Leukemia Cytogenetics in Children: Results of the German Therapy Studies

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From January 1984 to June 1988, 1179 bone marrow samples from children with acute lymphoblastic (ALL) and non-lymphoblastic leukemia (ANLL) were received for chromosomal analysis. More than 90% were sent by mail from more than 60 hospitals all over the Federal Republic of Germany, all of them registered in one of the multicenter therapy studies CoALL or BFM.

After arrival, the bone marrow was washed twice in RPMI 1640 and afterwards cultured in RPMI + 20% FCS for 24 h, including a synchronization of the cell cycle by methotrexate for 17 h. After release of the MTX block, the cells were cultured again for 4.5 h and chromosome preparation was performed with KCl and methanol:acetic acid 3:1. The cell suspension was then dropped onto a cold, wet slide and stained by G-banding after drying for 2–6 days.

Chromosomal analysis was carried out in 219 samples of patients with ANLL, 127 at diagnosis and 20 at relapse, and was successful in 147 cases (67.1%). Bone marrow of children with ALL was derived from 960 patients and successfully analyzed in 554 cases (57.7%).

A normal karyotype was found in the bone marrow of only 30% ($n=31$) of children with ANLL, whereas the majority showed structural ($n=38$), numerical ($n=16$), or a combination of both types ($n=19$).

In contrast to ALL, numerical changes were very rare, and chromosome numbers of more than 47 appeared in only a small percentage. Most frequently, the loss or gain of a single chromosome was found, and chromosomes 8 and 7 were very often involved in a trisomy or monosomy, respectively. Both of them are typical aberrations of ANLL.

Further consistent chromosomal abnormalities were found in this group of leukemia patients, most of them translocations (Fig. 1). T(8;21) is a very common aberration of ANLL and appears mainly in patients with FAB-type M_2, but it was also found in M_1 and M_4. All of the patients with t(8;21) in this study showed a FAB-type M_2. Whereas each of the other consistent aberrations was found to be very specific for only one FAB-group – e.g., inv(16) in M_4 and t(15;17) in M_3 – abnormalities involving the long arm of chromosome 11 (11q23) were found in subtypes M_1, M_2, M_4, and M_5 (Fig. 2a). This band is a very interesting breakpoint in the cytogenetics of leukemia, for it is also detected in the bone marrow of patients with ALL, and was involved in translocations and deletions, e.g., t(9;11), del(11q). One patient (M_5) had a t(4;11) (q21;q23), which is very rare in ANLL and is found mainly in ppB-ALL or hybrid leukemia.

In 41% ($n=167$) of patients with ALL, whose bone marrow was analyzed at diagnosis, a normal karyotype was found; 24% ($n=93$) of the patients showed only structural abnormalities (pseudodiploid), whereas in 31% ($n=118$) a gain (hyperdiploid) and in 3% ($n=12$) a loss (hypodiploid) of chromosomes was detected. Hyperdiploidy with chromosome numbers of 47–49 was found in only 9% ($n=34$) of the ALL patients, whereas

