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Contribution of Immunological Markers to the Diagnosis and Prognosis of Human Leukemia

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Abstract—*Surface markers have been of proven diagnostic and prognostic use in acute lymphoblastic leukemia (ALL). T cell ALL (T-ALL), where blasts possess receptors for sheep red blood cells (R-SRC+), is associated with an adverse prognosis in children and adults. The presence of common ALL antigen (CALLA)-positive blasts (i.e. common-ALL) in children is indicative of a good response to treatment, in contrast to the poor response shown by pre-B-ALL cases, where the blasts are also CALLA-positive but additionally contain cytoplasmic μ chains. Recently a subgroup of T-ALL, immature T-ALL, was identified, where the blasts lack R-SRC and T cell markers (such as T1, T3, T4, T8, T6) but carry a pan T cell antigen (p40) recognized by the monoclonal antibody LAU-A1 (12/103 ALL cases in our series). This new subgroup, immature T-ALL (R-SRC-/p40+), also seems to be associated with a poor prognosis, like T-ALL.*

THE CONTRIBUTION of immunologic surface markers to the diagnosis and prognosis of acute leukemias has been substantial mainly for acute lymphoblastic leukemia (ALL), whereas in acute myeloblastic leukemia (AML) surface markers have so far been of little help for the hematologist or the oncologist due to the lack of immunologic markers for immature myeloid cells. At the present time, for the diagnosis of AML or monoblastic leukemias, no surface marker has been shown to be superior to conventional cytochemical stains such as Sudan Black, myeloperoxidase and alpha-naphthyl acetate esterase, with or without the characteristic inhibition by sodium fluoride for the recognition of monoblasts [1]. Thus this paper focuses on the description of surface markers relevant to ALL where the phenotyping of the leukemic cells has already proven useful as an aid to diagnosis and prognosis. The classification of ALL is conveniently presented by briefly reviewing the historical steps that led to our current knowledge concerning this disease. For many years, the diagnosis of ALL relied upon the negativity for the Sudan Black and myeloperoxidase reactions of the leukemic blasts and on their morphologic features, such as the nuclear chromatin pattern, the absence of azurophilic granules and the high nucleus-cytoplasm ratio. The course of the disease thus defined was highly variable, ranging from cure to non-responsive disease, and no reproducible morphologic criterion was available to predict the outcome of a patient suffering from ALL.

In 1975 Sen and Borella [2] for the first time described a subset of ALL in which the leukemic blasts formed rosettes with sheep red blood cells (SRC), a feature which was and still is highly specific for cells belonging to the T-lineage. The formation of rosettes is due to the presence of membrane receptors for SRC recently characterized as a glycoprotein of an approximate molecular weight (mol. wt) of 50,000 [3] which can also be defined by commercially available monoclonal antibodies such as OKT11 or Leu 5. Thus the ALL surface phenotype characterized by the presence of receptors for SRC (R-SRC), which is often referred to as E+ (erythrocyte positive) phenotype, defines T cell ALL. It is of importance that this phenotype is associated with a poor prognosis in adult and childhood leukemia [2, 4, 5]. Most of these patients are over 10 yr of age at presentation, have a high WBC count and a mediastinal enlargement. Also in 1975, Flandrin *et al.* [6] described a rare form of ALL, termed B-ALL, in which the leukemic blasts expressed

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monomeric IgM on the cell surface. This surface immunoglobulin possessed either a kappa or lambda light chain, thus indicating that it was monoclonal. It is now evident that this ALL subset is also associated with a poor prognosis. Thus in 1975 (Fig. 1) ALL could be subdivided into three categories: a rare form called B-ALL (about 1% of the cases), a more frequent form called T-ALL (about 25% of the cases) and the most common form of ALL characterized by the absence of lymphoid markers and therefore called 'non-B, non-T' ALL.

