Phenotypic Heterogeneity of TDT + Cells in the Blood and Bone Marrow: Implications for Surveillance of Residual Leukemia

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Terminal deoxynucleotidyl transferase (TdT) is a useful marker for normal lymphocyte precursors and acute lymphoblastic leukemia (ALL). Our previous studies, however, have shown that for monitoring minimal residual disease in the circulation, assay for TdT alone is not sufficiently specific to distinguish leukemia cells from the background of rare normal blood TdT + cells. In an attempt to increase specificity for leukemic cells, we have used double and triple immunophenotypic analysis to characterize normal circulating and bone marrow TdT + cells. Overall, normal TdT + cells were about 1000-fold more frequent in the marrow than in the blood. More than 75% of TdT + cells in both the blood and marrow expressed the CD34, CD22, and HLA-DR antigens. However, circulating TdT + cells infrequently expressed CD19 (4.5%) and CD9 (2.3%), compared with their marrow counterparts (74% and 47%, respectively). The brightly staining CD10 + phenotype, frequently associated with ALL blasts, was significantly less common among normal blood (5.7%) than marrow (31%) TdT + cells. Although T-lineage markers were rarely expressed on TdT + cells in either site, CD7 + cells were far more prevalent within the circulating TdT + subset (4%) than among the marrow population (<0.2%). The results suggest a selective release of lineage-uncommitted and/or thymus-destined TdT + cells from the marrow into the circulation. Moreover, since CD19, CD9, and high-density CD10 are frequently found on ALL blasts, staining for these markers on TdT + cells in the circulation should improve the specificity of assay for residual common ALL cells. Likewise, assay for CD5 + and possibly CD7 + TdT + cells in either marrow or blood should provide a very sensitive method of detection of T-ALL blasts.

A CUTE LYMPHOBLASTIC leukemias (ALL) closely resemble normal lymphocyte precursors in their expression of lineage specific proteins and rearrangement of antigen receptor genes. Indeed, no truly leukemia-specific proteins that could serve as markers for minimal residual disease have been identified. In previous studies designed to identify residual leukemic cells, we focused on the minor subpopulation of cells that express terminal deoxynucleotidyl transferase (TdT). Because the background of TdT + lymphocyte precursors in the bone marrow is high, we examined the peripheral blood as a favorable site for monitoring disease. Although normal TdT + cells are exceedingly rare in the blood, our earlier observations suggested that normal circulating TdT + cells may fluctuate above the normal range under the stress of chemotherapy. Thus, because patients in remission generally receive chemotherapy, this single marker technique was not sufficiently specific for the leukemic phenotypes to predict relapse accurately.

Recently, dual and triple marker studies have suggested that in comparison to normal marrow B-cell precursors, blasts from patients with ALL frequently express early B-lineage markers in an asynchronous fashion. These observations raise the possibility that simultaneous analysis of multiple markers may discriminate between normal and leukemic cells, even though no single marker is unique to leukemic cells. These disordered patterns of expression could then be exploited to develop accurate assays for minimal residual disease.

In this report, we have used double and triple immunostaining procedures to define more precisely the phenotypes of normal circulating TdT + cells and to compare them to marrow TdT + cells. Indeed, in contrast to TdT + cells in the bone marrow, little is known of the phenotypes of these rare blood cells. The results reveal significant differences in the expression of B- and T-lineage markers on normal TdT + cells in blood and bone marrow. These differences suggest a selective release of marrow TdT + cells into the circulation. Moreover, certain commonly recognized ALL immunotypes were found to be infrequent among normal blood TdT + cells. Therefore, surveillance for these immunotypes may be a useful strategy in monitoring the peripheral blood for residual ALL blasts.

MATERIALS AND METHODS

Cells. Blood and bone marrow cells were obtained from consenting healthy volunteers. Blood donors (7 men, 4 women) ranged in age from 13 to 46 years. Marrow donors (4 men, 1 woman) were 24 to 35 years old. The study was approved by the Institutional Review Board, as approved by the Department of Health and Human Services. Mononuclear cells were obtained by centrifugation over Ficoll-Hypaque.