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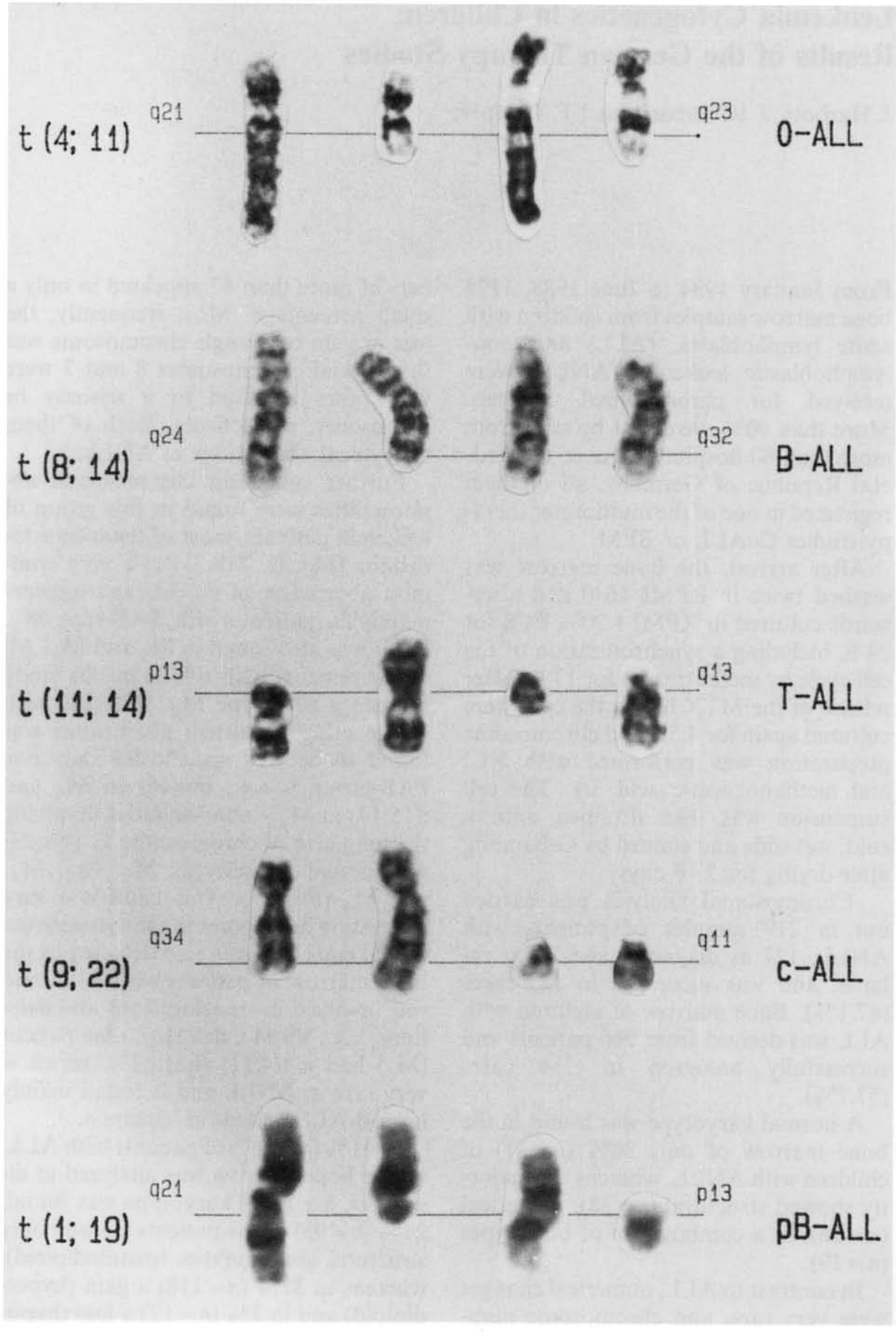


Fig. 1. Consistent aberrations of various immunophenotypes of ALL

Frequency of Consistent Aberrations in ALL

	B-cell ALL	T-cell ALL	common ALL	ppB-ALL	mixed
t(1;19)			8		
t(8;14)	14				
t(4;11) *				8	5
t(11;19)					1
t(9;22)			10		
t(11;14)		5			
t(1;14)		2			
t(10;14)		2			
t(12;14)		1			
inv(14q)		1		*) 1 patient with M5	

Frequency of Consistent Aberrations in ANLL

	M1	M2	M3	M4	M5
t(8;21)		15			
t(15;17)			4		
inv(16)				1	
11q23	1	2		2	9
+8	2	2		1	5

Fig. 2a, b. Frequency of consistent chromosomal abnormalities in ALL (**a**) and ANLL (**b**)

22% ($n=84$) had 50 and more chromosomes. The latter seems to be very typical, for it was found only in the bone marrow of patients with common ALL, a subtype in which pseudodiploidy is very rare.

