Prethymic Phenotype and Genotype of Pre-T (CD7+/ER-) Cell Leukemia and Its Clinical Significance Within Adult Acute Lymphoblastic Leukemia

By Eckhard Thiel, Bernd R. Kranz, Anand Raghavachar, Claus R. Bartram, Helmut Löffler, Dorle Messerer, Arnold Ganser, Wolf-Dieter Ludwig, Thomas Büchner, and Dieter Hoelzer

Pretreatment blast cells from 739 adults with acute lymphoblastic leukemia (ALL) were immunophenotyped as part of a prospective treatment protocol study. Among 192 patients (26%) with T lineage ALL, 47 (6%; 24% of T lineage ALL) had lymphoblasts without sheep erythrocyte rosette formation, but with pan-T antigen CD7 on the membrane and intracellular CD3 proteins mostly in peripheral accumulation. The T-cell surface antigens CD5 and/or CD2 and focal acid phosphatase were additional markers of this subgroup traditionally called pre-T ALL, whereas thymocyte antigen CD1 as well as CD4 and CD8 antigens were not expressed. Hematopoietic progenitor cell markers, namely terminal deoxynucleotidyl transferase (TdT), and in part common ALL antigen (CD10), HLA-DR antigens, and/or My-10 (CD34), a unique antigen of marrow cells absent in thymus cells, further characterized this immature T-ALL form of putative prethymocytic phenotype (CD7+/intracellular CD3+/TdT+/My-10+/HLA-DR+/CD10-). The prethymic T cell character was supported by germ-line T-cell receptor β genes found in 21 of 36 patients analyzed. In five cases only Tγ-chain genes were rearranged. Fifteen patients, however, had rearrangements of both Tβ and Tγ genes. Immunoglobulin heavy chain genes were rearranged only in two cases. Pre-T ALL differed significantly from E-rosette+ T-ALL in some presenting clinical features, namely mediastinal mass, lymphadenopathy, and platelet count, and independently of clinical factors in prognosis (P = .02, median remission duration: 15.7 ± 3.5 months, and P = .02, median survival time: 24.6 ± 50.7 months). We conclude that ALL classification based solely on T- or B-cell lineage affiliation is not sufficient but needs further subdivision according to relevant maturation stages as exemplified here within the T-cell axis. The putative prethymic T cell progenitor phenotype described might help elucidate the sequence of genetic events that commit normal hematopoietic cells to the T-cell lineage.

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protein. CD3 gene transcription as a very early event in T-cell differentiation can be demonstrated in the most immature, E-R thymic lymphocytes, which have been prepared by means of sheep erythrocyte rosette sedimentation procedure.\textsuperscript{31} Since the immature phenotype of E-R thymocytes appeared to be similar to E-R, T-cell antigen CD7\textsuperscript{+} ALL cells, we focused our attention in the present study on this ALL subgroup. Taking advantage of a large number of leukemias received as part of a prospective therapeutic study of ALL in adults, we addressed the following main questions. First, do patients with E-R T-ALL form a distinct subgroup concerning clinicopathologic correlations and outcome? Second, are there major differences in phenotype and genotype as compared with other T-ALL subgroups? Third, can we get more information on the putative phenotype and genotype of early thymic and especially prethymic cells via these clonal T-cell neoplasms?

**METHODS**

**Patients.** Pretreatment specimens (heparinized bone marrow and/or blood) were obtained from 837 patients who entered the prospective multicenter German BMFT-ALL/AUL study of adults between January 1979 and November 30, 1986. A diagnosis of ALL was made by local and central assessment of cytologic features according to the French-American-British (FAB) criteria,\textsuperscript{32} including cytomorphic examination of May-Grünewald-Giemsa-stained smears of bone marrow and blood, standard cytotoxic assays for myeloperoxidase, alpha naphthyl acetate esterase and acid phosphatase, the periodic acid Schiff stain, and indirect immunofluorescence for terminal deoxynucleotidyl transferase (TdT) on smears.\textsuperscript{33} FAB criteria were used for assigning of ALL to Li, L2, and L3 designations.\textsuperscript{34} In 739 of the 837 ALL cases, specimens were adequate for complete immunophenotyping as described below. Thirty-five of the 739 immunophenotyped patients were excluded from the therapeutic study for several reasons, eg, underlying disease with contraindication for the protocol, age over 65 years, no informed consent, or violation of induction therapy protocol.

All patients were treated according to a multimodal treatment protocol\textsuperscript{35,36} derived from a therapy regimen successfully used in childhood ALL.\textsuperscript{37} Clinical evaluations were performed as described elsewhere.\textsuperscript{35} At the time of evaluation (November 1986) remission rates and follow-up of 602 of the 704 phenotyped and study-qualified patients were available; 102 patients were too early in therapy for response evaluation.

