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Molecular pathogenesis of childhood acute lymphoblastic leukemia

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ABSTRACT

This concise review focuses on the most recent advances in understanding molecular genetic abnormalities in childhood acute leukemia (ALL). An increasing number of chromosomal translocations associated to distinct molecular genetic abnormalities have been described. Recurrent motifs have been recognized behind the great heterogeneity of genes involved in chromosomal translocations occurring in childhood ALL. The expression or activation of specific genes encoding for transcription factors have been recognized to be the most frequent recurring mechanism. In addition to the identification of genes involved in translocations, the analysis of deleted or mutated genes has provided new insights into the molecular pathogenesis of childhood ALL. The understanding of the genetic heterogeneity has turned out to have great impact on routine diagnosis and treatment. Molecular analysis has revealed that the t(12;21) translocation, barely detectable when searched for by conventional cytogenetic techniques, is the most frequent genetic lesion occurring in childhood ALL. Accumulating evidence clearly indicates that molecular characterisation at diagnosis represents the most relevant prognostic information for risk stratification of the patients at diagnosis. Several target genes are now available for the study of minimal residual disease and to evaluate its potential impact for tailoring treatment. Finally, our progress in understanding the relationships between genetic lesions and environmental etiologic agents will further contribute to delineating the natural history of pediatric ALL.

Key words: leukemia, children, translocation, gene, transcription factor

Recurrent chromosome abnormalities can be detected in most malignant diseases and are closely associated with particular tumor phenotypes.[1] Clonal abnormalities can be identified in approximately 65-70% of childhood acute lymphoblastic leukemia (ALL). In most instances the genes disrupted by these abnormalities have been identified, thus providing important insights into disease pathogenesis and normal cellular physiology.[2] Molecular assays have been developed and a more accurate diagnosis of disease subtypes is currently available for the translocations most frequently occurring in ALL as well as in acute myeloblastic leukemia (AML).[3,4]

Although several genes have been shown to be involved in chromosomal translocations occurring in childhood ALL, changes in the expression or activation of specific genes encoding for transcription factors (TF) have been recognized to be the most frequently recurring mechanism.[5] TF are proteins with a pivotal role in regulating gene expression.[6] They bind to regulatory elements in DNA known as promoters and enhancers stimulating (or sometimes inhibiting) gene transcription, and thus the formation of messenger RNA, through direct interactions with DNA. The DNA-binding domain of a TF has a helical shape (α helix) within or adjacent to which clusters of positively charged amino acids are located. Recurrent structural motifs in DNA-binding domains provide the basis for the classification of transcription factors. More than 80% of these factors contain one of the following motifs: helix-turn-helix (HTH), zinc-finger, leucine zipper, or helix-loop-helix (HLH). A physically separate domain in transcription factors, the transactivation domain, mediates the interaction with several other factors involved in the complex regulation of gene transcription.

The results of gene manipulation in mice have shown the profound effects on normal hemopoiesis of genes encoding TF proteins which are involved in chromosomal translocations.[7] In some cases gene disruptions affect multiple hematopoietic lineages while in others they result in lymphoid-specific phenotypes. Disruption of the TAL1/SCL gene, which codes for a b-HLH protein originally identified as the most frequent genetic alteration occurring in T-ALL,[8] results in embryonic lethality at day E10.5, owing to the absence of erythropoiesis. *In vitro* erythropoiesis from TAL1$^{-/-}$ yolk-sac cells was also blocked. In addition, myeloid colony formation was virtually absent, probably reflecting a defect at the myelo-erythroid progenitor level.[9] In contrast mice carrying a homozygous mutation in the E2A gene, specifically involved in the t(1;19) translocation of pre-B cell ALL, lacked detectable B cells, whereas T cells and other non-lymphoid cell types were present and phenotypically normal.[10,11] The block in B-cell differentiation in the E2A-deficient mice precedes the onset of the Ig-gene

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Table 1. Chromosomal translocation breakpoints and genes in childhood ALL. Non-fusion genes.

