Activating FLT3 Mutations in CD117/KIT Positive T-Cell Acute Lymphoblastic Leukemias

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Abstract

Activating FLT3 mutations are the most common genetic aberrations in acute myeloid leukemia (AML), resulting in the constitutive activation of this receptor tyrosine kinase (RTK), but such mutations are rarely found in acute lymphoblastic leukemia (ALL). Here we describe a unique subset of de novo adult T-cell ALL cases that coexpress CD117/KIT and cytoplasmic CD3 (CD117/KITpos ALL). Activating mutations in the \textit{FLT3} RTK gene were found in each of three CD117/KITpos cases that were analyzed, but not in 52 other adult T-ALL samples from the same series that lacked CD117/KIT expression. Our results indicate the need for clinical trials to test the efficacy of drugs that inhibit the \textit{FLT3} RTK in this subset of T-ALL patients.
Introduction

Early hematopoietic stem and progenitor cells express CD117/KIT, the stem cell factor receptor.\(^1\) Detection of CD117/KIT expression by lymphoblasts in acute lymphoblastic leukemia (ALL) is rare and is restricted to T-lineage disease.\(^1\) During normal lymphopoiesis, CD117/KIT is expressed by a fraction of CD3\(^{\text{pos}}\)CD3\(^{\text{neg}}\)CD4\(^{\text{neg}}\)CD8\(^{\text{neg}}\) (triple-negative) thymocytes, which have not yet rearranged their T-cell receptor (TCR) genes.\(^4\) In these thymocytes,\(^5\) as in normal bone marrow progenitors,\(^6\) expression of CD117/KIT coincides with that of CD135, the FLT3 receptor tyrosine kinase (RTK) that is activated by the FLT3 ligand. FLT3 and CD117/KIT share extensive structural homology.\(^7\) Treatment with FLT3-ligand and stem cell factor elicits \textit{in vitro} differentiation of CD117/KIT\(^{\text{pos}}\)CD135/FLT3\(^{\text{pos}}\) T-cell progenitors towards the myeloid lineage, while stem cell factor together with interleukin-7 induces development towards a mature T-cell phenotype.\(^5\)

Activating mutations of FLT3 are the most common genetic abnormality found in AML and encode a protein with constitutive RTK activity in the absence of ligand.\(^6\) While the majority of \textit{FLT3} mutations are internal tandem duplications (ITDs) located in the juxtamembrane domain of the receptor, some leukemias instead harbor point mutations in the activation loop of the kinase domain. By contrast, FLT3 is rarely mutated in leukemic lymphoblasts,\(^6,8-10\) with the exception of the FLT3 point mutations that are found in B-lineage ALLs containing MLL gene rearrangements.\(^11\)
In this study, we asked whether the subset of early T-lineage ALL cases that express CD117/KIT also express FLT3 and harbor activating mutations of this gene. By analyzing leukemic cells from 55 adult T-ALL patients (ECOG E2993), we identified 3 cases with high CD117/KIT expression, and demonstrated activating FLT3 mutations in each of them - a finding with therapeutic significance in view of the availability of new inhibitors of FLT3 tyrosine kinase activity.

**Patients, materials and methods**

**Patient samples**

Bone marrow or peripheral blood from 449 patients on ECOG trial, E2993, were centrally immunophenotyped using multiparameter flow cytometry, as reported.\(^1\)\(^,\)\(^12\) Consent for collection and testing of samples was obtained at study entry. B-lineage ALL was diagnosed in 380, T-lineage ALL in 69 cases. Sufficient material for RT-PCR studies was available from 341 B-precursor ALLs and 55 T-lineage T-ALLs. Morphology and cytogenetics were reviewed centrally by ECOG core facilities. To investigate the association of CD117/KIT expression with T-lineage ALL, the Fisher exact test was run.

