er, prospective, controlled studies are warranted to investigate whether PRV-1 downregulation correlates with clinical response. If so, quantification of this surrogate marker would allow more rapid assessment of the potential of new therapeutic strategies for PV.

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Acute myeloid leukemia with recurring chromosome abnormalities as defined by the WHO-classification: incidence of subgroups, additional genetic abnormalities, FAB subtypes and age distribution in an unselected series of 1,897 patients with acute myeloid leukemia

The classification of acute myeloid leukemia (AML) has been based on cytomorphology and cytochemistry since the introduction of the FAB-classification in 1976.[1] In 1999 the WHO proposed a classification for tumors of hematopoietic and lymphoid tissues.[2,3]

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The classification incorporated morphologic, immunophenotypic, genetic and clinical features in order to define biologically homogenous entities which have clinical relevance. Thus, the WHO classification of AML encompasses four major categories: (i) AML with recurring genetic abnormalities, (ii) AML with multilineage dysplasia, (iii) AML, therapy-related and (iv) AML not otherwise categorized.

The first category includes the following subcategories: a) AML with t(8;21)(q22;q22);AML1/ETO, b) AML with abnormal bone marrow eosinophils inv(16)(p13q22) or t(16;16)(p13;q22);CBFB/MYH11, c) acute promyelocytic leukemia (AML with t(15;17)(q22;q12); PML-RARα and variants and d) AML with 11q23/MLL abnormalities. The aim of the current study was to characterize this category further using data from an unselected series of 1,897 patients with ALL, cytogenetically analyzed at diagnosis at our institution between 1996 and 2001. Molecular studies, using fluorescence *in situ* hybridization (FISH) and/or reverse transcriptase polymerase chain reaction (RT-PCR) were also performed, especially in cases with 11q23 abnormalities.

While published data on frequencies of chromosome aberrations are mostly derived from clinical trials which are often restricted to patients with *de novo* AML and those in a certain age range, our cohort included 1,632 cases of *de novo* AML, 148 cases of AML after an antecedent hematologic disorder and 117 therapy-related AML (cases with balanced translocations were included in the analysis of the International Workshop on t-AML).[4] The median age of the patients was 61 years (range 16-88). In total, 87 cases with t(8;21) (4.6%), 99 with t(15;17) (5.2%) (no alternative translocations involving RARα but not PML were included in this series), 87 with inv(16)/t(16;16) (4.6%), and 53 with 11q23/MLL rearrangement (2.8%) were observed. These cytogenetic subgroups were observed in 17.6% of *de novo* AML and in 31.6% of t-AML, but in none of 148 cases of AML occurring after an antecedent hematologic disorder. The incidences of MLL abnormalities, and of inv(16) were significantly higher in t-AML than in *de novo* AML (8.5% vs. 2.6%, p=0.0005; 11.1% vs. 4.5%, p=0.0016), respectively (Table 1).

All 87 cases with inv(16)/t(16;16) showed an AML M4eo FAB subtype. Seventy of the cases with t(15;17) had AML M3 while in 29 patients an AML M3v was diagnosed. In patients with t(8;21) 67 had AML M2, 5 had M1 and one had AML M4 (no data on FAB subtype was available for 14 patients). In AML with 11q23/MLL rearrangement AML M5a, M5b and M4 were the most common morphologies (present in 38.5%, 21.2% and 21.2%, respectively) but M0, M1 and M2 cases were also observed (in 1.9%, 7.7% and 9.6%, respectively). Therefore, AML M4eo with inv(16)/t(16;16)-CBFB-MYH11 is the only subtype showing a 100% correlation between genetics and a unique cytomorphologic picture. In AML with t(15;17)-PML-RARα, two distinct cytomorphologic subtypes

Table 1. Incidence of recurring chromosome abnormalities as defined by the WHO-classification.

	de novo AML n=1632	*t-AML n=117*
t(8;21)	4.9%	6.8%
inv(16)/t(16;16)	4.5%*	11.1%*
t(15;17)	5.8%	4.3%
11q23/MLL	2.7%°	9.4%°
total	17.9%#	31.6%#

**p=0.001, °p=0.0005, #p=0.0002.*

Table 2. Incidence of t(8;21), inv(16), t(15;17) and 11q23/MLL-translocations within different age groups.