**Immunophenotype analysis.** Blast cells for immunophenotype determination were isolated by standard Ficol-Hispaque density gradient centrifugation. Rosette assays at 4°C with untreated and AE-treated sheep erythrocytes, direct immunofluorescence with goat anti-Ig sera, and double-label immunofluorescence with fluoresceinated anti-kappa and rhodamin-conjugated anti-lambda reagents were performed as described.\textsuperscript{4} The binding of monoclonal antibodies was assessed with fluorescein-labeled, affinity-purified IgG F(ab')\textsubscript{2}, fragments of goat anti-mouse Ig by means of fluorescence microscopy and in part by flow cytometry, as described.\textsuperscript{48} The following selected panel of mouse monoclonal antibodies according to WHO nomenclature\textsuperscript{49} was used in each case (Table 1): VIL-A1 (CD10); BA-1 (CD24); WT1 or Leu-9 (CD7); OKT6-NA134 mixture (CD1); and VIM-D5 (CD15) and VIM-2. The criterion for surface-marker positivity was expression in at least 20% of the leukemic blast population.

Twenty-eight cryopreserved specimens in a total of 47 CD7\textsuperscript{-} positive, E-rosette-negative ALLs were analyzed in more detail, using a sensitive immunocytochemical method\textsuperscript{50} and a broader panel of monoclonal antibodies (Table 1) to determine all major T-cell surface antigens, cytoplasmic CD3 as well as markers of hematopoietic progenitor cells, notably including My-10.\textsuperscript{48} Briefly, 1 to 3 × 10\textsuperscript{4} cells, suspended in 10 μL 0.01 mol/L HEPES-buffered protein-free MEM (GIBCO, UK), were attached electrostatically to poly-L-lysine–coated multislot slides (15 minutes, 20°C) and then fixed with glutaraldehyde (0.05%, seven minutes, 20°C). Spots assigned for testing cytoplasmic CD3 were subsequently incubated at 20°C for 15 minutes with a 0.04% solution of the nonionic detergent Brij 56 (Sigma, St Louis) to permeabilize cell membranes by extracting lipids from the meshwork of glutaraldehyde-crosslinked membrane proteins. Nonpermeabilized and permeabilized cells were then incubated with the various primary antibodies, followed by sequential incubations of 30 minutes each with peroxidase-labeled goat-anti-mouse and peroxidase-labeled swine-anti-goat–immunoglobulin antibodies (Tago, USA) as second and third layer, respectively. After each incubation, cells were washed by simply dipping the slides into PBS. The enzyme reaction was performed using 3-amino-9-ethyl-carbazole as chromogen, followed by nuclear counterstaining with acid hemalum. After mounting with phosphate-buffered glycerol, at least 400 cells were evaluated per antigen. In prior methodologic studies as well as in the present study, the latter method had proved to be more sensitive in detecting antigens than routine immunofluorescence. The increase in sensitivity was most pronounced in the detection of CALLA, due to glutaraldehyde-mediated immobilization of the antigen (unpublished observation), and of cytoplasmic CD3, due to avoiding dehydration and alcohol-based fixatives or acetone, all of which were found to adversely affect antigenic integrity, such as has recently been reported for TdT.\textsuperscript{40}

**Southern blot analysis.** High molecular weight DNA was prepared from cryopreserved mononuclear cells by standard techniques. Fifteen micrograms of DNA were digested with appropriate restriction enzymes (Boehringer, Mannheim), electrophoresed on a 0.7% agarose gel, blotted, and hybridized as described.\textsuperscript{42} To demonstrate Ig gene rearrangements EcoRI and HindIII digests were hybridized to a 2.4 kb Sau3a13-AH probe and BamHI and HindIII digests to a 1.3 kb EcoRI C\textsubscript{A} as well as a C-K probe.\textsuperscript{43} In addition, EcoRI digests were hybridized to a combined C\textsubscript{A} probe, which consisted of a 8.0 kb BamHI-EcoRI fragment containing the Ca\textsubscript{A} gene and to a 1.2 kb BamHI-EcoRI fragment containing the Ca\textsubscript{A} gene.\textsuperscript{54,55} EcoRI, BamHI, and HindIII digests were hybridized to a TCR-\gamma probe\textsuperscript{44} and to a TCR-\gamma probe (1.0 kb Psrl-EcoRI fragment) hybridizing to both, J\textsubscript{v} and J\textsubscript{a} segments.\textsuperscript{44} After hybridization, the filters were washed under stringent conditions and exposed to XAR-5 film (Kodak, Rochester, NY) using Dupont Lightning Plus intensifying screens for 14 to 28 hours at ~70°C.