**Qualitative and quantitative RT-PCR**

Total RNA was extracted\(^13\) from mononuclear cells of and tested by RT-PCR for **BCR-ABL**, **MLL-AF4**, **E2A-PBX1**, and **TEL-AML1** fusion transcripts.\(^14\) Real-time RT-PCR analysis of the expression levels of the T-cell oncogenes,
TAL1, LYL1, HOX11, HOX11L2, LMO1 and LMO2, and the control glyceraldehyde-3-phosphate dehydrogenase) was performed as described.\textsuperscript{15} Samples were considered positive for a given oncogene if they expressed >3x10\textsuperscript{5} (LYL1), >10\textsuperscript{4} (LMO1, TAL1, HOX11, HOX11L2, BHLHB1), or >10\textsuperscript{5} (LMO2) mRNA copies per 100ng of total RNA.\textsuperscript{15}

The regions of the FLT3 mRNA sequence encoding the juxtamembrane or the activation loop domains were amplified by RT-PCR.\textsuperscript{16} For ITD analysis, PCR products larger than wild type FLT3 were subcloned into the PUC18 plasmid vector and sequenced. To detect mutations in the FLT3 activation loop domain, PCR products corresponding to this region were digested with EcoRV; digestion is disrupted by the most frequent point mutations in this region. DNA resistant to digestion was subcloned into the PUC18 plasmid vector and sequenced.

**Results and discussion**

Among 449 adult de novo ALL patients, we identified 3 males (ages 22, 36, 55 years) with CD117/KIT expression on >90% of T-lymphoblasts. This corresponded to 3/69 T-ALL or 4% (90% confidence interval 1%-11%) and reflected a strong association of CD117/KIT expression with T- compared with B-lineage disease (0% CD117/KIT\textsuperscript{pos} cases) (p=0.005). Leukemic blasts accounted for ~95% of mononuclear cells in every patient. By flow cytometry, all blasts were classified as T-lymphoblasts based on intracytoplasmic CD3 expression. The remaining antigen profile was identical among the 3
CD117/KIT\textsuperscript{pos} cases and was not found in any of the other E2993 patients: surface CD3\textsuperscript{neg}, CD34\textsuperscript{pos}, CD62L\textsuperscript{pos}, CD56\textsuperscript{neg}, CD2\textsuperscript{pos}, CD7\textsuperscript{pos}, CD1\textalpha\textsuperscript{neg}, CD5\textsuperscript{neg}, CD4/CD8\textsuperscript{neg}, TdT\textsuperscript{pos}, expressing one myeloid antigen, CD13. This immunophenotype fits into the most immature category of T-ALL,\textsuperscript{17} resembling multipotent thymic precursors.

Morphology was exclusively lymphoid in case 2, while cases 1 and 3 were predominantly lymphoid but contained 2-10\% FAB M1 blasts with occasional Auer rods. By flow cytometric analysis, myeloperoxidase and intracytoplasmic CD3 were co-expressed in 7\%, 0\% and 6\% of intracytoplasmic CD3\textsuperscript{pos} blast cells in cases 1, 2 and 3, respectively. Myeloperoxidase and intracytoplasmic CD3 are expressed by cells of the myeloid and the T-lymphoid lineage, respectively.\textsuperscript{18} Because of the predominance of T-lymphoid characteristics, we classified the immunophenotype of these leukemias as T-lymphoid. According to the WHO classification, cases 1 and 3 would fit the category of biphenotypic acute leukemia.\textsuperscript{18} The presence of CD13 as the sole surface myeloid antigen in CD117/KIT\textsuperscript{pos} T-ALL has been previously noted.\textsuperscript{19,20}

We have shown that the oncogenic transcription factor genes, \textit{HOX11}, \textit{HOX11L2}, \textit{TAL1}, \textit{LYL1}, \textit{LMO2} and \textit{MLL-ENL}, identify discrete molecular groups of T-ALL.\textsuperscript{15,22} The three CD117/KIT\textsuperscript{pos} ALL cases shared high expression levels of \textit{LYL1} and \textit{LMO2} oncogenes. This agreed with our observation in pediatric T-ALL that \textit{LYL1} and \textit{LMO2} together are associated with an early CD34\textsuperscript{pos} thymocyte phenotype,\textsuperscript{15} comparable to that of our adult CD117/KIT\textsuperscript{pos} patients.
As expected, the CD117/KIT\textsuperscript{pos} cases were negative for leukemia transcripts typical for B-lineage ALL (BCR-ABL, MLL-AF4, E2A-PBX1, TEL-AML1).