Primary antibodies. Affinity-purified rabbit anti-TdT was purchased from Dupont (Baltimore, MD). CD10(J5-biotin), CD20(B1), CD1(T6), CD2(T1), and CD5(T1) antibodies were obtained from Coulter Immunology (Hialeah, FL). CD19(Leu12), CD22(Leu14), CD5(Leu1-biotin), and CD34(HPCA-1) antibodies were purchased from Becton Dickinson Immunocytometry Systems (Mountain View, CA). CD3(Dako-T3) was obtained from Dako Corp (Santa Barbara, CA). CD22(UV22-2 and RFB4) antibodies were gifts from Drs Guo-Liang Shen and Ellen Vitetta. Antibody UV22-2 is commercially available from Tex-Star Monoclonals (Dallas). CD9(BA-2) antibody was obtained from Hybritech Inc (San Diego). CD7(3A1) antibody was from the American Type Culture Collection; the purified biotin-conjugated antibody was a gift of Dr Patrick Reynold. UCLA School of Medicine. HLA-DR(L243) antibody was provided by Dr Peter Lipsky. A mixture of...
all murine IgG isotypes (Coulter) was used as a control. All antibodies were used at saturating concentrations as determined by titration.

Secondary antibodies. Gold-conjugated goat anti-mouse IgG (5 nm particle size) and the Intense-M silver enhancement system were from Jansen Life Sciences Products (Piscataway, NJ). Tetramethylrhodamine (TRITC)-conjugated streptavidin was obtained from Jackson Immunoresearch Labs Inc (Avondale, PA). FITC-goat anti-rabbit IgG (human serum absorbed) was purchased from Kirkegaard and Perry Labs, Inc (Gaithersburg, MD).

Staining methods. Mononuclear leukocytes (0.5-3 x 10^6) were washed with PBS, centrifuged onto poly-L-lysine coated slides using Cytobuckets (IEC Corp, Needham Hts, MA), fixed with 2% paraformaldehyde for 15 minutes at room temperature, and rinsed thoroughly with PBS.

For double marker assays, either unconjugated or biotinylated primary antibody was then added, followed by either gold-conjugated goat anti-mouse IgG or TRITC-streptavidin. Cell membranes were permeabilized by treating with cold 75% methanol for 20 minutes. After 5 minutes rehydration with PBS, rabbit anti-TdT (1/40 dilution) was added. The cell spots were washed again and FITC-goat anti-rabbit IgG was applied. Cells were fixed with 2% paraformaldehyde and rinsed thoroughly in glass distilled water in preparation for silver enhancement. Intense-M was added for 22 minutes at room temperature. The cells were then rinsed with distilled water and PBS and mounted with glycerol gelatin (Sigma Chemical Co, St Louis).

Triple marker assays were similarly performed except that cells were flooded with 10% normal mouse serum after addition of the gold conjugate. The biotin-conjugated monoclonal antibody (CD10, CD5 or CD7) was applied, followed by streptavidin-TRITC. All staining operations were done in the cold except as noted. Further details of this triple-stain procedure will be published separately.

Stain controls included a mixture of all isotypes of murine IgG and biotin-conjugated purified murine Ig. The common and T-ALL cell lines NALL-1 and RPMI 8402 were used as positive controls for TdT and membrane markers. Since normal volunteers were used, normal T and B lineage cells also served as internal positive and negative controls.

Cell spots were examined under a Zeiss Standard microscope equipped with mutually exclusive fluorescein and tetramethylrhodamine epifluorescence filter combinations and with epipolarization filters. The entire spot was scanned for evaluation of peripheral blood TdT+ cells. Bone marrow spots were scanned until 200-500 TdT+ cells were identified and characterized. Statistical significance was evaluated using the Student’s t-test.

RESULTS

Simultaneous staining for two or three markers in immature lymphoid cells. The primary aim of this study was to determine the surface phenotypes of TdT+ cells in normal blood and marrow in order to serve as a basis for comparison to ALL cells. TdT+ cells are rare in normal blood. Since flow cytometric assays with the sensitivity and specificity required for detection of rare TdT+ cells have not been developed, a method using combined epifluorescence and epipolarization microscopy was adopted. The sensitivity of this method for detection of TdT+ cells is limited only by the number of cells scanned, and specificity is confirmed by the nuclear location of staining. In the current study, up to 3 x 10^6 blood cells were analyzed for each double or triple stain. TdT+ cells were enumerated for each stain and the mean for each donor calculated. The mean frequency of blood TdT+ cells for all donors (n = 11) was 69 ± 40/10^6 mononuclear cells; in the bone marrow there were 6.0 ± 3.6 TdT+ cells/100 mononuclear cells (n = 5).