**Statistical methods.** Chi-square tests were used to evaluate whether E-R T-ALL differs from E-R T-ALL or common ALL concerning initial patient characteristics. The patients were stratified according to different T-ALL subtypes to extract prognostic favorable groups concerning remission duration and survival time. Remission duration and survival time was estimated for each stratum by the Kaplan-Meier method\textsuperscript{44} and the strata were compared by Mantel-Cox tests.\textsuperscript{46} The proportional hazards model was applied to evaluate prognostic variables simultaneously concerning remission duration such as T-ALL subtypes, age, WBC count, mediastinal tumor, and time to achieve complete remission (CR).

**RESULTS**

**Immunophenotypic Classification of the Consecutive Series**

As shown in Table 2, the 739 consecutively studied patients were classified into four major immunophenotypic groups of adult ALL. Non-T ALL as defined by negativity for all T-cell markers especially for WT-1 (CD7) was
diagnosed in 547 patients (74%), whereas 192 patients (26%) had blast cells with T-cell lineage marker profiles. The non-T ALL category was divided into three major subgroups that might correlate with distinct stages of B-cell differentiation, namely null-ALL, common ALL, and B-ALL, with their respective subsets. T-lineage ALL was subdivided in a pre-T ALL subset having E-R\textsuperscript{+}/CD7\textsuperscript{+} blast cells, in a thymocytic T-ALL subset with blast cells positive for thymocyte antigen HTA-1 (CD1), and a E-R\textsuperscript{+}/CD1\textsuperscript{+} T-ALL subset. Reactions with VIM-2 and in part with VIM-D5 were recorded in a proportion of cases tested.

In order to characterize more precisely E-R\textsuperscript{+} T-ALLs (pre-T ALLs) we carried out extended studies on their immunophenotype in 28 cases and their genotype in 36 cases, respectively.

### Immunophenotypic pattern

In all 28 pre-T ALL cases reanalyzed by immunocytochemistry, the majority of blast cells expressed CD7, associated with TdT in all cases tested (Table 3). Except case 1, their affiliation to the T lineage was further evidenced by a substantial proportion of blast cells coexpressing CD5 and/or CD2 and/or cytoplasmic CD3. The successive use of glutaraldehyde fixation and detergent permeabilization allowed differential assessment of cytoplasmic staining in nonpermeabilized cells and of both surface and cytoplasmic staining in permeabilized cells, as exemplified in Fig 1. Staining in permeabilized cells was either restricted to or enhanced in perinuclear location, presumably corresponding to the perinuclear cistern. In case 1, with strong expression of CD7 but of no other T-cell markers, blast cells coexpressed CALLA and the B lineage marker CD24. In contrast to c-ALL, however, no other B-cell markers, including CD19, CD20, and cytoplasmic immunoglobulin-\(\mu\)-chain, and no immunoglobulin heavy chain gene rearrangement were found. Furthermore, CD24 was also expressed on the majority of blast cells in case 23, which showed a more...
complete T phenotype, with overlap in percentage of CD24, CD2, CD5, and cytoplasmic CD3-positive cells, as well as Tβ and γ gene rearrangement, thus suggesting an aberrant rare expression of CD24 in T-lineage ALL. This holds similarly true for the expression of VIM-2 on a substantial proportion of blast cells in cases 3, 7, and 18, in which our diagnosis of T-lineage ALL relied on the demonstration of three independent T markers, with overlap in staining of VIM-2 and T-cell markers, supported by TCR gene rearrangements in case 18.

None of the leukemias tested showed the full composite phenotype, with overlap in percentage of CD24, CD2, CD5, and cytoplasmic CD3-positive cells, as well as Tβ and γ gene rearrangement, thus suggesting an aberrant rare expression of CD24 in T-lineage ALL. This holds similarly true for the expression of VIM-2 on a substantial proportion of blast cells in cases 3, 7, and 18, in which our diagnosis of T-lineage ALL relied on the demonstration of three independent T markers, with overlap in staining of VIM-2 and T-cell markers, supported by TCR gene rearrangements in case 18.

Table 3. Immunophenotypic Patterns of E-R− T-ALL (Pre-T ALL) in Relation to Genotype Constellation*

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Abbreviation: ND, not done.

*As determined by immunocytochemical method. Additional 19 cases (no. 29-47) were tested at diagnosis by immunofluorescence with the following results: 19/19 CD7⁺; 19/19 TdT⁺; 14/17 CD5⁺; 0/19 CD1⁺; 1/19 CD24⁺; 2/19 CD10⁺; 1/17 HLA-DR⁺; 0/19 CD15⁺; 2/19 Vim 2⁺.