Type	*Affected gene*	*Rearranged gene*	*Frequency (%) ALL overall*
Basic-helix-loop-helix			
t(8;14)(q24;q32)	c-MYC (8q24)	IgH, IgL	3
t(2;8)(p12;q24)			<1
t(8;22)(q24;q11)			<1
t(8;14)(q24;q11)	c-MYC (8q24)	TCR-α	<1
t(8;12)(q24;q22)	c-MYC (8q24) BTG (12q22)	–	<1
t(7;19)(q35;p13)	LYL1 (19p13)	TCR-β	<1
t(1;14)(p32;q11)	TAL1/SCL (1p32)	TCR-α	<1
t(7;9)(q35;q34)	TAL2 (9q34)	TCR-β	<1
LIM proteins			
t(11;14)(p15;q11)	RBTN1/Ttg1 (11p15)	TCR-γ	<1
t(11;14)(p13;q11)	RBTN2/Ttg2 (11p13)	TCR-γ/α/β	1
t(7;11)(q35;p13)			<1
Homeobox protein			
t(10;14)(q24;q11)	HOX11 (10q24)	TCR-α/β	1
t(7;10)(q35;q24)			<1
t(5;14)(q31;q32)	IL-3(5q31)	IgH	<1
t(7;9)(q34;q34.3)	TAN1 (9q34.3)	TCR-β	<1
t(1;7)(p34;q34)	LCK (1p34)	TCR-β	<1

Modified from Rabbits T.H. (Nature 1994; 372:143).

rearrangement, although the extent of the defect is allele specific.

Activation of genes encoding transcription factors by chromosomal translocations

There are two main consequences of translocations and inversions leading to altered gene transcription. As occurs for the translocations listed in Table 1, a proto-oncogene can be activated by the juxtaposition of the promoter and enhancer elements of a distinct gene. This is the case in Burkitt's lymphoma as well as in B-ALL with the t(8;14) translocation, where the enhancer for the immunoglobulin heavy chain gene (IgH) is joined to the coding region of the c-MYC gene, thus altering its expression.[12] The c-MYC protein has several functional domains, including a basic region for DNA binding, b-HLH and leucine zipper (ZIP) protein dimerization motifs. Activation of c-MYC by translocation compromises a transcriptional complex network involving at least three other factors, all of which have also b-HLH and ZIP domains, named MAX, MAD and Mxi-1.[13-16] All these proteins are probably in monomer-dimer equilibrium in normal cells, but while c-MYC-MAX dimers are transcriptionally active, the MAX-MAD or MAX-Mxi-1 ones are not. After chromosomal translocation, this equilibrium is disrupted by incorrect expression of c-MYC.[17]

Alternatively, as occurs for the translocations listed in Table 2, breakpoints may occur within a gene on each involved chromosome, creating a fusion gene encoding a chimeric protein. The t(1;19) translocation, which occurs in up to 25% of childhood pre-B cell ALLs, produces fusion of the E2A and PBX-1 genes.

Table 2. Chromosomal translocation breakpoints and genes in childhood ALL. Fusion genes.

Type	*Affected gene*	*Protein domain*	*Fusion protein*	*Frequency (%) ALL overall*
t(9;22)(q34-q11)	c-ABL(9q34) BCR(22q11)	tyrosine kinase serine kinase	serine + tyrosine kinase	3
t(1;19)(q23;p13.3)	PBX1(1q23) E2A(19p13.3)	HD AD-b-HLH	AD + HD	5-6
t(17;19)(q22;p13)	HLF (17q22) E2A(19p13)	bZIP AD-b-HLH	AD + bZIP	<1
t(4;11)(q21;q23)	MLL(11q23) AF4(4q21)	A-T hook/Zn-finger Ser-Pro rich	A-T hook + Ser-pro	2
t(X;11)(q13;q23)	MLL(11q23) AFX1(Xq13)	A-T hook/Zn-finger (Ser-Pro rich)	A-T hook + (Ser-pro)	<1
t(1;11)(p32;q23)	MLL(11q23) AF1P(1p32)	A-T hook/Zn-finger Eps-15 homologue	A-T hook + ?	<1
t(6;11)(q27;q23)	MLL(11q23) AF6(6q27)	A-T hook/Zn-finger myosin homologue	A-T hook + ?	<1
t(12;21)(p13;q22)	ETV6/TEL (12p13) AML1/CBF-α (21q22)	HLH dimerization/Ets-like DNA binding DNA binding-runt homology	HLH dimerization/DNA binding-runt homology	20-25

Modified from Rabbits T.H. (Nature 1994; 372:143).

