenotype ($CD1a^-/CD2^+/CD7^+/CD34^+/CD117^+/Tdt^+$) is a better predictor of *FLT3* mutation than sole CD117 expression.

Keywords: *FLT3* mutation; T lymphoblastic leukemia; Biphenotypic leukemia; Immunophenotype; CD117

1. Introduction

CD117 (c-KIT), the stem cell factor receptor, is expressed in early hematopoietic stem cells and progenitor cells. During normal T-cell lymphopoiesis, CD117 is transiently expressed in a fraction of triple negative ($CD3^-$, $CD4^-$, and $CD8^-$) early thymic progenitors before T-cell receptor gene rearrangement [1]. At this stage CD117 is coexpressed with CD135, also known as the *FMS-like tyrosine kinase 3* gene (*FLT3*) receptor [2].

Interestingly, *FLT3*-activating mutations, one of the most common genetic abnormalities in acute myeloid leukemia, are found rarely in T lymphoblastic leukemia/lymphoma (T-LL) and reported in only 5 cases (3.5%; 5/141) [3,4]. Testing all T-LL cases for *FLT3* mutations may thus not be necessary. An earlier study reported *FLT3* mutations only in $CD117^+$ T-LL and suggested that CD117 expression might identify a subset of T-LL cases harboring *FLT3* mutations [3]. However, lack of *FLT3* mutation has been reported in a single case report on $CD117^+$ T-LL [5]. Our study aimed to further define the immunophenotypic features that may predict *FLT3* mutations in T-LL with an emphasis on the predictive value of CD117 expression.

2. Materials and methods

Over a 5-year period (2006-2010), 38 cases of T-LL were diagnosed by flow cytometry at our institution. Nine cases (24%) expressed CD117. We focused on 7 $CD117^+$ cases that had available samples suitable for *FLT3* mutation analysis by polymerase chain reaction (PCR) followed by capillary electrophoresis, as described previously [6]. Polymerase chain reaction for the region of interest, including *FLT3* internal tandem duplications (ITDs) of the juxtamembrane domain and point mutations at aspartate 835 (D835) in the second kinase domain, was performed using the InVivoScribe Technologies *FLT3* Mutation assay

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(San Diego, Calif). D835 PCR products were then digested with *Eco*RV endonuclease. The PCR products were then evaluated by fragment analysis on an Applied Biosystems Inc. (Foster City, CA, USA) 3730xl DNA Analyzer. Specimens were scored positive if ITD amplicons were longer than 330 base-pair (bp) or if *Eco*RV digested contained 130 base-pair (bp) fragments.

Conventional cytogenetic analysis was performed on bone marrow aspirates using the standard methodology. All cases were immunophenotyped using a 4-color FACSCalibur flow cytometry instrument with CELLQuest software (Becton Dickinson, San Jose, Calif) and analyzed using cluster analysis with Paint-a-Gate Software (Becton Dickinson). Bone marrow processing and antibody staining were performed as previously described [7]. The following monoclonal antibodies (clones) were used in the panels: CD1a (SK9), CD2(S5-2), CD3 (SK7), CD4 (SK3), CD5 (L17F12), CD7 (4H9), CD8 (SK1), CD10 (W8E7), CD19 (SJ25C1), CD20 (L27), CD34 (8G12), CD38 (HB7), CD45 (2D1), CD45RO (UCHL-1), CD79a (HM47), myeloperoxidase (MPO) (H43-5), and Tdt ([terminal deoxynucleotidyl transferase]TdT-6). Four-color antibody combinations (FITC/PE/PerCP/APC [fluorescein isothiocyanate/phycoerythrin/peridinin chlorophyll protein/allophycocyanin]) were used: CD10/CD22/CD20/CD34, CD34/CD14/CD45/CD38, CD8/CD/CD5/CD3/CD/CD4, CD15/CD33/CD45/CD34, CD2/CD117/CD45/CD34, CD34/CD13/HLA-DR/CD19, CD7/CD1a/CD3/CD45RO, intracellular MPO/79C-Da/CD45/CD34, and intracellular TdT/CD22/CD3/CD45. Positivity for an antigen was defined as at least 20% of leukemia events exceeding a 2% isotype control threshold. For CD117, partial expression and uniform expression were defined as 20% to 70% and more than 70% of leukemia events expressing this antigen, respectively. The percentage of leukemia events expressing MPO was present quantitatively.