†A indicates <20%; a (+) denotes 20%-50%; and a + indicates ≥50% positive blast cells.

‡The following monoclonal antibodies were unreactive with the exceptions noted: BA-i (CD24) in no. 1 (53%) and no. 23 (81%); VIM-2 in no. 7 (44%) and no. 18 (29%).

§Denotes IgM heavy chain gene rearrangement. All other cases had Ig genes in germline position.
displayed a germline pattern for k and λ light chain loci. In 28 cases of E-R T-ALL studied in parallel, all but one revealed clonally rearranged bands of TCR-β as well as of TRG-γ genes (data not shown). In this exceptional case which had a CD7+/CD2+/CD5+/CD10+/CD1−/CD4−/CD8−/surface CD3− immunophenotype, 70% of the lymphoblasts formed rosettes with untreated as well as AET-treated sheep erythrocytes in spite of a germline position of Tβ and Tγ genes, as revealed in repeated experiments. Each of 20 CD1+ ALL patients analyzed had both Tβ and Tγ genes rearranged.

**Constellation of T-cell receptor genotype and immunophenotype in E-R T-lineage ALL.** The E-R T-ALL cases analyzed for both genotype and detailed immunophenotype were grouped in Table 3 according to Tβ and Tγ rearrangement. HLA-DR and My-10 were more often positive in the Tβ germline subset (29% and 71% of germline vs 9% and 36% of rearranged cases). CD5 antigen was positive in all cases with rearranged Tβ genes, but negative in four of 17 cases with germline Tβ genes.

CD3 membrane staining of blast cells was recorded in seven of 28 cases, irrespective of the configuration of TCR genes. Since CD3 membrane expression without TCR-β gene rearrangement indicates the presence of a T3 receptor complex without a classic αβ heterodimer, the pre-T ALL cases no. 14, 16, and 17 may represent a fortuitous transformation of T-cell subsets with an alternative T-cell receptor, which was described recently in a population of human lymphocytes. The reported CD3+/CD4+/CD8− immunophenotype of this subset as well as its Tγ gene activation resemble the features of ALL cases no. 16 and 17. In four additional cases with CD3 staining (no. 25 through 28), rearrangements of TCR-β genes indicate that a rather more mature T-cell type is involved that is deficient in CD4 and CD8 antigens as well as in E-rosetting capability; in two of these cases, this was correlated with a CD2− immunophenotype. The lack of hematopoietic progenitor cell markers (HLA-DR, My-10, and CALLA) in those cases supports the view of a more mature T phenotype.

**Clinical and Hematologic Characteristics, and Response to Therapy of T-Lineage ALL Subgroups**

Hematologic and clinical features as well as response to therapy of the patients with T-lineage ALL are described below. Patients with common ALL were chosen for comparison concerning initial patient characteristics, since this subgroup of non-T ALL appears to be well defined. Response to therapy was evaluated in T-lineage ALL patients as compared with non-T ALL patients.

**Clinicopathologic features.** The clinical characteristics at presentation of patients with T-lineage ALL subgroups are given in Table 4 in comparison with patients with common ALL. In all characteristics listed except for presence of hepatomegaly and thrombocytopenia, E-R T-ALL differed from common ALL, whereas E-R− T-ALL differed from common ALL only in six of the 12 features analyzed in Table 4. T-ALL and even more pre-T ALL patients were more likely to be under 35 years of age, and more frequently were males. They were far more likely to present with a mediasti-
nal mass, and this feature was significantly more often observed in T-ALL than in pre-T ALL. Lymphadenopathy was significantly more often observed in T-ALL as compared with pre-T and common ALL. Hepatomegaly, CNS involvement, and elevated WBC were more frequent in T-lineage ALL subgroups, but these factors of paramount importance in defining traditional risk groups occurred at a similar rate in T- and pre-T ALL. Anemia was more frequent in common ALL. Note that thrombocytopenia was significantly rarer in pre-T ALL compared with T-ALL and common ALL.

Cytology and cytochemistry. Central morphological review including cytochemical stains confirmed that none of the ALL patients was positive on peroxidase testing. There was a predominance of the FAB L2 type in the whole series of ALL patients (67%). T-ALL differed significantly from common ALL and to some extent from pre-T ALL in the distribution of the morphological subtypes L1 and L2 (Table 4). No differences were recorded for PAS staining positive in 88% of common ALL, 87% of T-ALL, and 84% of pre-T ALL (data not shown). Acid phosphatase reaction, however, was significantly more often positive in T-ALL (66%) and in pre-T ALL (46%) as compared with common ALL (11%); T-ALL also differed from pre-T ALL significantly (Table 4).

