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

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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 perinuclear 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 prothymocytic phenotype ($CD7^+$/intracellular $CD3^+/TdT^+/My\text{-}10^{\pm}/HLA\text{-}DR^{\pm}/CD10^{\pm}$). 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, lymphoadenopathy, and platelet count, and independently of clinical factors in prognosis ($P = .02$, median remission duration: 15.7 *v* 33.5 months, and $P = .02$, median survival time: 24.6 *v* 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.

DIAGNOSIS of T-cell acute lymphoblastic leukemias (T-ALL) by immunophenotype analysis has long been recognized to be of clinical importance.[1] Associations with high WBC counts and mediastinal mass, as well as predominance of male patients and a poorer prognosis have been reported.[1-7] Although the prognostic relevance of the T phenotype has been questioned when clinical factors of prognosis were analyzed simultaneously,[5,6] the small number of patients studied, due to the relatively low frequency of T-ALL (15% to 25% of ALL), as well as the diversity of treatments used often limit the value of these studies. Also, variations of the methods used to diagnose ALL of T-cell lineage have to be considered. Originally, T-ALL was identified by rosette formation of blast cell with sheep erythrocytes.[1] This E-rosette test became a worldwide used procedure, but is prone to misdiagnosis due to cell damage or due to admixture of nonneoplastic T lymphocytes. The application of T-cell specific heteroantisera improved diagnostic sensitivity and precision and, in some cases, allowed the detection of T-cell–associated antigens on E-rosette–negative ($E\text{-}R^-$) lymphoblasts.[8-10] The introduction of monoclonal antibodies not only improved the precision and standardization of immunophenotyping, but also provided a tool to subclassify T-ALL according to the level of thymic differentiation.[11] Several elements of this T-ALL subclassification have been confirmed in a number of reports reviewed recently,[12] but also differences concerning the distribution of subgroups have been noted,[13] and clinicopathologic correlations have been limited so far by the small numbers of patients. The most sensitive marker for T-ALL was identified by monoclonal antibodies (3A1, WT1, Leu 9) directed against the CD7 antigen.[14-16] This pan-T 40 kd antigen has been shown to be present on most thymocytes and T cells but not on non-T ALL or B-cell lymphomas/leukemias,[14-16] and was proved to be a T marker of high sensitivity and specificity in large clinical trials.[13,17] By the recent introduction of molecular probes that identify immunoglobulin and T-cell–receptor (TCR) genes it became clear that, similar to the ordered fashion of rearrangements of the immunoglobulin genes,[18] genes of the TCR-CD3 complex undergo rearrangement and expression in an ordered, developmentally regulated manner.[19,26-31] In short, TCR-β genes undergo rearrangement during thymic ontogeny before expression of the TCR-associated CD3 antigen on the cell surface, whereas TCR-α chain transcripts were found only in thymocytes of later stages when another T-cell rearranging gene (TRG-γ) falls to very low levels of expression. The earliest identifiable stages of intrathymic T-cell differentiation transcribe TCRβmRNA from either germ-line or partially rearranged genes; these cells do not produce TCRαmRNA, but do contain CD3 mRNA and accumulate intracellular CD3

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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.[31] Since the immature phenotype of E-R$^-$ thymocytes appeared to be similar to E-R$^-$, T-cell antigen CD7$^+$ 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,[32] including cytomorphologic examination of May-Grünwald-Giemsa-stained smears of bone marrow and blood, standard cytochemical 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.[33] FAB criteria were used for assigning of ALL to L1, L2, and L3 designations.[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[35,36] derived from a therapy regimen successfully used in childhood ALL.[37] Clinical evaluations were performed as described elsewhere.[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 Ficoll-Isopaque density gradient centrifugation. Rosette assays at 4°C with untreated and AET-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.[4] The binding of monoclonal antibodies was assessed with fluorochrome-labeled, affinity-purified IgG F(ab')$_2$ fragments of goat anti-mouse Ig by means of fluorescence microscopy and in part by flow cytometry, as described.[38] The following selected panel of mouse monoclonal antibodies according to WHO nomenclature[39] 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-positive, E-rosette–negative ALLs were analyzed in more detail, using a sensitive immunocytochemical method[40] and a broader panel of monoclonal antibodies (Table 1) to determine all major T-cell surface antigens, cytoplasmic CD3 as well as markers of hematopoeitic progenitor cells, notably including My-10.[41] Briefly, 1 to 3 × 10^4 cells, suspended in 10 μL 0.03 mol/L HEPES-buffered protein-free MEM (GIBCO, UK), were attached electrostatically to poly-L-lysine–coated multispot 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.[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.[42] To demonstrate Ig gene rearrangements *Eco*RI and *Hind*III digests were hybridized to a 2.4 kb *Sau*3a JH probe and *Bam*HI and *Hind*III digests to a 1.3 kb *Eco*RI Cμ as well as a C-K probe.[43] In addition, *Eco*RI digests were hybridized to a combined Cλ probe, which consisted of a 8.0 kb *Bam*HI-*Eco*RI fragment containing the $C\lambda_1$ gene and to a 1.2 kb *Bam*HI-*Eco*RI fragment containing the $C\lambda_2$ gene.[17,43] *Eco*RI, *Bam*HI, and *Hind*III digests were hybridized to a TCR-β probe[44] and to a TCR-γ probe (1.0 kb *Pst*I-*Eco*RI fragment) hybridizing to both, $J\gamma_1$ and $J\gamma_2$ segments.[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[45] and the strata were compared by Mantel-Cox tests.[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).[47]

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

Table 1. Monoclonal Antibodies Used for Adult ALL Phenotyping

Antibody (Source)	Cluster of Differentiation*	Predominant Reactivity†
MAS036 (SL); OKT6 (O)	CD1	Thymocyte antigen (HTA1)
OKT11 (O)	CD2	Sheep erythrocyte receptor protein
UCHT1 (Dr. Beverley)	CD3	T-cell receptor associated antigen
Leu-3a (BD)	CD4	T-helper/inducer
NEI-015 (NEN)	CD5	Pan-T and CLL
WT1 (Dr. W. Tax); Leu-9 (BD)	CD7	Pan-T
Leu-2a (BD)	CD8	T-cytotoxic/suppressor
VIL-A1	CD10	Common ALL antigen (CALLA)
VIM-D5 (Dr. W. Knapp)	CD15	Myeloid differentiation antigen (MyA)
VIM 2		Myeloid differentiation antigen (MyA)
B1 (C)	CD20	B lymphocyte
BA-1 (H)	CD24	Pan B and granulocytes
My-10 (BD)	CD34	Hematopoietic precursor cells
OK Ia1 (O)		HLA-DR (Ia)

Abbreviations: SL, Sera-Lab; O, Ortho; BD, Becton Dickinson; NEN, New England Nuclear; C, Coulter; H, Hybritech.