Using rabbit antibodies, Greaves *et al.* [7] identified in the same year a new surface antigen now referred to as the common acute lymphoblastic leukemia antigen (CALLA), which has since been characterized as a single polypeptide chain of an approximate mol. wt of 100,000 [8]. This surface antigen is present on the blasts of most patients with ALL and on those with lymphoid crisis of chronic myelocytic leukemia (CML). The antigen is also expressed on a small percentage of normal marrow cells [9] and more recently it has been found on non-hemopoietic tissues: renal tubular and glomerular cells, fetal small intestine epithelial cells, myoepithelial cells of adult breast, glioma and melanoma cells [10–12]. Nevertheless, due to the selective expression of CALLA within the hemopoietic tissue, the group of patients previously classified as non-B non-T ALL (Fig. 1) could be split into two subtypes depending on the presence or the absence of CALLA (Fig. 2):(a) a CALLA- and Ia (= HLA-DR)-positive subtype, accounting for about 60% of the cases, thus representing the most frequent phenotype and therefore called common-ALL (c-ALL); (b) a CALLA-negative and usually Ia-positive subtype, termed null-ALL, representing about 5% of the cases in childhood ALL and up to 35% of adult cases in some series. An early British study of 94 children with ALL has already shown the prognostic significance of this classification (Fig. 3, [13]), with c-ALL cases having the longest, T-ALL cases the shortest and null-ALL cases an intermediate remission duration.

In 1978 a further small but clinically relevant subgroup of c-ALL was identified by Vogler *et al.* [14], who discovered that about one-fifth of c-ALL cases expressed cytoplasmic μ chains in a variable percentage of their blasts in the absence of surface IgM. This subset of c-ALL was thus termed pre-B-ALL (Fig. 4), as the presence of cytoplasmic immunoglobulin clearly indicated the B cell lineage commitment of these blasts. Moreover, Korsmeyer and others [15] demonstrated immunoglobulin gene rearrangements in c-ALL cells, indicating that even though these cells have not acquired the capacity to synthetize immunoglobulins, they have undergone gene rearrangements that are characteristic of cells belonging to the B lineage. Thus, c-ALL, the most common form of ALL, is now considered as an early B cell malignancy.

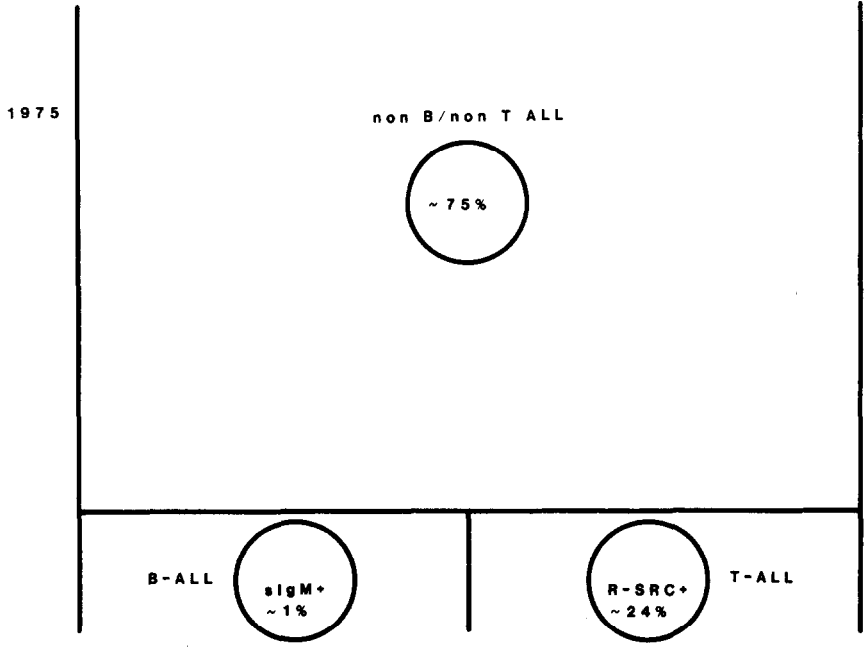

Fig. 1. Classification of ALL used in 1975. The percentages given in the circles refer to the approximate incidence of the different subtypes. Uncommitted subtypes are placed in the midline, B lineage and T lineage committed subtypes on the left and on the right side, respectively. sIgM+ refers to the presence of surface immunoglobulin type M on the leukemic blasts and R-SRC+ to the presence of receptors for sheep red blood cells.