Response to remission induction therapy. Responses to induction therapy are summarized in Table 5. CR was achieved in 88% of CD1+ T-ALL. CD1+ T-lineage ALL had a significantly lower remission rate; 76% for E-R- (pre-T) and 79% for E-R+ T-ALL. In pre-T ALL, 25% of the responders achieved remission to a delayed date; achievement of remission at first after the postinduction intensification regimen has been shown to be an adverse prognostic
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remission duration and survival. To insure that the immunophenotyped sample of ALL patients was representative of the whole series, follow-up data of phenotyped and unphenotyped patients were compared. As shown in Fig 3A, the survival curves of both groups did not differ. Overall, there was no difference in survival between CD7+ T-lineage ALL and CD7- non-T-ALL patients (Fig 3B). Among patients with CD7- ALL, there was a significant difference in survival between E-R+ (pre-T ALL) and E-R+ (P = 0.02; median 24.6 v 50.7 months) (Fig 3C). Among E-R+ T-ALL patients, there was no difference in survival between CD1+ and CD1- patients (P = 0.6395; median, 40 months v still not reached; data not shown). Likewise, subclassification of the whole series of CD7+ patients for CD1 expression revealed no significant difference in survival (data not shown). Also, subclassifications of thymocytic (CD1+) T-ALL for CD10 expression or for presence of mediastinal mass, and of pre-T ALL for Tβ gene rearrangement showed no difference in survival for the respective subsets (data not shown).

Table 4. Presenting Clinical and Hematologic Features of T-Lineage ALL Subgroups in Comparison With Common ALL

<table>
<thead>
<tr>
<th>Feature</th>
<th>T-ALL* (N = 143)</th>
<th>Pre-T ALL (N = 43)</th>
<th>Common ALL (N = 371)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P Value)</td>
<td>(P Value)</td>
<td>(P Value)</td>
</tr>
<tr>
<td>Age &gt;35 yr</td>
<td>34 (24) .41</td>
<td>7 (16) .02</td>
<td>130 (35) .02</td>
</tr>
<tr>
<td>Male</td>
<td>103 (72) .93</td>
<td>30 (70) .19</td>
<td>216 (58) .00</td>
</tr>
<tr>
<td>Mediastinal mass</td>
<td>77 (54) .04</td>
<td>15 (35) .00</td>
<td>7 (2) .00</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>117 (82) .02</td>
<td>27 (63) .16</td>
<td>186 (50) .00</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>77 (54) .5</td>
<td>20 (47) 1.0</td>
<td>171 (46) .14</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>96 (67) .08</td>
<td>22 (51) 1.0</td>
<td>192 (51) .00</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>21 (15) .99</td>
<td>7 (16) .01</td>
<td>18 (5) .00</td>
</tr>
<tr>
<td>WBC &gt;30 x 10^9/L</td>
<td>91 (64) .31</td>
<td>23 (54) .00</td>
<td>102 (30) .00</td>
</tr>
<tr>
<td>Hemoglobin &lt;8 g/dL</td>
<td>35 (25) .78</td>
<td>9 (25) .15</td>
<td>122 (33) .07</td>
</tr>
<tr>
<td>Platelets &lt;50 x 10^9/L</td>
<td>71 (50) .01</td>
<td>11 (26) .00</td>
<td>198 (54) .51</td>
</tr>
<tr>
<td>L1 type‡</td>
<td>48 (41) .08</td>
<td>8 (25) .60</td>
<td>79 (30) .02</td>
</tr>
<tr>
<td>L2 type‡</td>
<td>64 (55) .04</td>
<td>23 (70) .00</td>
<td>181 (69) .00</td>
</tr>
<tr>
<td>Focal acid phosphatase‡</td>
<td>77 (66) .04</td>
<td>15 (46) .00</td>
<td>29 (11) .00</td>
</tr>
</tbody>
</table>

* A WT-1 (CD7) and E-R+ group was made containing the CD1+ thymocytic T-ALL subgroup. Thymocytic T-ALL did not differ significantly from CD1- T-ALL in any feature.

‡ Only cases analyzed by central morphology and cytochemistry were considered (116 T-ALL patients; 33 pre-T ALL patients; 263 common ALL patients).

The prognostic relevance of the diagnosis and subclassification of T-lineage ALL was also evaluated by analysis of disease-free survival of remitters. Median remission duration (MRD) was significantly shorter for pre-T ALL patients as compared with E-R+ T-ALL patients (15.7 v 33.5 months, \( P = 0.0186 \)). However, the difference in MRD reached no significant level when the CD1+ subset of pre-T ALL is compared with pre-T ALL (P = 0.789), whereas the CD1+ subset differed significantly from pre-T ALL (Table 5). No differences in MRD were recorded for CD7+ T-ALL, among CD7+ for CD1-; among CD7+ for CD1+; among CD1+ for CD10+; and among E-R+ (pre-T ALL) for Tβ gene rearrangement v Tβ germline genes.