Representative examples of triple stains are shown in Fig 1. A CD19-/CD10− TdT+ cell in normal peripheral blood is shown in Fig 1(A-D). CD19+/CD10+ and CD19−/CD10− TdT+ cells in normal bone marrow were clearly distinguishable (Fig 1E-H). The independence of staining of two surface markers was demonstrated with antibody and cell controls. For example, CD19+ (immunogold)/CD10+ (biotin-streptavidin TRITC) and CD19+/CD10− TdT+ leukemic blasts could be seen in the same microscopic field (Fig 1I-L). Furthermore, the majority of peripheral blood TdT+ cells were of the CD34+/CD10− phenotype, while some leukemic cell populations were CD34−/CD10+ (data not shown).

Phenotypes of TdT+ cells in the blood: precursor and B-lineage markers. The surface characteristics of TdT+ cells found in normal peripheral blood are summarized in Tables 1 through 3. Table 1 contains data on cells stained with precursor and B-lineage markers. The majority (85 ± 12%) of the TdT+ cells bore the progenitor cell marker CD34 (Table 1). Conversely, a mean of 16% (range, 6% to 42%, n = 6) of all CD34+ cells were TdT+ (data not shown). HLA-DR antigen was detected on 65% to 100% of TdT+ blood lymphocytes. Presumptive mature B cells (TdT−) stained much brighter with the HLA-DR antibody than did TdT+ cells.

Another B-lineage antigen consistently found on the majority of TdT+ cells was CD22. Both the UV22-2 and Leu14 antibodies were used to identify this marker. The UV22-2 antibody stained 87% to 100% of blood TdT+ cells; the intensity of staining varied from dim to bright. In contrast, the Leu14 antibody stained 13% to 80% of TdT+ cells with an intensity which barely exceeded the background (data not shown). Both CD22 antibodies stained 4% to 11% of all mononuclear cells, the expected number of TdT− blood B lymphocytes; in each case the staining of these presumptive B lymphocytes was brighter than that of the TdT+ cells. Only the results obtained with the UV22-2 antibody are reported in Table 1.

To confirm that CD22 epitopes were present on the cell membranes of TdT+ cells, viable blood mononuclear cells from two normal donors were stained in suspension with UV22-2 antibody, washed, resuspended in either gold-conjugated goat anti-mouse IgG or FITC goat anti-mouse Ig, washed again, and spun onto slides. The cells were then fixed, permeabilized, and stained for TdT as described in Materials and Methods. This method detects only those CD22 epitopes exposed at the cell surface, as opposed to intracellular antigen. A mean of 93% of TdT+ cells were found to express CD22 molecules when the immunogold/silver method was used. The indirect immunofluorescence technique detected about one-third as many CD22+ TdT+ cells. The Leu14 antibody was not tested using this protocol. However, similar data were obtained when the anti-CD22 antibody RFB4 was substituted for UV22-2; a mean of 88% of the TdT+ cells were RFB4+. These results are nearly identical to the mean
obtained when cells were stained for CD22 after paraformaldehyde fixation (Table 1).

Two other B-cell precursor markers, CD10 and CD9, were expressed on a minority of blood TdT+ cells. CD10 was found on 42 ± 10% of the TdT+ subset; however, the staining was usually weak. Very few (2.3 ± 2.3%) of these cells expressed CD9.