E2A encodes the Ig enhancer binding protein, which is a member of the b-HLH family of transcription activators that have been mapped to 19p13; PBX-1, a homeobox gene of unknown function, maps to 1q23.[18,19]

One of the crucial issues in the delineation of the oncogenic properties of genes involved in chromosomal translocations is the identification of the target genes under the control of oncogenic transcription factors. Emerging evidence from analysis of progenitor cells in mice and humans suggests that the major clusters of homeobox containing HOX genes can be considered potential targets of encoding TF genes involved in chromosomal translocations.[5] Originally discovered in homeotic genes responsible for segment identity in *Drosophila* development, they were subsequently found in a number of evolutionarily distant organisms.[20] In addition to controlling embryonic development, evidence has been obtained that they also control ongoing differentiation processes, including normal hematopoiesis, in adults.[21] Furthermore, a contributory role of homeobox genes in tumorogenesis and leukemogenesis is consistent with data obtained by genetic manipulation of various homeobox genes.[7] The MLL gene, the mammalian counterpart of the thithorax (trx) gene of Drosophila, is suggested to act by disrupting the critical pattern of HOX genes in a way that selectively contributes to myeloid (for example, MLL-AF9) or lymphoid leukemias (MLL-AF4).[22-24] The small segment of the PBX1 gene that mediates interactions with HOX protein is critical for the transformation of the E2A-PBX1 fusion gene of the t(1;19) translocation.[25] The HOX11 gene, located on chromosome 10, band q24, is activated by the translocations t(10;14)(q24;q11) and t(7;10)(q35;q24) in T-ALL.[26] Murine Hox11 is required for spleen development through a mechanism that appears to affect survival of splanchnic precursors.[27-28] In addition, HOX11 interacts with phosphatases that normally function within a G2-phase checkpoint. As it is not expressed in normal T cells, its disregulation may interfere with this checkpoint and cause aberrant cells to enter into mitosis.[29]

The interference with the complex activation of the apoptotic pathway has been suggested to represent another mechanism of action of oncogenic transcription factors.[30] From the initial demonstration that BCL2 protein protects B and T cells from apoptosis in mice transgenic for BCL2 expression,[31-33] that subsequently develop B-cell hyperplasia and eventually B-cell malignancy, much attention has been drawn to the possible disregulation of programmed cell death in the acute leukemias. The E2A-HLF fusion protein, which is formed by the t(17;19) translocation, occurring in pediatric pro-B ALL[34] appears to exert a crucial role in a conserved cell death pathway that censors immature B lymphocytes.[34] Dominant-negative inhibition of E2A-HLF in transformed lymphocyte progenitors induces apoptosis, suggesting that the chimeric protein can increase the number of immature lymphoid cells by preventing cell death.[34]

The ETV6/AML1 fusion gene: the most frequent genetic lesion occurring in childhood ALL

Until recently, the translocation t(12;21) was considered to be of limited prognostic value due to its apparent rarity, being barely detectable in less than 0.05% of the patients.[35] Initial attempts to identify the t(12;21) translocation by the fluorescence *in situ* hybridization technique (FISH) have indicated that its prevalence is largely underestimated.[36,37] The ETV6 gene on chromosome 12p13[38] and the AML1 gene on chromosome 21q22 (reviewed in ref. #39) were recently shown to be involved in the t(12;21) translocation,[40] and a PCR technique has become available for the molecular detection of the translocation.[41-43]