*According to the Second International Workshop on Leukocyte Differentiation Antigens.[41]

†For references and detailed description see review by Foon and Todd.[12]

diagnosed in 547 patients (74%), whereas 192 patients (26%) had blast cells with T-cell lineage marker profiles. The non-T ALL category was devided 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$^-$/CD7$^+$ blast cells, in a thymocytic T-ALL subset with blast cells positive for thymocyte antigen HTA-1 (CD1), and a E-R$^+$/CD1$^-$ T-ALL subset. Reactions with VIM-2 and in part with VIM-D5 were recorded in some patients with T lineage or common ALL, but a further subgrouping for expression of myeloid antigens was not performed. We retained a diagnosis of a myeloid ALL phenotype for the situation of absence of any specific T or B cell marker; operationally, the latter subset was put together with Ig$^-$/CALLA$^-$/CD24$^+$ and with T- and B-antigen negative lymphoblastic leukemias in the poorly differentiated, traditionally called null-ALL category.

Leukemic Cell Characteristics of E-R$^-$ T-Lineage ALL

In order to characterize more precisely E-R$^-$ 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 surface 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-μ-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

Table 2. Occurrence of Major Subgroups in the Consecutive Series of 739 Patients With Adult ALL

Major Subgroup	Frequency	(No. of Cases)	Subsets	Distinctive Markers*
Null-ALL	19%	(144)†	Unclassified	TdT, HLA-DR
			B-lymphoid	TdT, CD24
			Myeloid	TdT, MyA
			Hybrid, mixed	TdT, CD24, MyA
Common ALL	52%	(385)	Pre-pre-B	CALLA, HLA-DR, CD24
			Pre-B	CALLA, cytoplasmic Ig
B-ALL	2%	(18)		Monoclonal SIg
T-lineage ALL	26%	(192)	Pre-T	CD7
			Thymocytic	CD7, CD1, E-R
			Mature T	CD7, E-R‡

*For the antibodies used see Table 1; only positivities relevant for differential diagnosis are given.

†The numbers relate to all patients immunophenotyped; the following numbers were qualified for treatment and evaluated for response, respectively: Null-ALL (131; 111), common ALL (371; 316), B-ALL (11; 5), T-lineage ALL (186; 166), comprising the subsets Pre-T (43; 38), thymocytic (94; 80), and mature T (49; 48).

‡CD1 negative; CD3 and/or CD4 and/or CD8 positivities were recorded in a proportion of cases tested.

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

Case No.	Hematopoietic Progenitor Cell Markers†				Cytoplasmic	T Cell Markers†‡ Surface Membrane						
	TdT	HLA-DR	My-10 CD34	CALLA CD10	CD3	CD7	CD5	CD2	CD1	CD3	CD4	CD8
TCR-β and TRG-γ genes in germline												
1	(+)	+	+	+	−	+	−	−	−	−	−	−
2	ND	+	+	−	(+)	+	−	+	−	−	−	−
3	+	+	+	−	(+)	+	−	+	−	−	−	−
4	(+)	(+)	−	−	(+)	+	(+)	−	−	−	−	−
5	+	(+)	(+)	+	+	+	+	(+)	−	−	−	−
6	+	−	(+)	−	+	+	−	−	−	−	−	−
7	+	−	(+)	−	+	+	−	+	−	−	−	−
8	+	−	(+)	−	+	+	+	−	−	−	−	−
9	(+)	−	+	+	+	+	+	−	−	−	−	−
10	+	−	(+)	+	+	+	+	−	−	−	−	−
11	+	−	(+)	−	+	+	+	+	−	−	−	−
12	+	−	−	−	(+)	+	+	+	−	−	−	−
13	+	−	−	+	+	+	+	+	−	−	−	−
14	+	−	(+)	−	+	+	+	+	−	(+)	−	−
TCR-β genes in germline, TRG-γ genes rearranged												
15	+	−	+	+	+	+	+	−	−	−	−	−
16§	+	−	(+)	−	+	+	(+)	−	−	(+)	−	−
17	+	−	−	+	+	+	+	(+)	(+)	(+)	−	−
TCR-β and TRG-γ genes rearranged												
18	+	+	+	−	(+)	+	+	−	−	−	−	−
19	+	−	+	−	+	+	+	+	−	−	−	−
20	ND	−	+	(+)	(+)	+	(+)	−	−	−	−	−
21	+	−	−	+	+	+	+	−	−	−	−	−
22§	+	−	+	+	+	+	+	−	(+)	−	−	−
23	+	−	−	+	(+)	+	+	+	−	−	−	−
24	+	−	−	+	+	+	+	(+)	−	−	−	−
25	+	−	−	−	+	+	+	+	−	+	−	−
26	(+)	−	−	−	+	+	+	−	−	(+)	−	−
27	+	−	−	−	+	+	+	−	−	+	−	−
28	(+)	−	−	−	+	+	+	+	−	(+)	−	−

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 additional monoclonal antibodies were unreactive with the exceptions noted: BA-1 (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.

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 that characterizes the vast majority of normal thymocytes, including CD1, CD4, and CD8 expressed simultaneously along with either CD10 (CALLA) or surface CD3. CD1 was detected in two cases in a minor subpopulation of blast cells, CD4 and CD8 were negative in all cases. Finally, in seven cases surface CD3 was found on a proportion of blast cells, the majority of which, however, again lacked other phenotypic components of intrathymic stages of T-cell maturation.

In 19 of the 28 cases tested, further evidence to suggest a prethymic level of maturation arrest was provided by the demonstration of HLA-DR and/or CD34 (My-10), both of which were found in our studies to be virtually absent from cells in normal infantile thymus. HLA-DR was expressed alone in one case (no. 4), and associated with CD34 in five cases (no. 1 through 3, 5, and 18). In seven further cases (no. 6 through 8, 11, 14, 16, and 19) CD34 was expressed alone, while in the remaining six such cases (no. 9, 10, 15, and 20 through 22) it was associated with CD10. Of the remaining nine cases with no expression of HLA-DR and/or CD34, six were CD10-positive (no. 12, 13, 17, and 23 through 25).