Only a small subset of blood TdT+ cells expressed the B-lineage markers CD19 and CD20. Although the four donors who underwent bone marrow aspirations just prior to blood sampling had 5% to 14% CD19+ TdT+ cells, six blood samples from donors who did not undergo prior marrow aspiration contained 0% to 6% CD19+ TdT+ cells ($P < .02$). Since the CD19+ TdT+ phenotype is common in

Fig 1. Triple immunostaining of TdT+ cells in normal blood (A-D), normal bone marrow (E-H), and in the blood of a patient with common ALL (I-L). In each panel of photographs, arrows point to TdT+ cells viewed under phase microscopy (A, E, and I), epifluorescence for fluorescein (TdT) (C, G, and K), epifluorescence for rhodamine (CD10) (B, F, and J), and epipolarization (CD19) (D, H, and L). (A-D) A normal CD19−/CD10− TdT+ blood cell is shown. The CD19+ TdT− B lymphocytes are clearly seen. (E-H) Two normal bone marrow TdT+ cells are shown; one is CD19+ /CD10+, and the other is CD19−/CD10−. Two additional unmarked TdT+ cells are present. Moderate fluorescein background staining is seen in the cytoplasm of some of the myeloid cells. (I-L) The blood cells from a patient with common ALL. One CD19+/CD10+ and one CD19+/CD10− cell are in close proximity; both are TdT+. A mixture of TdT− and TdT+ CD19+ cells is seen, representing both normal and leukemic B-lineage cells.
HETEROGENEITY OF TdT+ CELLS

the marrow (Fig 2) and TdT+ cells are about 1000-fold more prevalent in the marrow than in the blood, we cannot rule out the possibility that some of these CD19+ TdT+ cells may have escaped into the blood during marrow aspiration. When viable blood mononuclear cells were stained for CD19 in suspension, CD19+ TdT+ cells were again seen only rarely. The mature B-cell marker CD20 was found on 0% to 21% of blood TdT+ cells.

T-lineage markers on blood TdT+ cells. T-lineage markers were infrequently expressed on TdT+ blood cells (Table 2). CD5 was found on 1% to 2% of TdT+ blood cells from three of nine donors; in the remaining six donors CD5+ TdT+ cells were not found. Thus, the fraction of CD5+

TdT+ lymphocytes did not exceed 2 per 10⁶ blood mononuclear cells. CD7 was more prevalent in the TdT+ subset, being expressed on 2% to 12% of TdT+ cells from nine of 11 donors. In three of four donors examined, CD2 was on 3% to 15% of TdT+ cells. CD1 was rarely seen. No CD3+ cells were found in two blood samples examined (data not shown).

Simultaneous expression of surface markers on blood TdT+ cells. Certain combinations of B-lineage surface markers are commonly found on ALL blasts; these include CD19/10, CD9/10, CD34/10, and CD20/10. Therefore, normal peripheral blood TdT+ cells were evaluated for simultaneous expression of these antigens; these data are
shown in Table 3. As expected from the rarity of CD19, 9 and 20 expression on TdT+ blood cells (Table 1), the CD10+ subset of TdT+ blood cells seldom expressed any of these three markers (Table 3). The four donors who underwent bone marrow aspirations just prior to blood sampling had 3% to 13% CD19+ /10+ blood TdT+ cells, compared to 0% to 5% in donors not so manipulated (P < .05). The phenotype CD34+/10+ (h = high intensity or bright staining) was rarely found on normal blood TdT+ cells.

To a limited extent, simultaneous expression of markers on the infrequent CD7+ subset was also evaluated. HLA-DR was found on 33% and 100% of the CD7+ cells from two donors, and low-intensity CD22 was present on 90% of CD7+ cells from one donor (data not shown).

Comparison of phenotypes of blood and marrow TdT+ cells. Paired blood and bone marrow samples were obtained from five normal volunteers in order to reveal any differences in phenotypes between TdT+ cells in these compartments. As in peripheral blood, the majority of marrow TdT+ cells expressed CD34, CD22, and HLA-DR antigens (Fig 2). However, this analysis also revealed a number of phenotypic differences between TdT+ cells in these two sites. First, in the marrow, 74 ± 9.4% of TdT+ cells expressed CD19, compared to 6.8 ± 4.1% in the blood. Second, 47 ± 11% in the marrow were CD9+, compared to 3.2 ± 2.5% in the blood. Triple staining studies demonstrated that the majority of the marrow CD10+ subset expressed CD19 and CD9 (Fig 3). By contrast, CD19 and CD9 were infrequently found on blood TdT+ TdT+ cells. Third, CD10+ cells were represented nearly twice as often among marrow TdT+ cells as in the blood (77 ± 6.9% v 41 ± 14%). Moreover, only 5.8% of circulating TdT+ cells stained brightly for CD10+ (CD10h phenotype), compared to 31 ± 7.4% of their marrow counterparts. Fourth, the CD20+ fraction of TdT+ cells was greater in the marrow (13 ± 8.3%) than in the blood (5.4 ± 3.3%). Finally, the T-lineage markers CD5 and 7 were represented even less frequently on marrow than on blood TdT+ cells (Fig 2). Fewer than 0.2% of marrow TdT+ cells stained for either T-lineage marker, whereas 4.0 ± 2.8% of blood TdT+ cells expressed CD7.