Age group	*t(8;21)*	*inv(16)*	*t(15;17)*	*MLL*	*all*
< 60 years	7.1%	6.8%	7.4%	5.3%	26.6%
≥60 years	2.3%	2.5%	3.1%	0.8%	8.7%

can be distinguished.[5] This difference in phenotype is also reflected by differences in gene expression profiles.[6] The most cytomorphologically heterogeneous group is the subgroup of AML with 11q23/MLL-translocation. This group is also cytogenetically heterogeneous as 11 different partner genes of MLL were observed in our series (in order of frequency: 9p22, 10p12, 6q27, 19p13, 17q21, 17q25, 1q21, 15q15, 22q12, 2q37, 10q22) and more than 50 have been published. A correlation between specific partner genes and cytomorphology was not observed. Partial tandem duplications within the MLL gene were observed in 114 of 1,769 analyzed cases (6.4%).[7]

Additional cytogenetic abnormalities occurred in 69 of 87 cases (79.3%) with t(8;21), 44 of 99 (44.4%) with t(15;17), 38 of 87 (43.7%) with inv(16)/t(16;16) and in 20 of 53 (37.7%) patients with 11q23/MLL rearrangement. Recurring additional abnormalities in cases with t(8;21) were -X/-Y (n=59), del(9)(q22) (n=23) and +8 (n=6); recurring abnormalities in those with inv(16) were +22 (n=19), +8 (n=12), and +21 (n=5); those in cases with t(15;17) were +8 (n=15), ider(17)(q10)t(15;17) (n=8), and del(9)(q22) (n=3); and, finally, in cases with 11q23/MLL-rearrangements the recurring abnormalities were translocation +8 (n=5), -7 (n=3), +6 (n=3) and +20 (n=3).

In accordance with published data the incidence of t(8;21), t(15;17), inv(16)/t(16;16) and 11q23/MLL-rearrangements was significantly higher in patients younger than 60 years than in patients 60 years or older (7.1% vs. 2.3%, 7.4% vs. 3.1%, 6.8% vs. 2.5% and 5.3% vs. 0.8%, respectively, $p<0.0001$ for all) (Table 2).[8,9] While only 47% of all 1,897 AML patients were 60 years or younger in this cohort, in the group of patients with t(8;21), inv(16), t(15;17), or 11q23/MLL-abnormalities 72%, 70%, 67% and 89%, respectively, were 60 years or younger.

In conclusion, AML with recurring abnormalities as defined by the WHO classification account for 26.7% of cases of AML in patients under the age of 60, but for only 8.7% of cases of AML ion patients 60 years or older. It should be considered whether to include further genetic categories into the WHO classification in order to classify a larger proportion of AML on a genetic basis, although the common unbalanced karyotype abnormalities (+8, -5/5q-, -7/7q-), in particular, are neither genetically nor morphologically homogeneous. Molecular genetic defects including partial tandem duplications of the MLL gene, FLT3 length mutations and point mutations of genes (i.e.: AML1, CEBPα, FLT3) could be included to categorize a larger proportion of AML on a genetic basis, as these are frequently observed in cases of AML with a normal karyotype.[10,11]

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Thymidylate synthase gene polymorphism and its association with relapse in childhood B-cell precursor acute lymphoblastic leukemia

We investigated a tandem-repeat polymorphism within the promoter region of the thymidylate synthase gene in 40 matched pairs of relapsed and non-relapsed childhood B-cell precursor acute lymphoblastic leukemia patients. This polymorphism has previously been suggested to influence treatment outcome in childhood acute lymphoblastic leukemia. In our study, no association between thymidylate synthase genotype and risk of relapse was found.