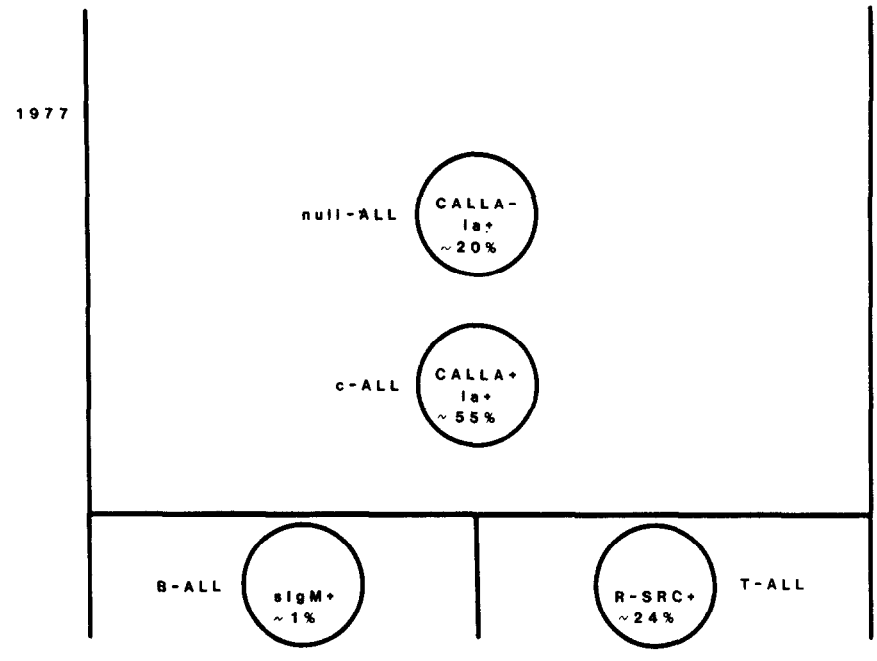

Fig. 2. Classification of ALL used in 1977. *Legend as for Fig.* 1 *except that CALLA refers to the common ALL antigen and Ia to common determinants of HLA-DR antigens.* + = *antigen present on leukemic cell surface,* - = *antigen absent.*

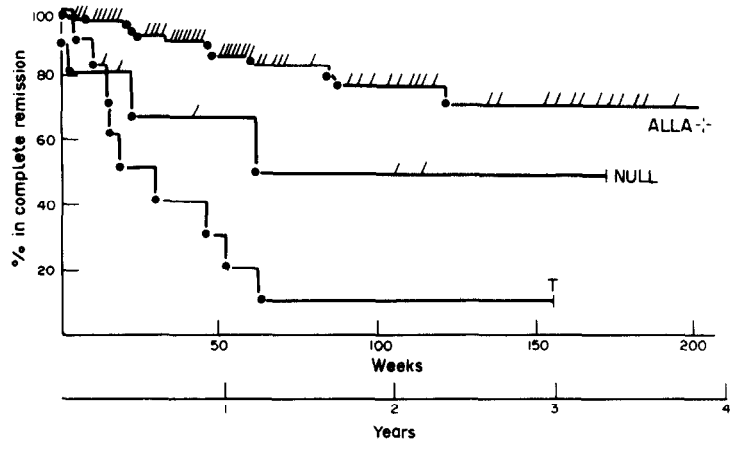

Fig. 3. Prognosis of T-ALL, null-ALL and common ALL (published in Lancet 1977. ii, 1308). *CALLA was termed ALLA by that time (= ALL antigen).*

The prognostic significance of the new subtype of pre-B-ALL was shown in a recent study from the Pediatric Oncology Group [16], where 78 cases of pre-B-ALL responded poorly to treatment compared to cases with c-ALL (Fig. 5). In particular, the clinical features of both the pre-B-ALL and c-ALL cases were identical, thus indicating the prognostic value of the phenotypic characterization independent of the WBC count at presentation—a matter that has been debated for a long time in childhood leukemia.

Some observations suggest that the definition of T-ALL should extend beyond the sole presence of the R-SRC. For example, it is known that as many as 40% of the patients with n-ALL can present with a mediastinal enlargement which is a prominent feature of T cell ALL and yet the leukemic cells are R-SRC-negative. Recently, Carrel *et al.* [17] defined a specific pan-T antigen also expressed on E rosette-negative T cells and recognized by a monoclonal antibody termed LAU-A1. Autoradiographs after SDS-polyacrylamide gel electrophoresis of ^{125}I-labeled surface proteins from a T cell line, from thymocytes and from peripheral T cells precipitated with this antibody showed an apparent mol. wt of 40,000 under reducing conditions. This molecule appears to be expressed by early T cells before the appearance of R-SRC and is similar, if not identical, to an antigen defined by monoclonal antibodies termed 3A1, 4H9 or WT1 [18-20].