The mechanism(s) by which the ETV6/AML1 fusion gene transforms cells is unknown (review in ref. #44). ETV6 is a new member of the ETS family of transcription factors that was cloned by virtue of its fusion to the platelet-derived growth factor β receptor (PDGFβR) in chronic myelomonocytic leukemia (CMML) with the t(5;12)(q33;p13) translocation. ETV6 contains the conserved carboxy terminal DNA binding domain which defines the ETS family of transcription factors. In addition ETV6 belongs to a subclass of ETS proteins which contain a highly conserved amino terminal domain. Because of its weak homology to the HLH unit of proteins such as MYC or MYO-D,[45-47] it has been suggested that the N-terminal region of ETV6 may be involved in protein-protein interactions which facilitate transcriptional activation.[48] However, direct evidence that the N-terminal HLH-like sequence mediates protein-protein interactions is still lacking.

In the t(12;21) translocation, it is possible that the fusion of two transcription factors leads to a protein with abnormally high transactivation activity compared to the corresponding wildtype ETV6 and AML1 gene products. Alternatively the fusion protein may result in a different activity. Indeed, it can be pointed out that AML1 functions as an activator of the TCRβ enhancer, while ETV6/AML1 is a repressor of that element.[49]

Patients with ETV6/AML1-positive ALL are usually young (more than three-quarters are younger than five years old), have relatively low white cell counts at diagnosis (64% of the patients have less than 20×10^9/L WBC) and leukemic blasts with non hyperdiploid DNA content (in 88% of the cases). Leukemic cells in these cases tend to express myeloid-associated antigens. In a study by Borkhardt *et al.*, expression of CD13, CD33 or CDw65 in > 20% of blasts was found in 15 out of 61 ETV6/AML1-positive (24.6%) cases versus 6 out of 199 (3.0%) without molecular abnormality.[50]

The presenting features associated with the expression of ETV6/AML1 fusion transcripts (age, WBC, phe-

notype) are known to be associated with a good prognosis, raising the issue of the clinical significance of this molecular lesion as an independent prognostic factor. The outcome of ALL patients carrying the t(12;21) translocation, retrospectively enrolled over a 10 years time, and treated by a variety of intensive chemotherapies, was recently evaluated.[50] The ETV6/AML1 positive group had a superior outcome, although the difference was not statistically significant because of the small number of patients studied.[42] More recently the association between ETV6/AML1 positivity and lack of relapse in ALL patients was reported in a series of 76 childhood ALL cases observed during a median follow-up of 8.3 years.[51] Although the study showed a statistically significant difference, a high proportion of the patients selected within the ETV6/AML1 negative group displayed high risk features, possibly amplifying the differences in clinical outcome observed with respect to ETV6/AML1 gene expression.[51]

In a recent series of children with ALL enrolled in the now closed German ALL-BFM90 and Italian AIEOP-91 protocols, ETV6/AML1 was detected in 99 of 342 (28.9%) cases studied.[50] The patients expressing the ETV6/AML1 fusion mRNA appeared to have a better event-free survival (EFS) than those lacking the chimeric product. In a Cox model, ETV6/AML1 remained a favorable prognostic indicator: patients with this abnormality were three-times less likely to relapse than those without it.[50] The prognostic impact of the t(12;21) translocation has been recently questioned by the observation that a similar incidence of ETV6/AML1 fusion transcript was observed in ALL relapsing patients as well as those in continuous complete remission (CCR).[52,53] These results are divergent, with the incidence of ETV6/AML1 rearrangement being 31% and 19%, probably due to the small number of patients. More recently Harbott *et al*. analyzed the bone marrow of 49 consecutive children with relapsed ALL and found a frequency of 19.6%, similar to the percentage reported for diagnosis in previous retrospective studies, as well as in a recent prospective German-Italian analysis.[50] On comparing the clinical features at diagnosis, no distinguishing features emerged among ETV6/AML1-positive children.[54] Along the same line, 29 of 142 children with ALL-relapse (20%) treated according to relapse trails ALL-REZ BFM 90-96 were found to be ETV6/AML1 positive.[55] ETV6/AML1-positive and ETV6/AML1-negative patients differed significantly with respect to duration of first complete remission, the time-point of relapse off/on therapy and age at initial diagnosis. At a median follow-up of 19.5 months, children positive for ETV6/AML1 had a significantly higher probability of survival and EFS compared to the negative children.[55] The authors concluded that the presence of ETV6/AML1 gene can potentially identify a large subgroup of children with relapsed-ALL with a favorable outcome and that this individual marker might be used for therapy stratification in future relapse trials.