Age, WBC count, and time to achieve CR were found to be of prognostic value in the univariate evaluations. Taking these variables as well as T-ALL subtypes simultaneously into account in the Cox model, pre-T ALL and CR later than 4 weeks after therapeutic onset were found to be of significantly independent adverse prognostic value (P = 0.04 and \( P = 0.02 \). T-ALL subtype and time to reach CR are more important than age, WBC count, or other potential patient characteristics concerning length of remission duration.

Table 5. Response to Induction Therapy in T-Lineage ALL Subgroups

<table>
<thead>
<tr>
<th>Feature</th>
<th>Pre-T ALL (P Value)</th>
<th>CD7+ T-ALL (P Value)</th>
<th>CD1+ T-ALL (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients treated</td>
<td>38</td>
<td>80</td>
<td>48</td>
</tr>
<tr>
<td>Complete remission (%)</td>
<td>76</td>
<td>0.03</td>
<td>88</td>
</tr>
<tr>
<td>Complete remission within 4 wk (%)</td>
<td>75</td>
<td>0.31</td>
<td>90</td>
</tr>
<tr>
<td>Induction death (%)</td>
<td>12</td>
<td>0.20</td>
<td>5</td>
</tr>
<tr>
<td>Median remission duration (mo)</td>
<td>15.7</td>
<td>0.02</td>
<td>NR</td>
</tr>
</tbody>
</table>

Abbreviation: NR, median not reached.

* P value for CD1+ T-ALL v pre-T ALL.
DISCUSSION

According to current knowledge, the heterogeneity of ALL in relation to cellular, biological, and clinical features can best be explained by leukemia cells, which arise in the B or T lymphocyte progenitor compartments and remain arrested at discrete, characteristic levels of early B or T cell maturation in a predominantly monoclonal expansion. After the first description of pre-B-cell ALL and of its normal precursor cell counterpart residing in the bone marrow, convincing data were accumulated indicating that the non-T ALLs reflect a developmental series of B cell precursors maintaining a hierarchical order of differentiation antigen and Ig receptor gene expression. Correspondingly, T-cell leukemias are ordered according to T-cell differentiation antigen and TCR gene expression closely related to discrete stages of intrathymic and postthymic maturation.

Although the source of precursor T lymphocytes is generally thought to be the bone marrow, from where they migrate to the thymic cortex to initiate T-cell differentiation, the frequencies of these prethymic T-committed lymphocytes or so-called prothymocytes are very low: 0.01% to 0.03% in murine and human bone marrow. Consequently, information on the phenotype of this small precursor pool is sparse or lacking, and the exceedingly low frequency makes DNA analysis of these cells impractical; likewise, the statistical risk of neoplastic transformation can be expected to be rather low. As phenotypic analysis of ALL in adult patients indicated an arrest at earlier stages of lymphoid differentiation, we reasoned that detailed scrutiny of T-lineage ALL in this group of patients might disclose neoplasias of prothymocytes.

Since nonrosetting thymocytes prepared by E-rosette separation have been shown to be the most immature thymus cells, we chose this marker as a prescreening criterion to dissect T-cell antigen positive ALL in rosetting and nonrosetting subgroups. In every instance, CD7 antigen was detected by using WT1 in nonrosetting T-ALL (pre-T ALL). To our surprise, the E-receptor associated T11 molecule (CD2) was expressed in 14 of 28 cases (Table 3), a finding already reported by Chen et al in three adult cases. Note that normal human prothymocytes comprised T11+/TdT and WT1+/TdT cells in the double staining analysis recently reported by van Dongen et al. It is not likely that the failure of E-rosette formation observed in T11+ pre-T lymphoblasts was due to technical reasons, since we performed two assays in every case using untreated erythrocytes as well as AET-treated erythrocytes to increase sensitivity. Recent work indicates that a T cell surface molecule different from CD2 is involved in spontaneous rosette formation. This molecule termed E2 is actively synthetized by T cells and strongly expressed by CD1+ thymocytes. Note that a CD4+/CD8+/CD1+ phenotype was associated with nonrosetting lymphoblasts in our study including those that were CD2+.