Rearrangements of T-cell receptor and immunoglobulin genes. Southern analysis was performed in 36 cases of E-R⁻ T lineage, including the 28 cases in which the immunophenotype was reanalyzed simultaneously (Table 3). TCR β

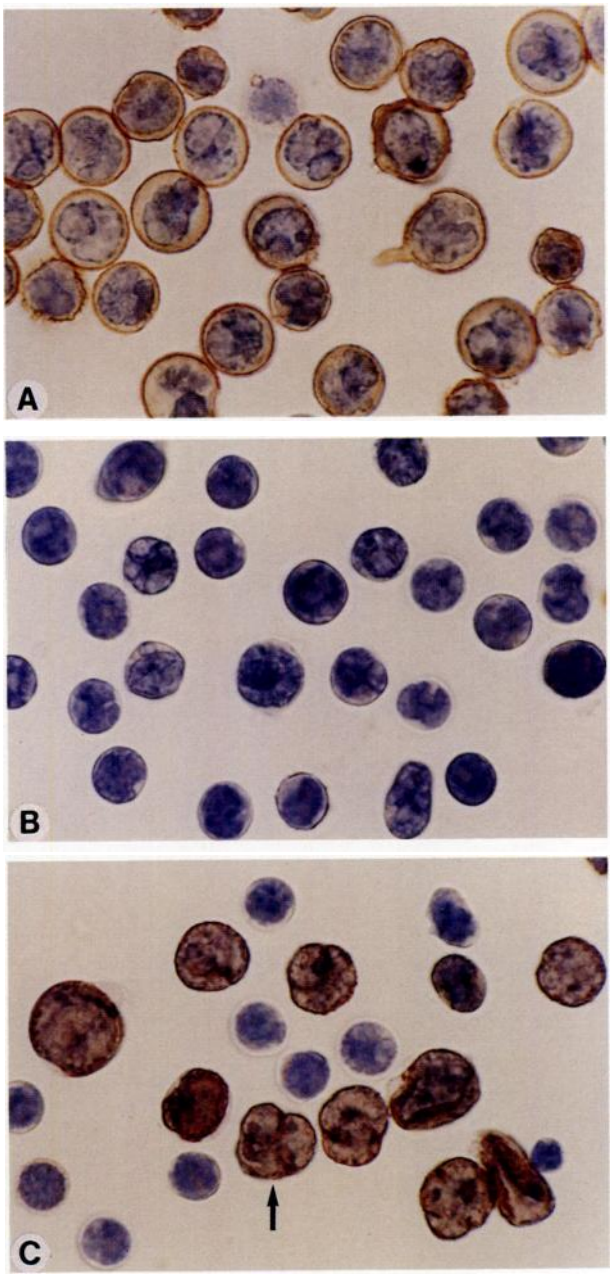

Fig 1. Immunocytochemical staining of lymphoblasts in case 12 (Table 13) with E-R⁻ T-lineage ALL (pre-T ALL). (A) Strong expression of the Pan-T antigen CD7 on the cell surface; (B) the background-free surface negativity for CD3 on nonpermeabilized cells; (C) the strong cytoplasmic expression of CD3 in a subpopulation of blast cells after detergent permeabilization. Note in panel *C* the predominantly perinuclear staining pattern with accumulation in nuclear folds of convoluted nuclei (arrow).

genes were found in germline position in 21 cases (58%) (Fig 2). In five of them, TRG-γ genes were rearranged, associated with Ig heavy chain gene rearrangement in one (no. 16, Table 3). Germline bands were confirmed in each case by using different restriction enzymes (*Bam*HI; *Eco*RI; *Hind*III). In 15 cases, rearrangements of Tβ and Tγ genes were detected, associated with IgM heavy chain rearrangement in one of them (no. 22, Table 3). All cases analyzed 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 *v* 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 α,β 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.[48] 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-