Searching for new genetic lesions

In addition to the identification of the genes involved in chromosomal translocations, the analysis of deleted or mutated genes in childhood ALL, has been an area of intense research.

In particular, a new class of small proteins involved in negative regulation of the cell-cycle, cyclin-dependent kinase inhibitor (CDI) has been shown to be altered in a large variety of malignancies, including leukemias.[56] A small protein, p16INK4, was initially isolated as a CDK4 binding protein and subsequently identified as a candidate tumor suppressor gene involved in familial melanomas.[57] This gene, as well as the closely linked p15INK4B, was found to be homozygously deleted in 30% of childhood B-cell precursor ALL and in most (up to 95%) of the cases of T-ALL.[58-60] Owing to the very high incidence of p16INK4 gene deletion especially in T-ALL, it is unlikely that it could be a clinically prognostic factor. Heyman *et al.* suggested that p16INK4 may be an independent prognostic factor in pediatric ALL,[61] but this is not confirmed in larger studies.[62] In contrast this genetic event might represent an initial important alteration which leads to premature entry into the S-phase of the cycle before completion of a critical process (mainly DNA repair), thus generating genetic instability. This in turn could favor the development of additional genetic mutations which represent the key steps towards the establishment of a definite malignant phenotype. p18 protein has a function similar to that of the p16INK4 protein although it is preferentially associated with inhibition of CDK6.[63] Analysis of the p18 gene in childhood T-ALL showed that the p18 homozygous deletion occurs less frequently than in p16INK4.[60] Moreover, all the p18 negative gene leukemias are also p16INK4 negative, thus suggesting that p18 gene inactivation may represent a late event which could contribute to the development of a more malignant phenotype.

Backtracking leukemia to birth: when does the first genetic lesion occur?

Some epidemiological evidence has suggested that prenatal events may play a role in the etiology of pediatric leukemia.[64,65] These findings have been further supported by the cases of leukemia in twins described so far. The high concordance rate of leukemia (20 to 25%), particularly in monozygotic twins raised the suspicions of a transformation event in utero even in the earliest studies.[66,67] More recently, Ford *et al.* reported concordant leukemia in three pairs of monozygotic infant twins, each pair with a clonal, nonconstitutional rearrangement of the MLL gene,[68] which is so frequently observed in infant leukemia.[69] These findings, demonstrated in cases with monochorionic placentas, were further confirmed in a pair of diplacental/dichorionic female infant twins with ALL and the t(11;19). In the latter case the transfer of the malignant clone occurred by crossing into the

maternal circulation and back to the second twin.[70]

Direct evidence for the prenatal origin has been recently provided by Gale *et al*. who described a PCR-method to analyse neonatal blood spots (Guthrie cards) for the presence of numerically infrequent leukemic cells at birth in individuals who subsequently developed leukemia. They developed sequence information of the MLL-AF4 fusion in diagnostic leukemic cells DNA prior to PCR analysis of the same Guthrie card. PCR amplification of a specific MLL-AF4 fragment containing the breakpoint region was obtained from all three patients analyzed. For a further verification that the breakpoint fusion sequence from the patient' blood spot was identical to that of their diagnostic leukemia DNA, PCR products amplified from blood spot DNA were purified and the sequences from both strands were found to be identical.[71] These findings may have profound implications for our understanding of the natural history of pediatric leukemia.[72] Most of the cases of infant leukemias carry MLL gene rearrangements.[69] In addition these abnormalities occur in therapy-related-AML (t-AML) and t-ALL after treatment with topoisomerase II inhibitors.[73] Whether prenatal exposure to natural or drug inhibitors of topoisomerase II may be critical to the pathogenesis of disease or not is of relevant interest and is currently being assessed in ongoing international cooperative epidemiological studies. That clonal origin in utero may represent a general pattern in the etiology of pediatric leukemia even beyond infancy is strengthened by the observation of a unique pair of older identical twins with the same clonal T cell malignancy and a 9 and 11 year preleukemic latent period before the onset of clinical disease.[74] More recently in a set of identical twins with ALL, aged 3.5 and 5 years at diagnosis and carrying the t(12;21) translocation, it has been shown that the size of the ETV6/AML1 fusion product and its sequence were identical in each case. Furthermore, the twins share an identical immunoglobulin heavy chain VDJ sequence including the N region.[75] Taken together, all these data strongly suggest that a first genetic hit may occur during fetal period followed by a pre-leukemic or latent period before the clinical manifestations.