**DISCUSSION**

A noteworthy finding of our study was the rarity of the CD19 marker on blood TdT+ cells. This antigen is expressed on very early B-lineage cells in the marrow, including TdT+ cells. The scarcity of this pan-B-lineage marker on blood TdT+ cells could imply that committed B-lineage precursors are infrequently released into the circulation. However, the CD22 antigen, which has been thought to mark late pre-B and B lymphocytes rather than TdT+ precursors, was present on nearly all of the blood TdT+ cells.

By staining viable cells prior to fixation, we verified that most blood TdT+ cells were membrane CD22+. Our data differ from those of Campana et al, who used indirect immunofluorescence to show that normal bone marrow TdT+ cells expressed CD22 determinants in the cytoplasm but not on the cell membrane. The most likely explanation for this discrepancy is the sensitivity of the immunogold/silver technique for the detection of low levels of cell membrane CD22 molecules. For example, using indirect immunofluorescence, Campana et al reported that the anti-CD22 antibody RFB4 stained several common ALL cell populations and cell lines only in the cytoplasm and not on the cell membrane. Subsequently, Boué and LeBien found that three anti-CD22 antibodies (Leu14, To15, and HD39) used at saturating concentrations stained the surface membranes of most B lineage ALL blast populations and of the

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**Table 1. Phenotypes of TdT+ Cells in Normal Blood Stained for B-Lineage Markers**

<table>
<thead>
<tr>
<th>TdT (x 10^6)</th>
<th>CD34</th>
<th>CD19</th>
<th>HLA-DR</th>
<th>CD9</th>
<th>CD10</th>
<th>CD20</th>
<th>CD22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>69</td>
<td>85</td>
<td>4.5</td>
<td>88</td>
<td>2.3</td>
<td>42</td>
<td>6.4</td>
</tr>
<tr>
<td>SD</td>
<td>40</td>
<td>12</td>
<td>3.7</td>
<td>14</td>
<td>2.3</td>
<td>10</td>
<td>6.1</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>11</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

Mononuclear leukocytes from peripheral blood (1-3 x 10^6) were stained for TdT and the other markers listed. CD designations are by number. TdT+ cells are expressed per million; CD+ cells are scored as percent of total TdT+ cells.

**Table 2. Phenotypes of TdT+ Cells in Blood Stained for T-Lineage Markers**

<table>
<thead>
<tr>
<th>TdT (x 10^6)</th>
<th>CD5</th>
<th>CD7</th>
<th>CD1</th>
<th>CD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>72</td>
<td>0.6</td>
<td>5.4</td>
<td>0.5</td>
</tr>
<tr>
<td>SD</td>
<td>39</td>
<td>0.9</td>
<td>3.5</td>
<td>1.0</td>
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<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Mononuclear leukocytes from peripheral blood (1-3 x 10^6) were stained for TdT and the other markers listed. TdT+ cells are expressed per million; CD+ cells are scored as percent of total TdT+ cells.

**Table 3. Simultaneous Expression of Surface Markers on CD10+ TdT+ Cells in the Blood**

<table>
<thead>
<tr>
<th>TdT (x 10^6)</th>
<th>CD10+</th>
<th>19+/10+</th>
<th>9+/10+</th>
<th>34+/10+</th>
<th>34-10h</th>
<th>20+/10+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>70</td>
<td>42</td>
<td>3.8</td>
<td>1.8</td>
<td>37</td>
<td>0.7</td>
</tr>
<tr>
<td>SD</td>
<td>40</td>
<td>10</td>
<td>4.0</td>
<td>1.8</td>
<td>11</td>
<td>0.6</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tr>
</tbody>
</table>

Mononuclear leukocytes from peripheral blood (1-3 x 10^6) were stained for TdT and the other markers listed. CD designations are by number. TdT+ cells are expressed per million; CD+ cells are scored as percent of total TdT+ cells.