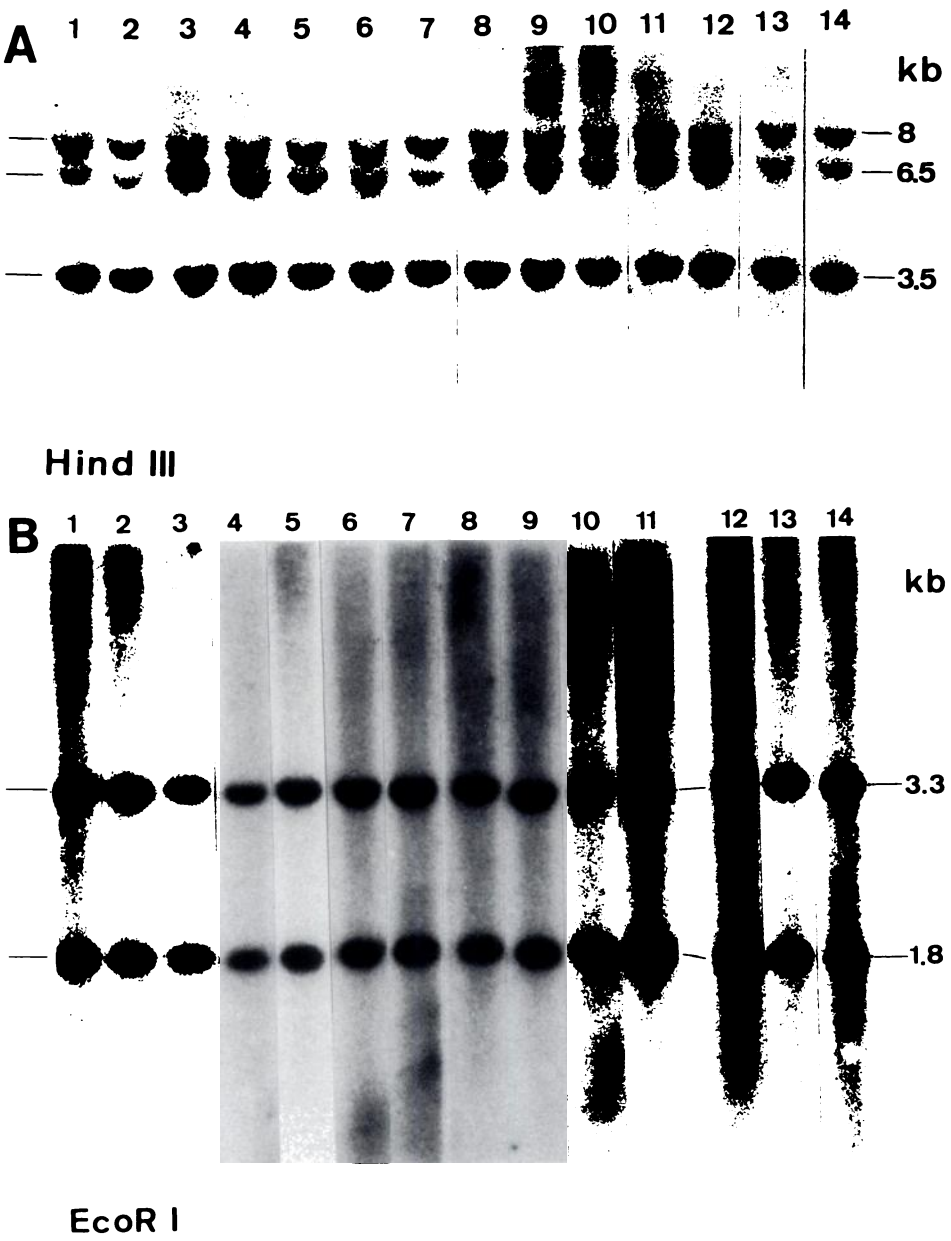

Fig 2. Southern blot analysis of 14 pre-T ALLs showing germline configuration of TCRβ (A) and TCR γ (B) genes. In panel *B* cases 1 through 11 and 12 through 14 were analyzed on separate gels.

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 peroxydase 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 $E\text{-}R^-$ (pre-T) and 79% for $E\text{-}R^+$ 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

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

Feature	Patient Subgroup: T-ALL* (N = 143) No. (%)	⟨P Value⟩	Pre-T ALL (N = 43) No. (%)	⟨P Value⟩	Common ALL (N = 371) No. (%)	⟨P Value†⟩
Age >35 yr	34 (24)	.41	7 (16)	.02	130 (35)	.02
Male	103 (72)	.93	30 (70)	.19	216 (58)	.00
Mediastinal mass	77 (54)	.04	15 (35)	.00	7 (2)	.00
Lymphadenopathy	117 (82)	.02	27 (63)	.16	186 (50)	.00
Hepatomegaly	77 (54)	.5	20 (47)	1.0	171 (46)	.14
Splenomegaly	96 (67)	.08	22 (51)	1.0	192 (51)	.00
CNS involvement	21 (15)	.99	7 (16)	.01	18 (5)	.00
WBC >30 < 10^9/L	91 (64)	.31	23 (54)	.00	102 (30)	.00
Hemoglobin <8 g/dL	35 (25)	.78	9 (25)	.15	122 (33)	.07
Platelets <50 × 10^9/L	71 (50)	.01	11 (26)	.00	198 (54)	.51
L1 type‡	48 (41)	.08	8 (25)	.60	79 (30)	.02
L2 type‡	64 (55)		23 (70)		181 (69)	
Focal acid phosphatase‡	77 (66)	.04	15 (46)	.00	29 (11)	.00

*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.

†*P* value common ALL *v* T-ALL.

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

factor,[35,49] which was relevant only for 10% of CD1$^+$ and 13% of CD1$^-$ T-ALL patients. A lower CR rate was associated with patients with the initial symptoms of bleeding or splenomegaly in this study[49]; pre-T ALL patients did not differ from other T-lineage ALL patients in splenomegaly (Table 4) nor in initial bleeding rate (data not shown). Death during induction therapy occurred at a lower rate in CD1$^+$ T-ALL as compared with the other T-lineage ALL subgroups.

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 = .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 = .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).

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 = .0186). However, the difference in MRD reached no significant level when the CD1$^-$ subset of E-R$^+$ T-ALL is compared with pre-T ALL (P = .789), whereas the CD1$^+$ subset differed significantly from pre-T ALL (Table 5). No differences in MRD were recorded for CD7$^+$ *v* CD7$^-$, among CD7$^+$ for CD1$^+$ *v* CD1$^-$, among CD1$^+$ for CD10$^+$ *v* 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 = .04 and P = .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

	Pre-T ALL	⟨P Value⟩	CD1$^+$ T-ALL	⟨P Value⟩	CD1$^-$ T-ALL	⟨P Value*⟩
No. of patients treated	38		80		48	
Complete remission (%)	76	.03	88	.02	79	1.0
Complete remission within 4 wk (%)	75	.31	90	.70	87	.73
Induction death (%)	12	.20	5	.17	12	1.0
Median remission duration (mo)	15.7	.02	NR	.42	28	.08

Abbreviation: NR, median not reached.