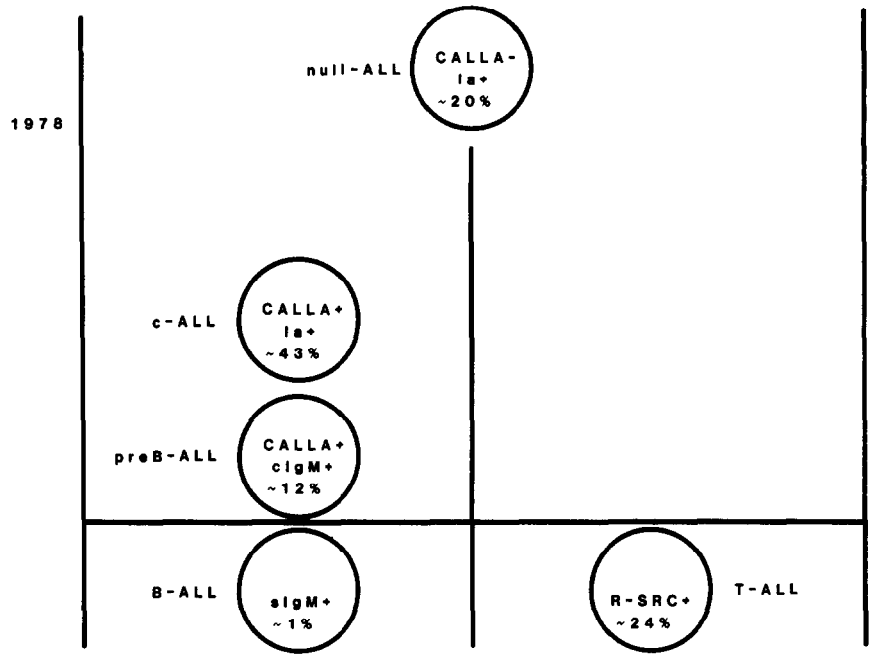

Fig. 4. Classification of ALL used in 1978. Legend as for Fig. 1 and 2 except that cIgM refers to cytoplasmic heavy μ chains.

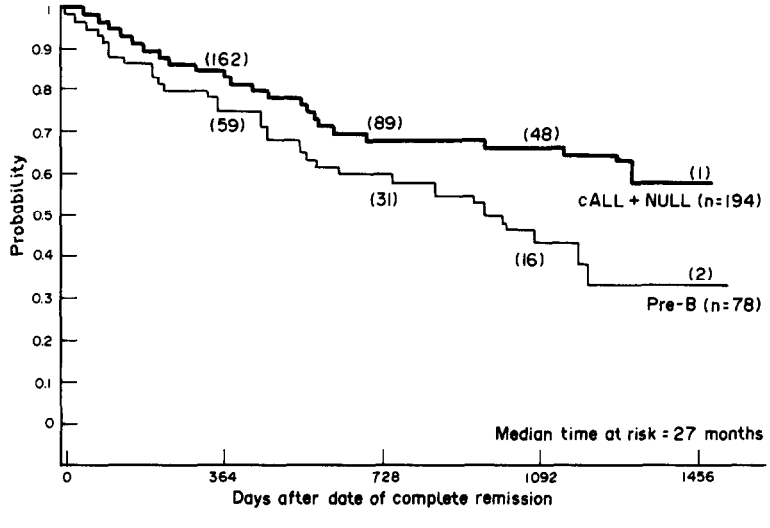

Fig. 5. Prognosis of pre-B-ALL in childhood leukemia (taken from Blood 1984 63, 409*(by permission of Grune & Stratton Inc.), with some modifications).*