In this immunophenotypical subgroup of ALL, however, two structural aberrations also appeared which were confined to this group: the Philadelphia chromosome, t(9;22), and the translocation (1;19) (Fig. 3). The t(8;14) were detected only in B-cell ALL, whereas the two variant forms of this aberration, namely t(2;8) and t(8;22), were never encountered. The t(4;11) is typical for very early stages of differentiation in hematopoiesis and was found in eight patients with ppB-ALL, five patients with a mixed leukemia, and, as mentioned above, in one child with ANLL FAB-type M_5. In T-cell ALL different aberrations were

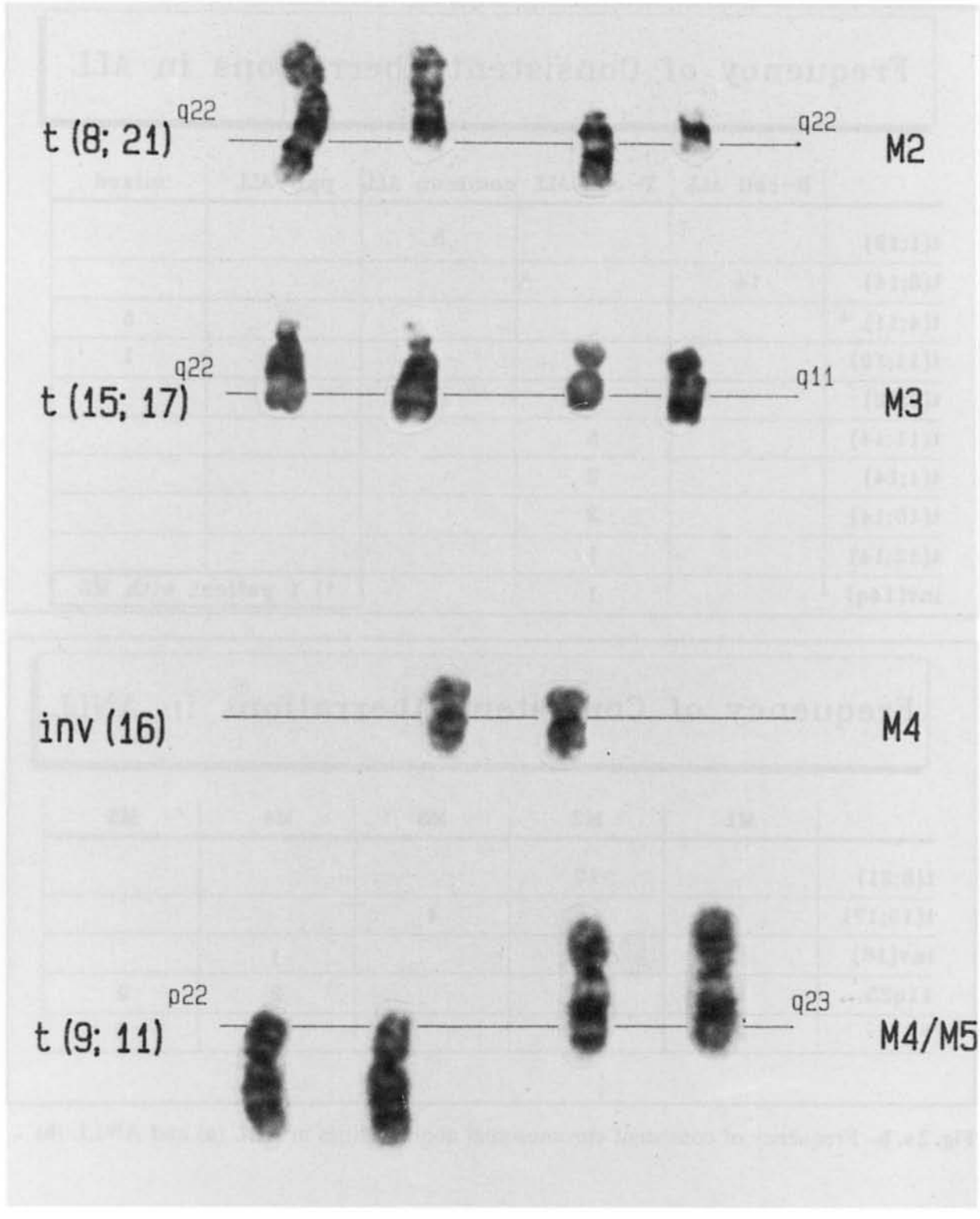


Fig. 3. Consistent aberrations of various FAB-groups of ANLL

found, all of them involving a single band of the long arm of chromosome 14 (14q11); t(11;14), t(1;14), t(10;14), t(12;14), and inv(14) (Fig. 2b). This band is the region where the TCR α and σ are located.