**P* value for CD1$^-$ T-ALL *v* pre-T ALL.

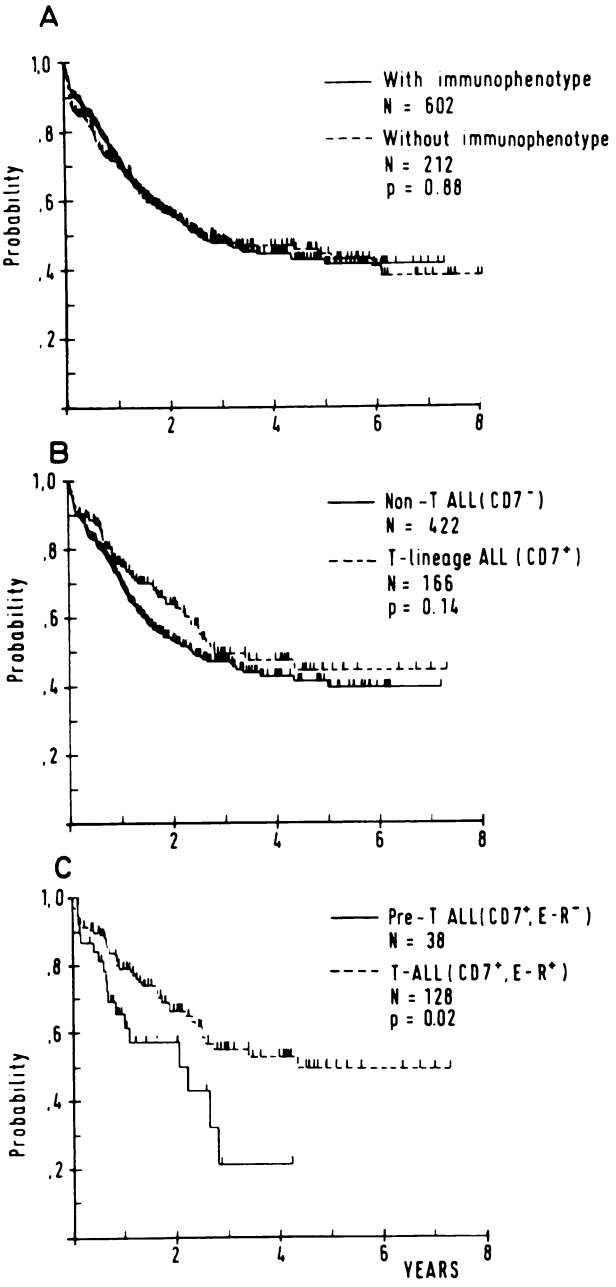

Fig 3. Survival probability of adult ALL patients of the BMFT study. Ticks represent surviving patients. (A) Immunophenotyped patients are compared with nonimmunophenotyped patients; (B) CD7-positive T-lineage ALL with CD7-negative non-T-lineage ALL; (C) E-R⁻ pre-T ALL with E-R⁺ T-ALL.

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,[50] 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.[18,51] 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.[11-13,19-22,25,26,31,44]

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,[52] 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.[53-56] Consequently, information on the phenotype of this small precursor cell 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,[57] 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,[31,58] 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.[59] Note that normal human prothymocytes comprised $T11^+/TdT^+$ and $WT1^+/TdT^+$ cells in the double staining analysis recently reported by van Dongen et al.[55] 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.[59a] This molecule termed E2 is actively synthetized by T cells and strongly expressed by $CD1^+$ thymocytes.[59a] Note that a $CD4^-/CD8^-/CD1^-$ phenotype was associated with nonrosetting lymphoblasts in our study including those that were $CD2^+$. Since WT1 reactions were recorded in a few cases of acute nonlymphocytic leukemias,[60] the T-cell specificity of CD7 appears to be equivocal, especially in the presence of HLA-DR antigens. The occurrence of the lymphoblast markers TdT and CALLA point for a lymphoid differentiation, but 5% to 10% of cases of AML were found to be TdT-positive and, very rarely, CALLA was demonstrated on myeloblastic leukemia cells.[61,62] 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.[31] Accordingly, by using CD3 cDNA probes in Northern blot analysis, van Dongen et al[44] succeeded in demonstrating normal size transcripts of the CD3-δ and CD3-ϵ genes in all 12 T-ALLs tested including two cases without Tβ gene rearrangement, which were interpreted as prothymocytic T-ALL. Recently, cytoplasmic T3δ and T3ϵ chains with the same perinuclear localization were demonstrated in immature E-R$^-$ thymus cells, which had a lymphoid morphology with a convoluted nucleus.[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,[41,63] 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$^-$ without exception, HLA-DR antigens appear to be a marker candidate for prethymic cells within the T-cell lineage. Accordingly, they were recently identified in CD7$^+$/TdT$^+$ normal human prothymocytes and in three cases of prothymocytic T-ALL.[44,55] My-10 (CD34), which is expressed specifically on immature normal human marrow cells,[41] has recently been shown to be inversely correlated with the expression of cytoplasmic μ in normal CD10-positive bone marrow lymphoid cells and in common ALL cells.[64] 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$^-$ 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,[56] this marker apparently is not restricted to prethymic cells within the T-cell series, because a fraction of thymocytes[58] 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,[18] was never observed.

So far, only a few cases have been reported where DNA analysis of T-ALL cells revealed TCR-β genes at germ-line. CD7 expression before Tβ gene rearrangement was first reported in two of 16 and in one of seven T-cell precursor leukemias,[31,65] in a single case report with a phenotype positive for TdT, CD7, CD5, CD1, and CD2,[66] in three HLA-DR$^+$ cases,[44] and in three of 23 patients in another recent report.[67] If we assume that all E-R$^+$ T-ALLs had rearranged Tβ genes, the frequency of T-lineage ALL with germline Tβ 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,[44] since rearrangement of TCR-β genes is believed to occur within the thymus as studied in hybridomas derived from murine fetal thymocytes.[68] 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, Tβ-chain rearrangements have not been found in the absence of γ-chain rearrangements. In five cases of pre-T ALL, however, only rearrangements of Tγ-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.[69,70] Recently, Tγ-gene activation has been demonstrated in CD4$^-$/CD8$^-$ lymphocytes that express T3 glycoproteins but not the T-cell receptor α- and β-subunits.[49] The composite phenotypes of cases 16 and 17 (Table 3), which were also C3$^+$/CD4$^-$/CD8$^-$, would rather fit to the T-cell subset described with a putative second T-cell receptor.[49] It is noteworthy that CALLA was expressed in four of the five cases, and a striking predominance of Tγ-rearrangements was recently found in the CALLA$^+$ B-lineage ALL.[71]

Distinct clinicopathologic features were recorded for adult patients with T-cell lineage ALL in this study. They differed significantly from patients with CALLA$^+$ 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$^+$ 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 thrombocytopoietic cells are less likely to be exerted by CD8$^-$ pre(thymic)-T lymphoblasts than by CD8$^+$ thymocytic lymphoblasts. Note that other T-ALL subgroups, eg., CD1$^+$ ν CD1$^-$ or CALLA$^+$ ν CALLA$^-$, did not differ significantly in any feature. Likewise, there was no difference detectable in between the relatively small pre-T ALL subsets with germline and rearranged TCR genes; eg, mediastinal mass was also observed in five cases with Tβ 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.[35,48] 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.[35,49] 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^+/$HLA\text{-}DR^+$/$CALLA^-$ phenotype of null-ALL mostly relates to early B-cell differentiation,[18,42] 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[72] 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.[42] 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 germline 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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REFERENCES

1. Sen L, Borella L: Clinical importance of lymphoblasts with T markers in childhood acute leukemia. N Engl J Med 292:828, 1975