Towards risk classification according to genetic lesions?

The availability of molecular methods to detect the most prognostic relevant chromosomal translocations in childhood ALL, has suggested the use of genetic features of leukemic cells as a tool for patients' risk classification. To date, the only subset of B-lineage ALL cases with a favorable prognosis has been the hyperdiploid group, and DNA index (> 1.16) is currently included as a criteria to identify a subgroup of ALL patients considered for less intensive chemotherapy.[76] As previously mentioned, no definitive conclusions can be drawn on the prognostic impact of ETV6 gene rearrangements. By contrast, the t(9;22), the t(4;11) and other MLL gene rearrangements are associated with a poor prognosis in patients treated with a variety of regimens.[77] MLL gene rearrangements occur in most of the cases of infant leukemia (60% to 70% of the cases)[69] and may account for the dismal prognosis in this subgroup.[69,78,79] Recently, Behm *et al.* demonstrated that the rearrangement of the MLL gene confers a poor prognosis even in children over 1 year of age, in whom it occurs less frequently (30% of the cases) than in infancy.[80] In a stratified statistical analysis adjusted for age and treatment protocol, the 4-year EFS was 10% for cases with a rearranged MLL gene and 64% for other cases. When infants were excluded from the analysis, MLL rearrangement was still associated with a poor outcome ($p = 0.02$), and remained this way with the exclusion of the t(4;11)-positive cases, although the number of patients included were very small.[80] The prognostic significance of the t(1;19) appears to be dependent on the intensity of the treatment used; contemporary intensive chemotherapy regimens have abolished the poor prognosis once ascribed to this translocation.[81]

Parallel to the recent research on molecular markers, investigation of cellular and clinical response to therapy has significantly contributed to the identification of subsets of patients with higher probability for therapy resistance or relapse.[82] A recent, interesting demonstration is that, even within genetically homogeneous subgroups – such as the t(9;22)-positive ALL – the pattern of early clinical response to treatment might be significantly different. In order to identify subsets of Ph+ ALL that might need different therapeutic approaches, the Ph+ ALL cases enrolled in the Italian and German consecutive trials from 1986 to 1995, were evaluated with respect to presenting clinical features, early response to prednisone, and clinical outcome.[83] Thirty-five percent of the evaluable patients who were poor prednisone responders (PPR=presence of more than 1,000 leukemic blasts per μL on day 8 of induction)[83] were older (10.0 vs 6.88 yrs) and had a higher WBC (144 vs 29×10^9/L) than patients with good response to prednisone (PGR; < 1,000 blasts/L). Only 2/20 patients (10%) with PPR remained in CR1 and alive, in contrast to 70% of the 37 patients with PGR who survived. Thus, two thirds of Ph+ childhood ALL cases can be identified early by PGR which, when treated with intensive BFM chemotherapy with or without BMT, have a significantly lower risk of treatment failure.[83]

Overall it is likely that the identification of discrete genetic lesions, shown to be prognostically relevant, in combination with the evaluation of early response to treatment, will help to modulate the treatment of childhood ALL in future clinical trials. It is still controversial whether reverse-transcription-PCR (RT-PCR) of the fusion transcripts created by the t(9;22), t(4;11) and t(1;19), may be used to identify patients with different outcomes within the same genetically identified subgroup. In patients with the t(4;11) translocation

the sequential analysis of the MLL-AF4 hybrid transcript showed a persistently negative RT-PCR in the five cases of continuous complete remission (CCR) long-term survivors.[84] By contrast, the PCR analysis resulted persistently positive in the remaining seven cases including the four cases who relapsed after the achievement of clinical CR. In ALL patients with the t(1;19) translocation, preliminary data in small cohorts of patients[85,86] have indicated a good correlation between the presence of this transcript and relapse. More recent data did not confirm the predictive value of MRD detection of E2A-PBX1 chimeric mRNA at the end of consolidation for patient outcome.[87]

Minimal residual disease in childhood ALL: a new tool for risk classification?