Preliminary studies performed by our group on 73 cases of ALL (presented in 1982 at the first monoclonal antibody workshop in Paris [21]) demonstrated that the LAU-A1 monoclonal antibody was able to define a new subgroup of T-ALL that is R-SRC negative. The reactivity of LAU-A1 was further studied in 240 cases of acute and chronic leukemias (Table 1). All T-ALL cases with R-SRC-positive blasts and Sezary T cell leukemias were found to be LAU-A1-positive. In addition, leukemic blasts from 12 cases previously classified as null-ALL but exhibiting clinical characteristics similar to classical T-ALL, i.e. mediastinal enlargement and high WBC count, were also found to be LAU-A1-positive. The LAU-A1 antibody did not react with any other diagnostic subgroup of acute or chronic leukemias. It should be emphasized that in our series none of the conventional monoclonal antibodies used to define T cell markers such as those detecting T1, T3, T4, T8 or T6 antigens [22] reacted with this newly defined subtype of T cell ALL. By recognizing all types of T-ALL and thus overriding the pronounced antigenic diversity of this form of cancer, this antibody (or its equivalents WT1, 4H9 or 3A1) will prove to be extremely useful for the detection of T cell disease, which is known to bear an unfavorable prognosis.

The classification of ALL which is currently in use is shown in Fig. 6. The major achievement

Table 1. Reactivity of LAU-A1 antibody in acute and chronic leukemias (240 cases)

Diagnosis	*n*	LAU-A1*	p50 (R-SRC)
c-ALL	77	-	-
B-ALL	3	-	-
T-ALL	11	+	+
T-ALL (p50-)	12	+	-
AUL	22	-	-
AML, AMoL	79	-	-
B-CLL	11	-	-
CML	4	-	-
BC(CML)	7	-	-
Sezary	4	+	+

Abbreviations: AUL, acute undifferentiated leukemia; AMoL, acute monoblastic leukemia; B-CLL, B cell chronic lymphocytic leukemia; BC, blastic crisis; Sezary, Sezary T cell leukemia; for other abbreviations see text.

*The presence of the LAU-A1-defined antigen (p40) was determined by immunofluorescence and complement-dependent cytotoxicity using a ^{51}Cr-release assay. In positive cases (+) virtually all leukemic cells stained brightly in immunofluorescence.

includes the recognition of what can more accurately be called immature T-ALL, where the blasts lack T cell differentiation markers like R-SRC and the conventional T cell markers cited above (T1-8) but express the LAU-A1-defined antigen (p40). Thus an immature T-ALL can now be distinguished from the classical or mature T-ALL, where the blasts always express R-SRC and the antigen p40, as well as various patterns of conventional T cell markers. In addition, it seems safe to consider as null-ALL only those cases which are Ia (HLA-DR)-positive and reveal an elevated activity of the terminal transferase enzyme (TdT), a marker highly characteristic of lymphoid tissue [23]. This also allows a better delineation of truly undifferentiated leukemia (AUL) which may contain currently unrecognizable cases of immature mono-myeloid, erythroid or megakaryoblastic proliferations. It should be stressed that neither CALLA nor TdT markers are indicative of B or T lineage commitment, whereas up to the present time the antigen p40 exhibits an exclusive specificity for T lineage within the lymphoid compartment. Thus, before cases are classified as c-ALL, n-ALL or AUL they must be negative for the pan-T cell antigen p40. This is further substantiated by a more recent study showing that the antigen p40 expression and immunoglobulin gene rearrangements were mutually exclusive [24]. In addition, these authors demonstrated B lineage commitment at the genetic level in null-ALL blasts as defined in Fig. 6.

In summary, it is now established that, in childhood ALL, c-ALL cases have the best prognosis whereas pre-B-ALL, B-ALL and T-ALL (R-SRC+) cases respond poorly to treatment and require different therapeutic approaches. The prognosis of n-ALL (as defined in Fig. 6), which accounts for only about 5% of the cases in childhood leukemia, and of AUL has been difficult to assess mainly due to the low incidence of cases and therefore needs further investigation. Preliminary data seem to indicate that immature