2. Tsukimoto I, Wong KY, Lampkin BC: Surface markers and prognostic factors in acute lymphoblastic leukemia. N Engl J Med 294:245, 1976

3. Sallan SE, Ritz J, Pesando J, Gelber R, O'Brien C, Hitchcock S, Coral F, Schlossman SF: Cell surface antigens: Prognostic implications in childhood acute lymphoblastic leukemic. Blood 55:395, 1980

4. Thiel E, Rodt H, Huhn D, Netzel B, Grosse-Wiede H, Ganeshaguru H, Thievfelder S: Multimarker classification of acute lymphoblastic leukemia: Evidence for further T subgroups and evaluation of their clinical significance. Blood 56:759, 1980

5. Greaves MF, Janossy G, Peto J, Kay H: Immunologically defined subclasses of acute lymphoblastic leukemia in children: Their relationship to presentation features and prognosis. Br J Haematol 48:179, 1981

6. Bowman WP, Melvin SL, Aur RJA, Mauer AM: A clinical perspective on cell markers in acute lymphocytic leukemia. Cancer Res 41:4794, 1981

7. Pullen DJ, Sullivan MP, Falletta JM, Boyett JM, Humphrey GB, Starling KA, Land VJ, Dyment PG, Vats T, Duncan MH: Modified LSA_2L_2 treatment in 53 children with E-rosette positive T-cell leukemia. Results and prognostic factors. Blood 60:1159, 1982

8. Kersey J, Nesbit M, Halligren H, Sabad A, Yunis E, Gajl-Peczalska K: Evidence of origin of certain childhood acute lymphoblastic leukemias and lymphomas in thymus-derived lymphocytes. Cancer 36:1348, 1975

9. Thiel E, Rodt H, Netzel B, Huhn D, Wündisch GF, Haas RJ, Bender-Götze C, Thievfelder S: T cell antigen positive, E-rosette negative acute lymphoblastic leukemia. Blut 36:363, 1978

10. Anderson JK, Metzgar RS: Detection and partial characterization of human T and B lymphocyte membrane antigens with antisera to HSB and SB cell lines. J Immunol 120:262, 1978

11. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF: Discrete stages of human intrathymic differentiation. Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci USA 77:1588, 1980

12. Foon KA, Todd RF: Immunological classification of leukemia and lymphoma. Blood 68:1, 1986

13. Roper M, Crist WM, Metzgar R, Ragab AH, Smith S, Starling K, Pullen J, Leventhal B, Bartolucci AA, Cooper MD: Monoclonal antibody characterization of surface antigens in childhood T-cell lymphoid malignancies. Blood 61:830, 1983

14. Haynes BF, Mann DL, Hemler ME, Schroer JA, Shelhamer

JH, Eisenbart GS, Strominger JL, Thomas CA, Mostowski HS, Fauci AS: Characterization of a monoclonal antibody that defines an immunoregulatory T cell subset for immunoglobulin synthesis in humans. Proc Natl Acad Sci USA 72:214, 1980

15. Link M, Warnke R, Finlay J, Amylon M, Miller R, Dilley J, Levy R: A single monoclonal antibody identifies T-cell lineage of childhood lymphoid malignancies. Blood 62:722, 1983

16. Vodinelich L, Tax W, Bai Y, Pegram S, Capel B, Greaves MF: A monoclonal antibody (WT1) for detecting leukemias of T-cell precursors (T-ALL). Blood 62:1108, 1983

17. Borowitz MJ, Dowell BL, Boyett JM, Falletta JM, Pullen DJ, Crist WM, Humphrey GB, Metzgar RS: Monoclonal antibody definition of T cell acute leukemia: A pediatric oncology group study. Blood 65:785, 1985

18. Korsmeyer SJ, Arnold A, Bakhshi A, Ravetch JV, Siebenlist U, Hieter PA, Sharrow SO, Le Bien T, Kevsey JH, Poplack DG, Leder P, Waldmann TA: Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T cell and B cell precursor origins. J Clin Invest 71:301, 1983

19. Minden MD, Mak TW: The structure of the T cell antigen receptor genes in normal and malignant T cells. Blood 68:327, 1986

20. Minden MD, Toyonaga B, Ha K, Yanagi Y, Chin B, Gelfond E, Mak T: Somatic rearrangement of T-cell antigen receptor gene in human T-cell malignancies. Proc Natl Acad Sci USA 82:1224, 1985

21. Flug F, Pelicci PG, Bonnetti F, Knowles DM, Dalla-Favera R: T-cell receptor gene rearrangements as markers of lineage and clonality in T cell neoplasms. Proc Natl Acad Sci USA 82:3460, 1985

22. Waldmann TA, Davis MM, Bongiovanni KF, Korsmeyer SJ: Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. N Engl J Med 313:776, 1985

23. Tawa A, Hozumi N, Minden MD, Mak TW, Gelfand EW: Rearrangements of the T-cell receptor β-chain gene in non–T-cell, non–B-cell acute lymphoblastic leukemia in childhood. N Engl J Med 313:1033, 1985

24. Kitchingman GR, Rovigatti U, Mauer AM, Melvin S, Murphy SB, Stass S: Rearrangement of immunoglobulin heavy chain genes in T cell acute lymphoblastic leukemia. Blood 65:725, 1985

25. Greaves MF, Mizutani S, Furley AJW, Sutherland DR, Chan LC, Ford AM, Molgaard HV: Differentiation linked gene rearrangement and expression in acute lymphoblastic leukaemia. Clin Haematol 15:621, 1986

26. Royer HD, Acuto O, Fabbi M, Tizard R, Ramachandran K, Smart J, Reinherz EL: Genes encoding the Ti β subunit of the antigen/MHC receptor undergo rearrangement during intrathymic ontogeny prior to surface T3-Ti expression. Cell 29:261, 1984

27. Raulet DH, Garman RD, Saito H, Tonegawa S: Development regulation of T cell receptor gene expression. Nature 314:103, 1985

28. Collins MKL, Tanigawa G, Kissonerghis A-M, Ritter M, Price KM, Tonegawa S, Owen MJ: Regulation of T-cell receptor gene expression in human T-cell development. Proc Natl Acad Sci USA 82:4603, 1985

29. Royer HD, Ramarli D, Acuto O, Campen TU, Reinherz EL: Genes encoding the T-cell receptor and β subunits are transcribed in an ordered manner during intrathymic ontogeny. Proc Natl Acad Sci USA 82:5510, 1985