The study of minimal residual disease (MRD) is of great interest in clinical oncology because of the potential of tailoring treatment and the possibility of gaining insights into the nature of cure. Several methods to detect MRD in leukemias have been proposed and extensively reviewed.[88,89]

In ALL rearrangements where Ig and TCR genes result in unique recombinations of variable (V), diversity (D) and joining (J) gene segments,[90] the junctional regions between these gene segments can be regarded as *fingerprint-like* sequences due to the deletion and random insertion of nucleotides during the rearrangement process.[90] PCR-based MRD detection via clone-specific junctional regions generally reaches sensitivity of 10^{-4} to 10^{-5}. For this purpose, oligonucleotide primers are designed at opposite sides of the junctional region. To discriminate between the leukemia-derived PCR products and PCR products of normal cells with comparable rearrangements, the amplification products are generally hybridized to a patient-specific junctional region probe, which is designed according to the junctional region sequences of the leukemic cells at diagnosis.[91]

Several retrospective and limited prospective studies indicate that the detection of MRD in childhood ALL has prognostic value, although the results of these studies are not fully concordant (reviewed in ref. #88) Absence of residual disease after remission induction is associated with a good prognosis.[92,93] However, approximately one half of the patients still remain MRD positive at that time point. Therefore the level of MRD positivity was evaluated and found to correlate with the outcome.[93,94] If multiple BM samples are analyzed during the follow-up, steady decreases of MRD levels to negative are associated with a favorable prognosis,[95,98] whereas persistence of MRD generally leads to clinical relapse.[99-101] A crucial issue to be considered in the interpretation of the data is the level of sensitivity reached by the method used. A recent study by Roberts *et al*.[102] claims that the MRD may persist at low levels (10^{-5} to 10^{-7}) up to 35 months after cessation of therapy in many patients in CCR.[102]

In order to achieve more information on how MRD can be applied to the clinical decision process, a large prospective multicenter study was performed in 240 children with ALL, treated according to national protocols of the I-BFM Study group (I-BFM-SG) in Germany, Austria, Italy and the Netherlands. MRD negativity at the various time points was associated with low relapse rate (3-16%), but five to fourteen-fold higher relapse rates (41-86%) were found in MRD positive patients. By using the combined MRD information at the first two follow-up time points, it was possible to recognize a low risk group comprising 43% of the analyzed patients with a relapse rate of only 2% and a high risk group of 15% of patients with a relapse rate of 84%.[103] These data are likely to provide a rationale for the design of MRD-based stratification of treatment protocols for childhood ALL.

Conclusions and Perspectives

Treatment of childhood ALL is likely to progress from uniform strategies to more refined protocols tailored to the risk of relapse in discrete subgroups. This is challenging not only the need for international cooperation in order to achieve significant data in a short time (due to the limited size of each subgroup), but the need to develop and explore more targeted therapy. So far, the use of all-trans retinoic acid for acute promyelocytic leukemia (APL) is the only example of effective treatment for a chimeric transcription factor. Alternative strategies would be the use of antisense oligonucletides, ribozymes designed to inactivate the mRNAs encoding chimeric proteins or the use of small molecules that repress the transcription of specific genes. It is likely that new strategies will link the knowledge on the mechanisms shown to be altered in different pathways controlling apoptosis, differentiation and proliferative capacity of hemopoietic cells, thus providing more effective treatment for childhood ALL.

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AB was primarily responsible for the conception of this review article and the writing of the paper. GM contributed to the analysis of the literature and writing of the paper.

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