In this situation, the identification of additional T-cell surface markers (CD5 in 24 and/or CD2 in 14 of 28 cases, Table 3) and of CD3 in the cytoplasm of blast cells in each case except one of pre-T ALL (Fig 1, Table 3) appear to be reliable markers for differential diagnosis, because non-T leukemia cells studied in comparison were
negative for surface CD5 and for cytoplasmic CD3 staining. Recently, CD3 gene expression has been shown to be a very early event in T-cell differentiation.\textsuperscript{34} Accordingly, by using CD3 cDNA probes in Northern blot analysis, van Dongen et al\textsuperscript{44} succeeded in demonstrating normal size transcripts of the CD3-\alpha and CD3-\epsilon genes in all 12 T-ALLs tested including two cases without T\beta gene rearrangement, which were interpreted as prothymocytic T-ALL. Recently, cytoplasmic T\beta and T\epsilon chains with the same perinuclear localization were demonstrated in immature E-R\textsuperscript{+} thymus cells, which had a lymphoid morphology with a convoluted nucleus.\textsuperscript{31} Note that convoluted nuclei were also characteristic for many of the pre-T ALL lymphoblasts (Fig 1).

In addition to TdT we looked for the expression of the hematopoietic precursor cell markers HLA-DR and My-10,\textsuperscript{41,42} in order to substantiate the precursor cell attribute more precisely. HLA-DR antigens were expressed in six of 28 cases; notably five of these six had germline TCR genes. Since 22 cases of thymocytic T-ALL were HLA-DR\textsuperscript{−} without exception, HLA-DR antigens appear to be a marker candidate for prethymic within the T-cell lineage. Accordingly, they were recently identified in CD7\textsuperscript{+/−}/TdT\textsuperscript{−} normal human prothymocytes and in three cases of prothymocytic T-ALL.\textsuperscript{44,45} My-10 (CD34), which is expressed specifically on immature normal human marrow cells,\textsuperscript{41} has recently been shown to be inversely correlated with the expression of cytoplasmic \( \mu \) in normal CD10-positive bone marrow lymphoid cells and in common ALL cells.\textsuperscript{44} We report here for the first time that CD34 is also a precursor cell marker within the T-cell lineage, as 18 of 28 pre-T ALL cases had blast cells positive in overlapping percentage with T-cell markers (Table 3). Since 22 thymocytic T-ALL cases studied in parallel were CD34\textsuperscript{+} and since <0.1% of thymus cells were stained in three childhood thymus samples tested (data not shown), My-10 can be regarded as a marker of the prethymic phase within the T-cell lineage. Although CALLA (CD10) has been shown to be expressed in a population of fetal bone marrow cells that developed T-cell markers in cloning experiments,\textsuperscript{44} this marker apparently is not restricted to prethymic cells within the T-cell series, because a fraction of thymocytes\textsuperscript{44} as well as 36% of thymocytic T-ALL patients of our series did express CALLA; the CD10 antigen can, therefore, be taken as a marker of T precursor cells of prothymocyte and immature thymocyte phenotype. With one exception, CALLA was expressed in the absence of HLA-DR, and its simultaneous occurrence with B-cell antigens and Ig gene rearrangement, which is obligatory for common ALL,\textsuperscript{18} was never observed.

So far, only a few cases have been reported where DNA analysis of T-ALL cells revealed TCR-\beta genes at germ-line. CD7 expression before T\beta gene rearrangement was first reported in two of 16 and in one of seven T-cell precursor leukemias,\textsuperscript{34,31} in a single case report with a phenotype positive for TdT, CD7, CD5, CD1, and CD2,\textsuperscript{44} in three HLA-DR\textsuperscript{−} cases,\textsuperscript{44} and in three of 23 patients in another recent report.\textsuperscript{47} If we assume that all E-R\textsuperscript{+} T-ALLs had rearranged T\beta genes, the frequency of T-lineage ALL with germine T\beta genes would be 11% within T-ALL and 3% within the whole ALL series of this study. According to the current conception, the other part of pre-T ALL cases would be classified as immature thymocytic,\textsuperscript{46} since rearrangement of TCR-\beta genes is believed to occur within the thymus as studied in hybridomas derived from murine fetal thymocytes.\textsuperscript{47} However, the immunophenotype of some cases of this pre-T ALL subset of presumptive thymocytic genotype reflect rather a bone marrow derived origin as exemplified by HLA-DR and/or My-10 positivity (Table 3, no. 18 through 22).