30. Hayday A, Saito H, Gillies SD, Kranz DM, Tanigawa G, Eisen HN, Tonegawa S: Structure, organization and somatic rearrangement of the T cell gamma genes. Cell 40:259, 1985

31. Furley AJ, Mizutani S, Weilbaecher K, Dhaliwal HS, Ford AM, Chan LC, Molgaard HV, Toyonaga B, Mak T, van den Elsen P, Gold D, Terhovst C, Greaves MF: Developmentally regulated rearrangement and expression of genes encoding the T cell receptor-T3 complex. Cell 46:75, 1986

32. Bennett JM, Catovsky D, Daniel MT, Flanchrin G, Galton DAG, Gralnick HR, Sultan C: Proposals for the classification of the acute leukemias. Br J Haematol 33:451, 1976

33. Stass SA, Schumacher HR, Kenelteis TP, Bollum FJ: Terminal deoxynucleotidyl transferase immunofluorescence on bone marrow smears. Experience in 156 cases. Am J Clin Pathol 72:898, 1979

34. Bennett JM, Catovsky D, Daniel MT, Flanchrin G, Galton DAG, Gralnick HR, Sultan C: The morphological classification of acute lymphoblastic leukemia: Concordance among observers and clinical correlations. Br J Haematol 47:553, 1981

35. Hoelzer D, Thiel E, Löffler H, Bodenstein H, Plaumann L, Büchner T, Urbanitz D, Koch P, Heimpel H, Engelhardt R, Müller U, Wendt FC, Sodomann H, Rühl H, Herrmann F, Kaboth W, Dietzfelbinger H, Pralle H, Lunscken C, Hellriegel KP, Spors S, Nowrousian RM, Fischer J, Fülle H, Mitrou PS, Pfreundschuh M, Görg C, Emmerich B, Queisser W, Meyer P, Labedzki L, Essers U, König H, Mainzer K, Herrmann R, Messerer D, Zwingers T: Intensified therapy in acute lymphoblastic and acute undifferentiated leukemia in adults. Blood 64:38, 1984

36. Hoelzer D, Thiel E, Löffler H, Büchner T, Ganser A, Heil G, Kurrle E, Heimpel H, Koch P, Lipp T, Kaboth W, Kuse R, Küchler R, Sodomann H, Maschmeyer G, Freund M, Diedrich H, von Paleske A, Weh J, Kolb H, Müller U, Bross K, Fuhr G, Gassmann W, Gerecke D, Kress M, Busch FW, Nowrousian RM, Schneider W, Aul C, Rühl H, Bartels H, Harms F, Weiss A, Löffler B, Glöckner W, Fülle H, Pralle H, Ho AD, Bonfert B, Emmerich B, Braumann D, Brenner-Serke M, Planker M, Straif K, Meyer P, Greil R, Petsch S, Görg C, Grüneisen A, Vaupel HA, Bodenstein H, Overkamp F, Schlimock G, Augener W, Öhl S, Nowicki L, Raeth U, Zurborn KH, Neiss A, Messerer D: Teniposide (VM-26) and cytosine as consolidation therapy in adult high-risk patients with acute lymphoblastic leukemia. Semin Oncol 14:92, 1987

37. Riehm H, Gadner H, Langermann HV, Odenwald E: The Berlin childhood acute lymphoblastic leukemia therapy study, 1970-1976. Am J Pediatr Hematol Oncol 2:299, 1980

38. Thiel E, Kummer U, Rodt H, Stünkel K, Munker R, Majdic O, Knapp W, Thievfelder S: Comparison of currently available monoclonal antibodies with conventional markers for phenotyping of one hundred acute leukemias. Blut 44:95, 1982

39. IUIS-WHO Nomenclature Subcommittee on human leukocyte differentiation antigens. Bull World Health Org 5:809, 1984

40. Kranz BR, Thierfelder S: Improved detection of terminal transferase (TdT): The use of detergents on glutaraldehyde-fixed non-dehydrated cells prevents denaturation and diffusion artifacts. Leukemia Res 10:1041, 1986

41. Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH: Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 133:157, 1984

42. Rhagavachar A, Bartram CR, Ganser A, Heil G, Kubanek B: Acute undifferentiated leukemia (AUL): Implications for cellular origin and clonality suggested by analysis of cell surface markers and immunoglobulin gene rearrangement. Blood 68:658, 1986

43. Bakhshi A, Minowada J, Arnold A, Cossman J, Jensen JP, Whang-Peng J, Waldmann TA, Korsmeyer SJ: Lymphoid blast crises of chronic myelogenous leukemia represent stages in the development of B-cell precursors. N Engl J Med 309:826, 1983

44. van Dongen JJM, Quartermous T, Bartram CR, Gold DP, Wolvers-Tettero ILM, Comans-Bitter M, Hooijkas H, Adnaansen HJ, deKlein A, Rhagavachar A, Ganser A, Duby AD, Seidman JG,

vanden Elsen P, Terhorst C: The T cell receptor CD3 complex during early T cell differentiation: Analysis of immature T cell acute lymphoblastic leukemia (T-ALL) at DNA, RNA and cell membrane level. J Immunol 138:1260, 1987

45. Kaplan EL, Meier P: Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457, 1958

46. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163, 1966

47. Cox CR: Regression models and life-tables. J R Stat Soc 34:187, 1972

48. Brenner MB, McLean J, Dialynas DP, Strominger JL, Smith JA, Owen FL, Seidman JG, Ip S, Rosen F, Krangel MS: Identification of a putative second T-cell receptor. Nature 322:145, 1986

49. Hoelzer D, Thiel E, Löffler H, Büchner T, Ganser A, Heil G, Koch P, Freund M, Diedrich H, Rühl H, Maschmeyer G, Lipp T, Nowrousian MR, Burkert M, Gerecke D, Pralle H, Müller U, Lunscken C, Fülle H, Ho AD, Küchler R, Busch FW, Schneider W, Görg C, Emmerich B, Braumann D, Vaupel HA, von Paleske A, Bartels H, Neiss A, Messerer D: Prognostic factors in a multicenter study for treatment of acute lymphoblastic leukemia in adults. Blood 71:123, 1988

50. Vogler LB, Crist WM, Bockman DE, Pearl ER, Lawton AR, Cooper MD: Pre–B-cell leukemia. A new phenotype of childhood lymphoblastic leukemia. N Engl J Med 298:872, 1978