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Expression of thymidylate synthase (TS) has been suggested to influence the effect of cancer chemotherapeutic agents such as methotrexate and 5-fluouracil. TS catalyzes the intracellular conversion of deoxyuridylate monophosphate to deoxythymidylate monophosphate, which makes it an essential enzyme in proliferating cells.[1,2] A genetic tandem-repeat polymorphism within the promotor region downstream of the cap site of the TS gene has been described.[3] Recently, the TS genotype of this tandem-repeat polymorphism was suggested to influence treatment outcome in a cohort of 205 French-Canadian children with acute lymphoblastic leukemia (ALL), including 32 who relapsed or died due to the disease.[4] In that study, patients homozygous for the variant TS allele (3R/3R) had a 4.6-fold higher risk of an event than did those expressing the 2R TS allele (2R/2R and 2R/3R) in multivariate analysis. In the same cohort, heterozygous patients (2R/3R) did not show an increased risk of an event compared to the risk in those homozygous for the 2R TS allele.

The present study was aimed at investigating the association of the above mentioned TS tandem-repeat polymorphisms and risk of relapse in a homogeneously treated group of patients with standard and intermediate risk childhood B-cell precursor ALL. The study was performed as a case-control study on 40 relapsed ALL patients with pre-B cell or common ALL immunophenotype, derived from the Berlin-Frankfurt-Münster trials ALL-BFM 86 and ALL-BFM 90. Cases were individually matched to successfully treated ALL patients. The minimum follow-up for the control subjects was 5 years. Matching criteria were gender, age at diagnosis, immunophenotype, initial white blood cell count and risk group. Our study could not consider genetic aberrations with prognostic impact on treatment outcome as potential confounding variables, because these data are not available for the majority of patients in trials ALL-BFM 86 and ALL-BFM 90. In particular, TEL/AML1 rearrangement was not investigated in these trials. All patients included were standard (SR) or intermediate risk (MR) patients. Treatment for SR and MR patients was similar in trials ALL-BFM 86 and ALL-BFM 90.[5,6] The cumulative dosage of methotrexate (MTX) applied was the same for all the patients in the current case-control study.[7] Polymerase chain reaction was performed using sense and antisense primers according to Horie *et al.*[3] Conditional logistic regression analysis to calculate odds ratios and their 95% confidence intervals (CI) was performed to assess the association of genotype with risk of ALL relapse. Genotypes were used as categorical variables in the analyses. The distribution of the patients' matching criteria are shown in Table 1.

The frequency of the 3R/3R TS genotype within the control sample of our study was similar to that observed in the control sample investigated by Krajinovic *et al.* The risk of relapse in the investigated subset of matched patients was not associated with the variant 3R/3R TS genotype, neither with reference

Table 1. Distribution of matching criteria in 80 children with acute lymphoblastic leukemia with (cases) and without (controls) relapse selected from trials ALL-BFM 86 and ALL-BFM 90.

	Cases (n=40) n (%)	*Controls (n=40) n (%)*
Sex		
male	26 (65.0)	26 (65.0)
female	14 (35.0)	14 (35.0)
Age at diagnosis		
<1 year	–	–
1 - <10 years	36 (97.5)	36 (97.5)
≥ 10 years	4 (10.0)	4 (10.0)
Initial WBC		
< 50,000/μL	37 (92.5)	37 (92.5)
≥ 50,000/μL	1 (2.5)	1 (2.5)
Immunophenotype		
common ALL	37 (92.5)	36 (90.0)
pre-B ALL	3 (7.5)	4 (10.0)
Risk group		
standard	13 (32.5)	13 (32.5)
intermediate	27 (67.5)	27 (67.5)

Table 2. Thymidylate synthase genotype in 40 matched pairs of children with standard and intermediate risk B-cell precursor ALL with and without relapse.

	Cases (n=40) n (%)	*Controls (n=40) n (%)*	*Odds ratio (95% CI)*	*p*
Genotype				
2R/2R	20 (50.0)	16 (40.0)	1*	
2R/3R	11 (27.5)	16 (40.0)	0.4 (0.13-1.94)	0.208
3R/3R	9 (22.5)	8 (20.0)	1.0 (–)	
2R/2R and 2R/3R	31 (77.5)	32 (80.0)	1*	
3R/3R	9 (22.5)	8 (20.0)	1.1 (0.70-2.98)	0.795

**Reference category.*