In all cases of this study, T\beta-chain rearrangements have not been found in the absence of \( \gamma \)-chain rearrangements. In five cases of pre-T ALL, however, only rearrangements of \( \gamma \)-gene were detected (three of them are shown in Table 3). This constellation supports a similar hierarchy of TCR gene rearrangements also in humans as described in the developing murine thymus.\textsuperscript{48,70} Recently, \( \gamma \)-gene activation has been demonstrated in CD4\textsuperscript{+}/CD8\textsuperscript{−} lymphocytes that express T3 glycoproteins but not the T-cell receptor \( \alpha \)- and \( \beta \)-subunits.\textsuperscript{48} The composite phenotypes of cases 16 and 17 (Table 3), which were also C3\textsuperscript{+}/CD4\textsuperscript{−}/CD8\textsuperscript{−}, would rather fit to the T-cell subset described with a putative second T-cell receptor.\textsuperscript{48} It is noteworthy that CALLA was expressed in four of the five cases, and a striking predominance of \( \gamma \)-rearrangements was recently found in the CALLA\textsuperscript{+} B-lineage ALL.\textsuperscript{71}

Distinct clinicopathologic features were recorded for adult patients with T-cell lineage ALL in this study. They differed significantly from patients with CALLA\textsuperscript{+} B-cell lineage ALL (common ALL) in age, sex, mediastinal mass, lymphadenopathy, hepatomegaly, splenomegaly, CNS involvement, leukocyte count, and hemoglobin level at diagnosis (Table 4). The pre-T and E-R\textsuperscript{+} T-ALL subgroups had many features in common, but differed significantly in mediastinal mass, lymphadenopathy, and platelet count, and to some extent in splenomegaly. The lower incidence of extramedullary involvement observed in pre-T ALL may result from a more pronounced bone marrow homing of pre(thymic)-T leukemia cells. The reason for the relatively rare complication of thrombocytopenia is unclear, but possible suppressive effects on thrombocytopenic patients are less likely to be exerted by CD8\textsuperscript{−} (pre(thymic)-T) lymphoblasts than by CD8\textsuperscript{+} thymocytic lymphoblasts. Note that other T-ALL subgroups, eg, CD1\textsuperscript{+} CD10\textsuperscript{−} or CALLA\textsuperscript{+} CALLA\textsuperscript{−}, did not differ significantly in any feature. Likewise, there was no difference detectable in between the relatively small pre-T ALL subsets with germine and rearranged TCR genes; eg, mediastinal mass was also observed in five cases with T\beta genes at germ-line.

Of utmost importance for the evaluation of the prognostic significance of the ALL subgroups was the fact that all patients were treated in the same way, as therapy itself is an important factor of prognosis. For adult patients with ALL treated in this study, the following independent prognostic factors were established and reconfirmed in the ongoing trial, namely age, leukocyte count, time to achieve remission, and the immunological subgroup.\textsuperscript{34,44} With regard to the latter risk factor, the null ALL subgroup proved to be of substantially worse prognosis, whereas T-ALL as a whole had a good prognosis.\textsuperscript{34,48} We now dissected a small subset out of the
latter subgroup that is distinct in leukemia cell features, clinicopathologic properties, and in prognostic outcome, the pre-T ALL; consequently in the ongoing trial with risk-adapted stratification of therapy intensity, pre-T ALL is used, like null-ALL, as a poor risk factor. As the TdT+/HLA-DR+/CALLA− phenotype of null-ALL mostly relates to early B-cell differentiation,14 ALL of early primitive lymphoid stem cell phenotype appears to be of worse prognosis in adults irrespective of T or B differentiation. Correspondingly, the recently demonstrated expression of myeloid antigens on lymphoblasts of B or T lineage phenotype in some adult ALL cases might become explained as an indicator of immaturity. Although this was not clarified, a lower complete remission rate was noted for My− patients.72 On clinical grounds, the recognition of primitive stem cell characteristics appears to be of greater importance in ALL management than T- or B-cell affiliation as exemplified now here within T-lineage ALL. Of many unknown reasons, one could be that oligoclonality is more often encountered in T- or B-stem cell type ALL.49 Clonal variation at relapse as detected by different rearrangement pattern of Ig or TCR genes was recently reported by us, eg, one case with Tβ gerline gene pre-T ALL at diagnosis had lymphoblasts with Tβ gene rearrangement together with a more mature T-phenotype at relapse.73 Obviously, poorly T- or B-differentiated ALL of lymphoid stem cell type appears to be more prone to switch over into clonal variants that resist current therapy. This became recently exemplified in a case with a stem cell leukemia of T lymphoid phenotype, which converted to a myeloid phenotype induced by the adenosine deaminase inhibitor 2′-deoxycoformycin.74 In view of therapeutic implications such as intensification of chemotherapy, application of new drugs or of bone marrow transplantation, a proper diagnosis of poor-risk-associated ALL subgroups is of clinical importance. The immunophenotype of pre-T ALL is of particular significance for this purpose, as southern analysis using currently available gene probes does not result in the diagnosis of a monoclonal leukemia cell population in approximately half of the patients.

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