What Significance Should We Attribute to the Detection of MLL Fusion Transcripts?

By Stephen P. Hunger and Michael L. Cleary

Translocations involving the mixed lineage leukemia (MLL) gene (also termed ALL-1, HRX, HTRX1) at chromosome band 11q23 occur in 5% to 10% of acute lymphoblastic and acute myeloid leukemias (ALLs and AMLs) and serve as particularly illustrative examples of the clinical, biological, and epidemiological implications of contemporary molecular oncology investigations.\(^1,2\) Remarkably, various translocations in ALL and AML fuse MLL to more than two dozen different partner genes, one dozen or more of which have been cloned and identified at the current time.\(^3,4\) MLL also undergoes partial tandem duplication in some patients with AML.\(^5\) In ALL, the t(4;11)(q21;q23), which fuses MLL to AF4 (FEL), occurs most frequently and accounts for about half of all MLL translocations. Cytogenetic data, supported by gene transfer studies and knock-in mouse models, indicate that the critical product is the der(11)-encoded fusion protein that consists of amino terminal ~1,200 amino acids of MLL fused to carboxy terminal polypeptides of various sizes specified by the different partner genes.\(^6-8\)

Epidemiologically, MLL translocations occur at greatly increased frequency in two clinical settings: infant leukemias and secondary leukemias that arise after treatment with chemotherapeutic agents classified as inhibitors of DNA topoisomerase II.\(^1,2\) Molecular studies have shown that MLL translocations are present in 70% to 80% of ALLs and 50% to 60% of AMLs that occur in infants less than 1 year of age. The occasional diagnosis of leukemias with MLL translocations in the immediate neonatal period, twin studies, and retrospective analysis of cord blood specimens indicate that MLL translocations can occur in utero.\(^9,10\)

Clinically, MLL translocations have aroused particular interest because of their prognostic significance. Infants with ALL and MLL gene rearrangements have an extremely poor outcome when treated with various different chemotherapy regimens.\(^9\) Older children and adults with ALL carrying MLL translocations also tend to fare poorly.\(^11\)

How does this information affect clinical practice? Many centers and cooperative groups are beginning to routinely screen patients with ALL, particularly infants, for MLL abnormalities, and consider altering therapy if these are detected. For example, the Children’s Cancer Group (CCG) and the Pediatric Oncology Group (POG), which collectively treat almost all infants with ALL in the United States, are currently conducting trials that explore the use of allogeneic bone marrow (BM) transplant, using either matched sibling or unrelated donors, in first remission for infants with ALL bearing an MLL translocation.

Against this backdrop, the report by Uckun et al\(^12\) in this issue of Blood presents fascinating, and occasionally perplexing, information that has significant implications for understanding the molecular details of leukemogenesis, and for interpreting molecular analyses that clinicians must now consider in treating patients with leukemia. These investigators used single-step and nested reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify MLL-AF4 fusion transcripts from infants and children with ALL. The salient points of this manuscript include the following:

1. MLL-AF4 fusion transcripts were amplified by single-step RT-PCR from only 6 of 8 infants and 1 of 2 older children with a cytogenetically evident t(4;11) (we will refer to these as standard PCR\(^+\)). This is quite similar to results previously published in Blood by Downing et al,\(^13\) who, using the same primers and reaction conditions, found that MLL-AF4 fusion transcripts were amplified by single-step RT-PCR from 17 of 23 children with t(4;11)\(^+\) ALL. In both studies, MLL-AF4 transcripts were amplified from each of the remaining t(4;11)\(^+\) patients when a second round of PCR was performed (nested PCR\(^+\)).

2. Fifteen of 125 children (12%) with ALL who lacked cytogenetic evidence of a t(4;11) were found to have an MLL-AF4 fusion transcript by nested PCR. Overall, these children did not have the clinical features and poor treatment outcome typically associated with the t(4;11), but rather were similar to the larger group of t(4;11)\(^+\)/nested PCR\(^-\) children.
with ALL. However, for some of these nested PCR \(^+\) leukemias (3 of 5 tested), Southern blot studies showed \(\text{MLL}\) gene rearrangements, indicating that a cryptic \(t(4;11)\) was present in the leukemic cells. Conversely, 2 of 5 nested PCR \(^-\) leukemias had no evidence of \(\text{MLL}\) gene rearrangements, suggesting that the \(t(4;11)\) was present in only a minor (<5\%) subpopulation of the cells.