Cytogenetic analysis of the CD117/KIT\textsuperscript{pos} ALL cases showed the presence of a der(16)t(1;16)(q12;q11.2) in case 1, a t(7;14)(q22;q32) in case 2, and a complex karyotype including t(7;11)(p22;q23) and an add(11)(q23) in case 3, two abnormalities potentially involving the \textit{MLL} gene. B-lineage\textsuperscript{11} but not T-lineage ALL with \textit{MLL} rearrangements\textsuperscript{21} has been associated with high levels of \textit{FLT3} expression and the presence of \textit{FLT3} mutations.

Antibody staining for FLT3 (CD135) indicated strong cell surface expression of this protein in each of the three CD117/KIT\textsuperscript{pos} ALL. To determine whether \textit{FLT3} was mutated in CD117/KIT\textsuperscript{pos} T-ALL, we performed RT-PCR analysis looking for abnormalities in the FLT3 juxtamembrane region. \textit{FLT3} transcripts were readily detected and showed the \textit{FLT3}-ITD abnormality in cases 1 and 2. Sequence analysis of RT-PCR products confirmed the presence of an insertion of 21 base pairs encoding 7 amino acids in the juxtamembrane region of \textit{FLT3} in case 1 and of a 60 base pair insertion resulting in 20 extra amino acids in case 2. Sequence analysis of the activation-loop coding region of \textit{FLT3} showed that case 3 harbored a point mutation in this region, resulting in a D835Y amino acid change, which is the most frequently reported activating point mutation of \textit{FLT3}\textsuperscript{22} (Figure 1).
Figure 1: FLT3 tyrosine kinase activating mutations in CD117/KIT<sup>pos</sup> T-ALL samples. RT-PCR analysis of the juxtamembrane region suggested length mutations (ITDs) in cases 1 (A) and 2 (B). Sequence analysis of PCR products demonstrated the insertion of 21 (case 1) and 60 base pairs (case 2) in positions 1863 and 1894 of the juxtamembrane region of the FLT3 kinase gene respectively. In case 3, the D835Y mutation (MUT) in the activation loop of FLT3 was detected when compared to the wild-type allele (WT). Mutation position annotation is based in the FLT3 reference sequence NM_004119.
The mutant-to-wild-type ratio was 1.0 in cases 1 and 3, while the mutant level approached 75% in case 2, suggesting that in every case the FLT3 mutation was present in all blast cells, and that in case 2 heterozygosity was partially lost. FLT3 gene mutations were not detected in any of the CD117/KIT\textsuperscript{neg} T-lineage ALLs analyzed. In the majority of these cases, CD135 expression was weak or undetectable. The relationship between FLT3 mutation status and CD135 expression has not yet been established.

Thus, our results suggest that CD117/KIT expression in T-ALL lymphoblasts identifies a subset of patients in whom FLT3 gene mutations play an essential part of the multistep mutational pathway to oncogenesis. To date, FLT3 inhibitors have been considered for clinical trials exclusively in AML, due to the high frequency of activating FTL3 mutations in this disease. Based on our findings, we suggest that the analysis of CD117/KIT expression should be included in the immunophenotypic workup of all T-ALL patients who relapse on current therapy. Importantly, CD117/KIT\textsuperscript{pos} patients should be tested for FLT3 activating mutations so that these patients can be enrolled on trials to determine the efficacy of FLT3 inhibitors as single agents in refractory patients with this disease.

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References


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