Abbreviations: +, detectable membrane staining regardless of intensity; -, undetectable staining; h, high intensity staining; SD, standard deviation; n, number of normal donors studied.
same common ALL cell lines used in the Campana study.\textsuperscript{15} This requirement for high concentrations of antibody suggests that lower densities of CD22 molecules are present on these ALL cells than on mature B cells. When viable cells were stained, our immunogold/silver detection system was more sensitive in revealing CD22 determinants on normal TdT+ cells than with indirect immunofluorescence. We conclude that most normal TdT+ cells express CD22 molecules both on the membrane and in the cytoplasm, and in this respect resemble B-lineage ALL cells. Our routine staining technique may detect CD22 molecules in both these sites.

The nature of these CD19−CD22+ blood TdT+ cells is uncertain. We favor the interpretation that the presence on normal blood TdT+ cells of low-density CD22 does not imply commitment to the B lineage. Consistent with this hypothesis was the finding that CD7+ TdT+ blood cells were also found to bind UV22-2, albeit again at low levels. We further suggest that the CD19−CD7− majority subset of circulating TdT+ cells may be uncommitted to either major pathway of lymphocyte differentiation.

Bone marrow TdT+ cells were found to differ from their blood counterparts in several important ways. The results are consistent with the idea that the majority of the bone marrow TdT+ cells occupy early stages of B-lineage development, while their blood counterparts are either uncommitted or are destined for the T lineages, presumably in the thymus.\textsuperscript{16} Since the circulating TdT+ compartment is ultimately derived from the bone marrow, the results further suggest that the release of TdT+ cells from the marrow or their survival in the circulating selectively favors the CD19−, CD9− phenotypes.

Regarding the small CD7+ subset of normal TdT+ cells, van Dongen and collaborators suggested that such cells may represent prothymocytes.\textsuperscript{16} HLA-DR determinants were found on these rare CD7+ TdT+ cells in both van Dongen’s study and our own. We detected only rare CD7+ cells in the bone marrow TdT+ subset. In the earlier study, such cells accounted for 0% to 0.2% of marrow TdT+ cells from normal adults.\textsuperscript{16} Clearly, the fraction of CD7+ cells in the TdT+ subset is larger in blood than in marrow. This difference is consistent with the selective release of marrow prothymocytes into the blood and supports the notion that...
the CD7+ cells do indeed represent these precursors. Further evidence for this pathway comes from the observation that treatment of marrow cells with anti-CD7 antibodies plus complement specifically eliminates most clonogenic T-cell precursors.

Since the majority of B-lineage ALLs bear the marker pairs CD19/10, CD9/10, and CD20/10, the phenotypes of these ALL populations resemble those of bone marrow, rather than blood, TdT+ cells. Therefore, surveillance in the marrow for these frequent ALL phenotypes will not provide a sensitive strategy for detection of residual disease. Furthermore, in our hands the CD22 marker did not distinguish normal from leukemic TdT+ lymphocytes. However, under certain circumstances, surveillance for frequent B lineage ALL phenotypes in the blood may prove to be a useful strategy for surveillance of minimal residual disease. These phenotypes include the CD19+/CD10+, CD9+/CD10+, CD20+/CD10+ and CD34−/CD10+ TdT+ combinations. In this regard, the most discriminating marker is CD19, which is found on more than 95% of B lineage ALLs but was seen on less than 15% of normal blood TdT+ cells. The brighter the staining of ALL blasts for CD10, the easier it would be to distinguish circulating normal and leukemic TdT+ cells from each other.

Our data suggest that prospects for accurate surveillance of T-ALL are even more favorable. The CD5, CD7, and CD2 markers are the most consistently expressed lineage-specific antigens on T-ALL cells. Less than 15% of the TdT+ cells or fewer than 15 per 10^6 mononuclear cells in either normal blood or marrow expressed any of these T-lineage markers. Therefore, assay for TdT+ cells bearing the CD5 and, to a lesser extent, the CD7 and CD2 markers may provide a useful strategy for surveillance of T-ALL.

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Phenotypic heterogeneity of TDT+ cells in the blood and bone marrow: implications for surveillance of residual leukemia [see comments]

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