In order to propose prognostic meaning, we compared only patients who were treated by the same uniform therapy, as treatment is the most important prognostic factor. When the clinical outcome of patients with common ALL and different karyotypes was compared, a relapse became visible in five of 12 children with pseudodiploidy. In contrast, relapse was less often seen when a normal or hyperdiploid karyotype was diagnosed (Fig. 4). With regard to the meaning of

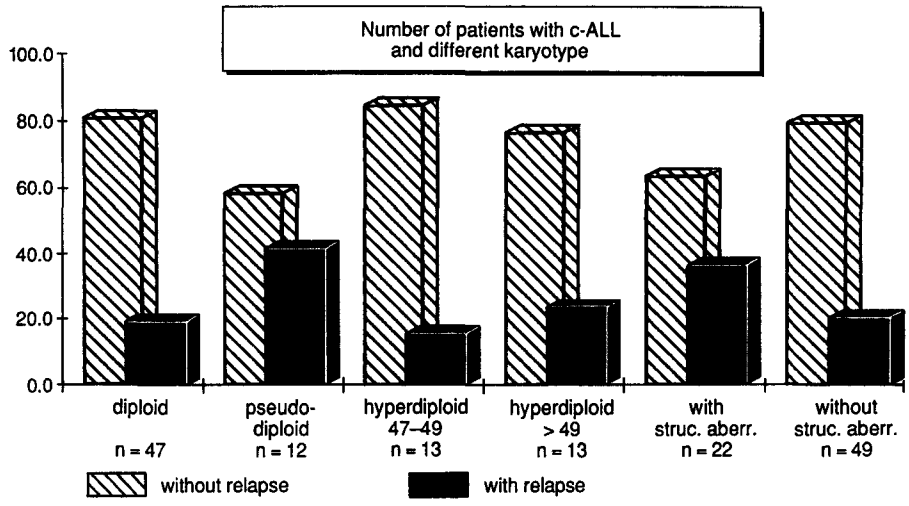
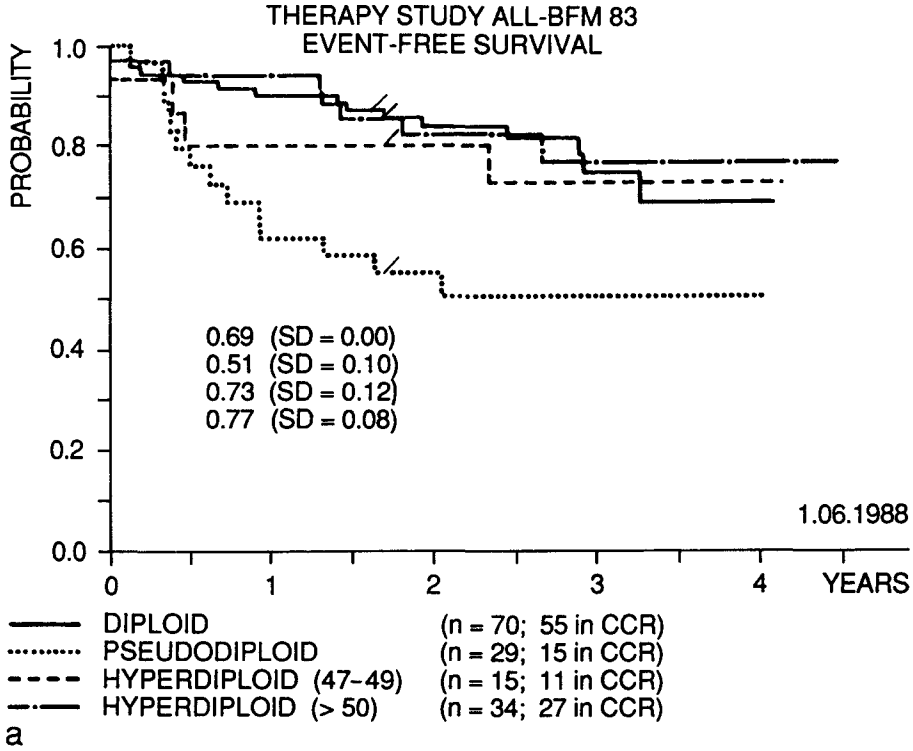
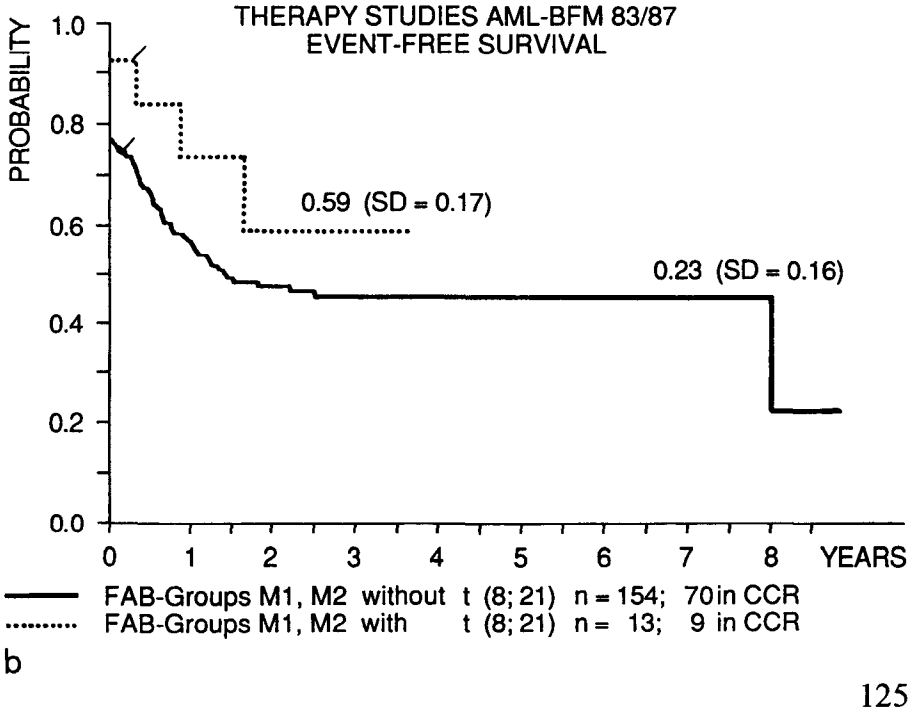