(3) The investigators detected \(\text{MLL-AF4}\) fusion transcripts by nested PCR in a substantial proportion of samples from nonleukemic sources (4 of 16 fetal BMs, 5 of 13 fetal livers, and 1 of 6 normal infant BMs). All of the nested PCR \(^+\) normal samples that were tested by Southern blot lacked \(\text{MLL}\) gene rearrangements.

One important message of this study is that standard PCR, as used by the investigators, lacks sufficient sensitivity to identify all patients with a \(t(4;11)\). Furthermore, nested PCR, while identifying all patients with a \(t(4;11)\), lacks acceptable specificity since it was positive in some children with ALL that lacked cytogenetic evidence of a \(t(4;11)\) and an adverse treatment outcome. In addition, nested PCR was positive in a subset of samples obtained from normal children and apparently healthy fetuses. Although the investigators found that none of the children with standard-risk ALL who were in remission at the end of induction were nested PCR\(^+\), the study also raises concerns about the use of RT-PCR for detection of minimal residual disease in patients with \(t(4;11)\). One possibility is problems with the PCR reaction itself. Mutations may be present in the regions to which the first-round primers anneal, but not affecting the second-round primers. However, this seems highly unlikely. It is important to note that no mention is made in this report of the PCR threshold of detection for Southern blot studies. Therefore, we must hypothesize that the \(t(4;11)\) is present in only a small percentage of cells in some children with ALL. The fact that the \(t(4;11)/\text{nested PCR}^+\) leukemias in this study did not have the clinical features and poor treatment outcome typically associated with the \(t(4;11)\) seems to support this hypothesis. If correct, then these patients might represent the counterparts of the healthy fetuses/infants with rare \(t(4;11)^+\) cells (see below). However, this would not explain why \(\text{MLL-AF4}\) transcripts are not detected once patients enter remission unless the \(t(4;11)\), in the absence of other mutations, renders cells more sensitive to chemotherapy.

The detection of \(\text{MLL-AF4}\) transcripts by RT-PCR in BM from one presumably healthy infant and normal fetal liver and BM adds the \(t(4;11)\) to the list of molecular abnormalities—\(t(14;18)\), \(t(9;22)\), \(t(8;14)\) and \(\text{MLL}\) tandem duplication—that are present, at low levels, in hematopoietic cells of normal individuals, and apparently tolerated without adverse consequences.\(^{19-22}\) Perhaps these results should no longer be surprising. All available data (statistical models, transgenic animal studies, latency following in utero mutations) indicate that development of leukemia, like solid tumors, is a multistep process that requires cooperative mutations in more than one oncogene and/or tumor suppressor gene. Thus, it is likely that cells with mutations typically found in leukemias and lymphomas frequently arise in normal individuals. However, additional mutations necessary for progression to clinical malignancy must ostensibly occur in only a subset of individuals. In others, “single-hit” mutations might provide the cell with a survival advantage that allows long-term persistence, i.e., Bcl-2 overexpression in a \(t(14;18)^+\) cell. In other cases, it might render the cell more susceptible to spontaneous or exogenously induced cell death, as postulated above for \(\text{MLL-AF4}\).

What then should we conclude from this and similar reports? First, molecular analyses, like all other clinical information, must be interpreted cautiously and in the context of other available data. At the present time, it seems prudent to use tests other than RT-PCR, such as Southern blot analysis, to screen for \(\text{MLL}\) abnormalities, or to insist that \(\text{MLL}\) abnormalities be confirmed by another technique in those patients that are nested PCR\(^+\) but not standard PCR\(^-\). Nested PCR assays must be rigorously tested to ensure that they reliably distinguish between leukemia/lymphoma patients that have low levels of disease, and healthy individuals that carry rare nonmalignant cells that possess a specific molecular defect. Biologically, we must move beyond focusing exclusively on translocations and
other “sentinel” molecular defects toward a more comprehensive characterization of the spectrum of mutations present in malignant cells.

REFERENCES

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