

SPECIAL TECHNICAL REPORT



Heterogeneity in junctional regions of immunoglobulin kappa deleting element rearrangements in B cell leukemias: a new molecular target for detection of minimal residual disease

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Virtually all immunoglobulin kappa (*IGK*) gene deletions are mediated via rearrangements of the so-called kappa deleting element (Kde). Kde rearrangements occur either to $V\kappa$ gene segments ($V\kappa$ -Kde rearrangements) or to the heptamer recombination signal sequence in the $J\kappa$ - $C\kappa$ intron. Kde rearrangements were analyzed by the polymerase chain reaction (PCR) and heteroduplex analysis in 130 B-lineage leukemias: 63 precursor-B-acute lymphoblastic leukemias (ALL) and 67 chronic B cell leukemias. To obtain detailed information about Kde rearrangements, we sequenced 109 of the 189 detected junctional regions. $V\kappa$ gene family usage in the $V\kappa$ -Kde rearrangements in our series of B-lineage leukemias was comparable to $V\kappa$ gene family usage in functional $V\kappa$ - $J\kappa$ rearrangements in normal and malignant mature B cells, except for a higher frequency of $V\kappa$ family usage in precursor-B-ALL. Junctional region sequencing of the Kde rearrangements in precursor-B-ALL revealed a mean insertion of 4.7 nucleotides and a mean deletion of 9.5 nucleotides, resulting in an extensive junctional diversity, whereas in chronic B cell leukemias the insertion (1.9) and deletion (6.0) were significantly lower. The relatively extensive junctional diversity of the Kde rearrangements in precursor-B-ALL allowed us to design leukemia/patient-specific oligonucleotide probes, which were proven to be useful for detection of minimal residual disease (MRD) with sensitivities of 10^{-4} to 10^{-5} . Kde rearrangements occur in approximately 50% of precursor-B-ALL cases and are likely to remain stable during the disease course, because Kde rearrangements are assumed to be 'end-stage' rearrangements, which cannot easily be replaced by continuing rearrangement processes. These findings indicate that junctional regions of Kde rearrangements in precursor-B-ALL represent new valuable patient-specific PCR targets for detection of MRD.

Keywords: *IGK* genes; kappa deleting element (Kde); rearrangements; precursor-B-ALL; B-lineage leukemias; MRD

Introduction

The various types of B-lineage leukemias, ie precursor-B-acute lymphoblastic leukemias (ALL) and chronic B cell leukemias

are regarded as malignant counterparts of immature and mature B cells, respectively.^{1–5} Based on leukemia studies, it has been found that immunoglobulin (Ig) gene rearrangements occur in sequential order: Ig heavy-chain (*IGH*) genes rearrange first, followed by Ig kappa (*IGK*) gene rearrangements, which precede Ig lambda (*IGL*) gene rearrangements.^{2–6} *IGL* gene rearrangements occur after or coincide with *IGK* gene deletions, which involve deletion of $J\kappa$ and/or $C\kappa$ gene segments.^{6,7}

The vast majority (>98%) of *IGK* gene deletions are mediated via site-specific rearrangements of the kappa deleting element (Kde), which is located approximately 24 kb downstream of the $C\kappa$ gene segment.^{7–11} Kde rearranges via its heptamer–nonamer recombination signal sequence (RSS) either to the heptamer RSS located in the $J\kappa$ - $C\kappa$ intron (intron RSS), resulting in $C\kappa$ deletions, or to the heptamer–nonamer RSS at the 3' side of a $V\kappa$ gene segment, resulting in $J\kappa$ - $C\kappa$ deletions (Figure 1).^{7–11} In principle $V\kappa$ -Kde rearrangements can involve any of the approximately 76 $V\kappa$ gene segments,^{12,13} which are located either adjacent to the $J\kappa$ - $C\kappa$ region (indicated as proximal) or located further upstream from the $J\kappa$ - $C\kappa$ region in an inverted orientation (indicated as

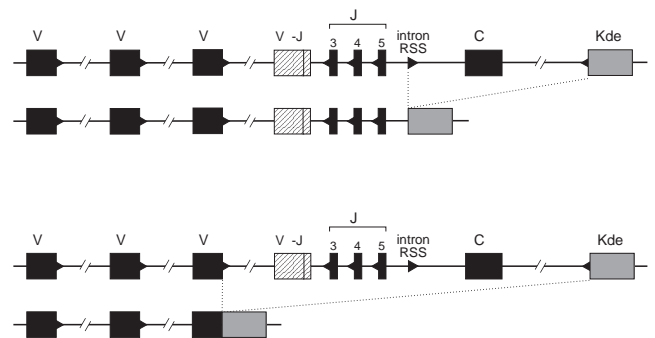


Figure 1 Schematic diagrams of *IGK* gene deletions mediated by Kde rearrangements. Two types of Kde rearrangements can occur: (upper diagram) Kde rearrangement to the intron RSS; (lower diagram) Kde rearrangement to a $V\kappa$ gene segment.