51. Nadler LM, Korsmeyer SJ, Anderson KC, Boyd AW, Slaughenhoupt B, Park E, Jensen J, Corae F, Mayer RJ, Sallan SE, Ritz J, Schlossman SF: B cell origin of non-T cell acute lymphoblastic leukemia. A model for discrete stages of neoplastic and normal pre-B cell differentiation. J Clin Invest 74:332, 1984

52. Ford CE, Micklem HS: The thymus and lymph-nodes in radiation chimaeras. Lancet 1:359, 1963

53. Lepault F, Weissman IL: An in vivo assay for thymus homing bone marrow cells. Nature 293:151, 1981

54. Boersma W, Betel I, Daculsi R, van der Westen G: Post-irradiation thymocyte regeneration after bone marrow transplantation. I. Regeneration and quantification of thymocyte progenitor cells in the bone marrow. Cell Tissue Kinet 14:179, 1981

55. van Dongen JJM, Hooijkaas H, Comans-Bitter M, Hählen K, deKlein A, van Zanen G, van 'T Veer MB, Abels J, Benner R: Human bone marrow cells positive for terminal deoxynucleotidyl transferase (TdT), HLA-DR, and a T cell marker may represent prothymocytes. J Immunol 135:3144, 1985

56. Hokland P, Hokland M, Daley J, Ritz J: Identification and cloning of a prethymic precursor T lymphocyte from a population of common acute lymphoblastic leukemia antigen (CALLA)-positive fetal bone marrow cells. J Exp Med X:1749, 1987

57. Sobol RE, Royston I, LeBien T, Minowada J, Anderson K, Darcy FR, Cuttner J, Schiffer C, Ellison RR, Bloomfield CD: Adult acute lymphoblastic leukemia phenotypes defined by monoclonal antibodies. Blood 65, 730, 1985

58. Neudorf SML, LeBien T, Kersey JH: Characterization of thymocytes expressing the common acute lymphoblastic leukemia antigen. Leukemia Res 8:173, 1984

59. Chen P-M, Chiang H, Chou C-K, Hwang T-S, Chiang BN, Greaves MF: Immunological classification of T-cell acute lymphoblastic leukemia using monoclonal antibodies. Leukemia Res 7:339, 1983

59a. Bernard A, Anbrit F, Raynel B, Pham D, Bousell L: T cell surface molecule different from CD2 is involved in spontaneous rosette formation with erythrocytes. J Immunol 140:1802, 1988

60. Vodenelich L, Tax W, Bai Y, Pegram S, Capal P, Greaves MF: A monoclonal antibody (WT1) for detecting leukemias of T-cell precursors (T-ALL). Blood 62:1108, 1983

61. Jani P, Verbi W, Greaves MF, Bevan D, Bollum F: Terminal deoxynucleotidyl transferase in acute myeloid leukemia. Leukemia Res 7:17, 1983

62. Mirro J, Zipf TF, Ching-Hon P, Kitchingman G, Williams D, Melvin S, Murphy SB, Stass S: Acute mixed lineage leukemia: Clinicopathologic correlations and prognostic significance. Blood 66:115, 1985

63. Falkenberg JHF, Jansen J, van der Vaart-Duinkerken N, Veenhof FJ, Blotkamp J, Goselink HM, Parleolet J, van Rood JJ: Polymorphic and monomorphic HLA-DR determinants on human hematopoietic progenitor cells. Blood 63:1125, 1984

64. Ryan DH, Chapple CW, Kossover SA, Sandberg AA, Cohen HJ: Phenotypic similarities and differences between CALLA-positive acute lymphoblastic leukemia cells and normal marrow CALLA-positive B cell precursors. Blood 70:814, 1987

65. Pittagula S, Raffeld M, Lipford EH, Cossman J: 3A1 (CD7) expression precedes T_β gene rearrangements in precursor T (lymphoblastic) neoplasms. Blood 68:134, 1987

66. Greenberg JM, Kersey JH: Terminal deoxynucleotidyl transferase expression can precede T-cell receptor β chain and chain rearrangement in T-cell acute lymphoblastic leukemia. Blood 69:356, 1987

67. Felix CA, Wright JJ, Poplack DG, Reaman GH, Cole D, Goldman P, Korsmeyer SJ: T-cell receptor, β-, and y-genes in T-cell and pre–B-cell acute lymphoblastic leukemia. J Clin Invest 80:545, 1987

68. Born W, Yagüe J, Palmer E, Kappler J, Marrack P: Rearrangement of T-cell receptor β-chain genes during T-cell development. Proc Natl Acad Sci USA 82:2925, 1985

69. Raulet DH, Garman RD, Tonegawa S: Developmental regulation of T-cell receptor gene expression. Nature 314:103, 1985

70. Pardoll DM, Fowlkes BJ, Bluestone JA, Kruisbeck A, Maloy WL, Coligan JE, Schwartz RH: Differential expression of two distinct T-cell receptors during thymocyte development. Nature 326:79, 1987

71. Goorha R, Bunin N, Mirro J, Murphy SB, Cross AH, Behm FG, Quertermous T, Seidman J, Kitchingman GR: Provocative pattern of rearrangements of the genes for the γ and β chains of the T-cell receptor in human leukemias. Proc Natl Acad Sci USA 84:4547, 1987

72. Sobol RE, Mick R, Royston I, Davey FR, Ellison RR, Newman R, Cuttner J, Griffin JD, Collins H, Nelson DA, Bloomfield CD: Clinical importance of myeloid antigen expression in adult acute lymphoblastic leukemia. N Engl J Med 316:1111, 1987

73. Raghavachar A, Thiel E, Bartram CR: Analyses of phenotype and genotype in acute lymphoblastic leukemias at first presentation and in relapse. Blood 70:1079, 1987

74. Hershfield MS, Kurztberg J, Harden E, Moore JO, Whang-Peng J, Haynes BF: Conversion of a stem cell leukemia from a T-lymphoid to a myeloid phenotype induced by the adenosine deaminase inhibitor 2'-deoxycoformycin. Proc Natl Acad Sci USA 81:253, 1984

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Prethymic phenotype and genotype of pre-T (CD7+/ER-)-cell leukemia and its clinical significance within adult acute lymphoblastic leukemia [see comments]

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