2.1. Statistical analysis

Statistical analysis was performed using Prism software (version 5.0a; GraphPad Software, Inc, La Jolla, Calif). The differences of sex, antigen expression in *FLT3*-mutated vs *FLT3*-unmutated groups were tested by Fisher exact test. The difference of age was tested by t test. $P < .05$ was considered as statistically significant.

3. Results

Of 7 evaluable $CD117^+$ T-LL cases, 3 (43%) cases harbored *FLT3*-ITD mutations. Four cases had neither ITD nor D835 *FLT3* mutations. There were no significant differences in age and sex between *FLT3*-mutated and *FLT3*-unmutated patients with T-LL (age, 6-74 years vs 7-59 years; 1 woman/2 men vs 1 woman/3 men).

All *FLT3*-mutated cases expressed CD2, CD7, CD34, CD117, and Tdt but lacked CD1a (Table 1 and Fig. 1). Although each of this antigen expression was not significantly different between *FLT3*-mutated and *FLT3*-unmutated groups, this distinctly combined immunophenotype, $CD1a^-/CD2^+/CD7^+/CD34^+/CD117^+/Tdt^+$ and was present only in *FLT3*-mutated cases (3/3 vs 0/4 in *FLT3*-unmutated cases, $P = .03$). Notably, none of *FLT3*-unmutated cases showed coexpression of CD34 and Tdt.

Furthermore, the pattern of CD117 expression was distinctly different between the 2 groups: uniform CD117 expression in *FLT3*-mutated cases in contrast to partial CD117 expression in *FLT3*-unmutated cases (Fig. 2). Myeloperoxidase was expressed at the 5% to 10% range in *FLT3*-mutated cases, which was considered negative by criteria defined in this study. All *FLT3*-unmutated cases expressed MPO at the lower-than-1% level. The expression profiles for surface CD3, intracellular CD3, CD4, CD5, CD8, CD10, and CD13/33 were similar in the 2 groups.

There were no recurrent cytogenetic abnormalities found in either *FLT3*-mutated or *FLT3*-unmutated T-LL cases. In *FLT3*-mutated cases, all 3 cases possessed a normal karyotype. Of the *FLT3*-unmutated T-LL cases, 1 possessed a normal karyotpye; 1 had 46,XX,del(5)(q31q35), add(12)(p11.2) [3] .ish del(9)(q34q34)(ABL1-); and 1 had a *BCR-ABL1* rearrangement. Although the latter case is

Table 1
Immunophenotypic profile of $CD117^+$ T-LL cases

	Age/sex	*FLT3*	CD1a	CD2	sCD3	icCD3	CD4	CD5	CD7	CD8	CD10	CD13/33	CD34	CD117	Tdt	MPO
FLT3 mutated																
Case 1	16/F	ITD	−	+	−	+	−	−	+	−	−	+/+	+	+	+	6%
Case 2	6/M	ITD	−	+	−	+	−	−	+	−	−	+/+	+	+	+	10%
Case 3	74M	ITD	−	+	−	+	−	−	+	−	−	+/−	+	+	+	5%
Sum			0/3	3/3	0/3	3/3	0/3	0/3	3/3	0/3	0/3	3/3	3/3	3/3	3/3	
FLT3 unmutated																
Case 4	7/F	Wild	−	−	+	+	−	+	+	+	−	+/−	−	pt+	+	<1%
Case 5	59/M	Wild	+	+	−	+	−	+	+	−	−	−/−	−	pt+	+	<1%
Case 6	33/M	Wild	−	−	−	+	−	−	+	−	+	−/+	+	pt+	−	<1%
Case 7	10/M	Wild	−	−	+	+	−	+	+	+	+	−/−	−	pt+	+	<1%
Sum			1/4	1/4	2/4	4/4	0/4	3/4	4/4	2/4	2/4	2/4	1/4	4/4	3/4	
P	1.00/1.00		1.00	.14	.43	1.00	1.00	.14	1.00	1.00	1.00	.43	.14	1.00	1.00	

F indicates female; M, male; pt, partial.