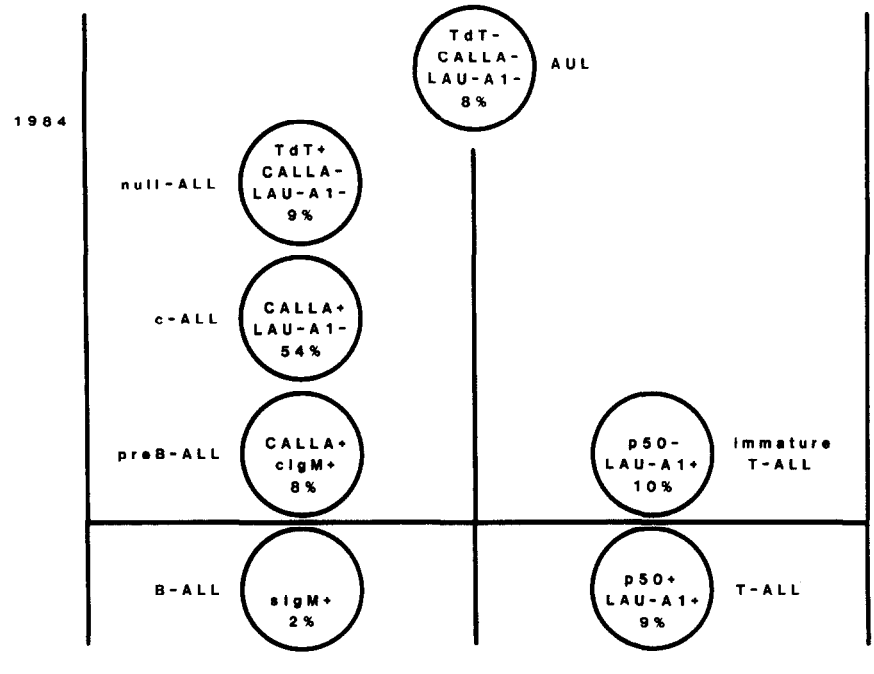

Fig. 6. Current classification of ALL. Legend as for Figs 1, 2 and 4, except that: S-RBC is replaced by p50; LAU-A1 refers to the pan-T antigen recognized by this monoclonal antibody and TdT to the terminal transferase enzyme; the percentages given in the circles refer to the incidence of the different subtypes recorded in our own series of 125 cases of ALL and AUL, including children and adults.

T-ALL bears an equally bad prognosis as mature T-ALL. However, further studies are needed to prove this important point.

In adult ALL the situation is much less clear: cases with c-ALL have the best prognosis [25] unless they carry the philadelphia chromosome [26], which may occur in up to 25% of adult c-ALL cases and which is indicative of a bad prognosis. B-ALL and T-ALL, as in childhood leukemia, respond poorly to treatment. Little is known on the prognostic value of pre-B-ALL, immature T-ALL, null-ALL and AUL phenotypes in adults and several current trials are designed to answer this important question, especially as null-ALL and AUL are fairly common in adults (22% of non-myeloid acute leukemia in our series of 84 adults).

There is no doubt that the application of surface marker analysis to the acute leukemias during the past 10 yr has led to considerable progress in the phenotyping of ALL and has been of proven diagnostic and prognostic use in children. However, much more work is needed to assess the prognostic value of surface markers in adult ALL. Moreover, new markers are required for the definition of AUL and of the immature myeloid, monocytic, erythroid and megakaryocytic series. Hopefully, further progress along this line will provide the clinician with additional means to optimize therapy for the different subgroups of acute leukemia.