Fig. 4. Numbers of patients with and without relapse of common ALL. All of them were treated with the therapy protocol BFM-83, but different karyotypes were found in their leukemic cells at diagnosis of ALL



a



b

Fig. 5 a, b. Life-table analysis according to Kaplan-Meier. (CCR = Continuous Complete Remission) **a** Patients with pseudodiploidy in their leukemic cells have a significantly poorer prognosis than those with other karyotypes. **b** Prognosis of patients with ANLL M_1 and M_2. Children with t(8;21) seem to have a better prognosis than those without this aberration

structural abnormalities, the patient group with only numerical aberrations or normal karyotypes had only half the percentage of relapses compared with the patients with translocations, deletions, or other abnormalities (Fig. 4).

Life-table analysis by Kaplan-Meier also showed the poor prognosis of children with pseudodiploidy (Fig. 5a). By this analysis, however, it can also be demonstrated that patients with structural aberrations do not always have a poorer prognosis. The survival probability for children with translocation (8;21) in the leukemia karyotype seems to be significantly better than for the other patients with ANLL FAB-type M_2 (Fig. 5b).

By cytogenetic analysis of 554 children with ALL and 147 with ANLL it could be shown that chromosomal aberrations of acute leukemias are closely connected with single subgroups of leukemia and can be used for diagnostic classification. The comparison of relapse data showed a poorer prognosis for ALL patients with a pseudodiploid leukemia karyotype at diagnosis.