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REFERENCES

1. Bennett JM, Catovsky D, Daniel MT *et al.* Proposals for the classification of the acute leukaemias. *Br J Haematol* 1976, **33**, 451-458.
2. Sen L, Borella L. Clinical importance of lymphoblasts with T markers in childhood acute leukemia. *N Engl J Med* 1975, **292**, 828-832.
3. Kamoun M, Martin PJ, Hansen JA, Brown MA, Siadak AW, Nowinski RC. Identification of a human T lymphocyte surface protein associated with the E-rosette receptor. *J Exp Med* 1981, **153**, 207-212.
4. Lister TA, Roberts MM, Brearley RL, Woodruff RK, Greaves MF. Prognostic significance of cell surface phenotype in adult acute lymphoblastic leukemia. *Cancer Immunol Immunother* 1979, **6**, 227-230.
5. Brouet JC, Valensi F, Daniel MT, Flandrin G, Preud'homme JL, Seligmann M. Immunologic classification of acute lymphoblastic leukemias: evaluation of its clinical significance in a hundred patients. *Br J Haematol* 1976, **33**, 319-382.
6. Flandrin G, Brouet JC, Daniel MT, Preud'homme JL. Acute leukemia with Burkitt's tumor cells: a study of six cases with special reference to lymphocyte surface markers. *Blood* 1975, **45**, 813-818.
7. Greaves MF, Brown G, Rapson N, Lister TA. Antisera to acute lymphoblastic leukaemia cells. *Clin Immunol Immunopathol* 1975, **4**, 67-84.
8. Sutherland R, Smart J, Niaudet P, Greaves M. Acute lymphoblastic leukaemia associated antigen II. Isolation and partial characterization. *Leuk Res* 1978, **2**, 115-126.
9. Janossy G, Bollum F, Bradstock K, McMichael A, Rapson N, Greaves MF. Terminal transferase-positive human bone marrow cells exhibit the antigenic phenotype of common acute lymphoblastic leukemia. *J Immunol* 1979, **123**, 1525-1529.
10. Metzgar RS, Borowitz MJ, Jones NH, Dowell BL. Distribution of common acute lymphoblastic leukemia antigen in non hematopoietic tissues. *J Exp Med* 1981, **154**, 1249-1254.
11. Carrel S, de Tribolet N, Gross N. Expression of HLA-DR and common acute lymphoblastic leukemia antigens on glioma cells. *Eur J Immunol* 1982, **12**, 354-357.
12. Carrel S, Schmidt-Kessen A, Mach JP, Heumann D, Girardet C. Expression of common acute lymphoblastic leukemia antigen (CALLA) on human malignant melanoma cell lines. *J Immunol* 1983, **130**, 2456-2460.
13. Chessels JM, Hardisty RM, Rapson NT, Greaves MF. Acute lymphoblastic leukemia in children: classification and prognosis. *Lancet* 1977, **ii**, 1307-1309.
14. 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* 1978, **298**, 872-878.
15. Korsmeyer SJ, Hieter PA, Ravetch JV, Poplack DG, Waldmann TA, Leder P. Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B-cells. *Proc Natl Acad Sci USA* 1981, **78**, 7096-7100.

16. Crist WM, Boyett J, Roper M *et al.* Pre-B cell leukemia responds poorly to treatment: a Pediatric Oncology Group study. *Blood* 1984, **63,** 407-414.
17. Carrel S, Gross N, Heumann D *et al.* Monoclonal antibody against a pan-T cell antigen expressed by thymocytes, peripheral T-lymphocytes and T-cell leukemias. *Mol Immunol* 1984, **21,** 831-840.
18. Haynes BF, Eisenbarth G, Fauci A. Human lymphocyte antigens: production of a monoclonal antibody that defines functional thymus-derived lymphocyte subsets. *Proc Natl Acad Sci USA* 1979, **76,** 5829-5833.
19. Link M, Warnke R, Finaly J *et al.* A single monoclonal antibody identifies T-cell lineage of childhood lymphoid malignancies. *Blood* 1983, **62,** 722-728.
20. Vodinelich L, Tax W, Bai Y, Pegram S, Capel P, Greaves MF. A monoclonal antibody (WT1) for detecting leukemias of T-cell precursors (T-ALL). *Blood* 1983, **62,** 1108-1113.
21. von Fliedner V, Carrel S, Heumann D *et al.* Multimarker classification of acute lymphoblastic leukemias: evidence for new subgroups. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, eds. *Leuocyte Typing*. Berlin, Springer Verlag, 1984, 691-692.
22. Rheinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-lineage. *Proc Natl Acad Sci USA* 1980, **77,** 1588-1592.
23. Bollum FJ. Terminal deoxynucleotidyl transferase as a hematopoietic cell marker. *Blood* 1979, **54,** 1203-1215.
24. Korsmeyer SJ, Arnold A, Bakhshi A *et al.* Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T cell and B cell precursor origins. *J Clin Invest* 1983, **71,** 301-313.
25. Greaves MF. Analysis of the clinical and biological significance of lymphoid phenotypes in acute leukemia. *Cancer Res* 1981, **41,** 4752-4766.
26. Bloomfield CD, Peterson LC, Yunis JJ, Brunning RD. The Philadelphia chromosome (Ph') in adults presenting with acute leukemia: a comparison of Ph'+ and Ph'- patients. *Br Haematol* 1977, **36,** 347-358.