Table 1 Oligonucleotide primers for PCR analysis and sequencing of Kde gene rearrangements

Primer code	Size of primer (bp)	Position in bp primer included (excluded) ^a	Application (PCR, SEQ) ^b	5'	Sequence	3'
V κ I-5'	24	-246 (-222)	PCR	GTAGGAGACAGAGTCCACCATCACT		
V κ I-3'	20	-105 (-85)	SEQ	GTTCAGCGGCAGTGGATCTG		
V κ II-5'	21	-262 (-241)	PCR	TGGAGAGCCGGCCTCCATCTC		
V κ II-3'	23	-83 (-60)	SEQ	ACAGATTTCACTGAAAATCAG		
V κ III-5'	22	-244 (-222)	PCR	GGGAAAGAGCCACCCTCTCCTG		
V κ III-3'	20	-86 (-66)	SEQ	GGGACAGACTTCACTCTCAC		
V κ IV-5'	21	-260 (-239)	PCR	GGCGAGAGGGCCACCATCAAC		
V κ IV-3'	20	-86 (-66)	SEQ	GGGACAGATTTCACTCTCAC		
V κ V-5'	24	-245 (-221)	PCR/SEQ	CCAGGAGACAAAAGTCAACATCTCC		
V κ VI-5'	23	-253 (-230)	PCR/SEQ	CTGTGACTCCAAGGAGAAAAGTC		
V κ VII-5'	22	-255 (-233)	PCR/SEQ	AGGACAGAGGGCCACCATCACC		
Ig κ RS-5'	27	-326 (-299)	PCR	GTTATTCCTAAAAGTCAATCTCAAAG		
Ig κ RS-3'	20	-116 (-96)	SEQ	CGATTGAGTGGCTTTGGTGG		
Kde-3'	23	+185 (+162)	PCR	CCCTTCATAGACCCCTCAGGCAC		
Kde-5'	20	+91 (+71)	PCR/SEQ	TTCTAGGGAGGTCAGACTC		

Primers for the V κ I, V κ II and V κ III were designed based on the alignment presented in the review of Schable and Zachau.¹² Sequence information used to design primers for V κ IV, V κ V, V κ VI and V κ VII primers were derived from Refs 36–39. The J κ -C κ intron and Kde primers were developed according to sequences described in Refs 8 and 40.

^aThe position of the 3' side of the primers is indicated upstream (+) or downstream (-) of the closest RSS. In case of the Ig κ RS (intron) primers the position is indicated relative to the heptamer RSS in the J κ -C κ intron sequence.

^bThe application of the primers is indicated: PCR, primer for PCR amplification; SEQ, primer for sequencing.

distal).¹⁴ The V κ gene segments are clustered in seven families on the basis of sequence homology.^{12,13}

Comparable to V-(D)-J rearrangements,^{15–18} the Kde rearrangements probably also result in junctional regions with deletion of nucleotides by trimming of the gene involved segments,¹⁹ P-region nucleotides,²⁰ and N-region nucleotides.^{18–21} P-region nucleotides are template dependent, whereas N-nucleotides are randomly inserted at junctions by the enzyme terminal deoxynucleotidyl transferase (TdT).^{18–21}

Junctional regions of rearranged *IGH*, T cell receptor gamma (*TCRG*), and T cell receptor delta (*TCRD*) genes are being used as targets for detection of minimal residual disease (MRD) in ALL by use of the polymerase chain reaction (PCR) technique.^{22–26} However, it should be noted that the applicability of these patient-specific sequences as targets for the MRD-PCR technique is dependent on the occurrence, sensitivity and stability of the involved Ig/TCR gene rearrangements.^{4,26–33} So far, junctional regions of rearranged Ig light chain genes have not been evaluated as MRD-PCR targets in ALL, because initial reports suggested that *IGK* and *IGL* gene rearrangements occur at low frequency in ALL (approximately 20%) and because it was assumed that the junctional diversity of rearranged *IGK* and *IGL* genes is restricted as observed in normal blood B-lymphocytes.^{2,34}

The aim of our study was to obtain more detailed information about Kde rearrangements during B cell differentiation. Therefore, we examined the junctional regions of V κ -Kde rearrangements and intron RSS-Kde rearrangements in a large series of 130 immature and mature B-lineage leukemias. Furthermore, we evaluated the applicability of the Kde rearrangements as MRD-PCR targets in precursor-B-ALL.

Materials and methods

Cell samples

A large group of 130 B-lineage leukemias was selected based on the presence of Kde rearrangements as detected by South-

ern blotting in previous studies.^{4,11} This group of leukemias comprised 63 precursor-B-ALL (one pro-B-ALL, 41 common-ALL, and 21 pre-B-ALL) and 67 chronic B cell leukemias (58 chronic lymphocytic leukemias (CLL), five prolymphocytic leukemias (PLL), and four hairy cell leukemias (HCL)).

Mononuclear cells (MNC) were obtained from peripheral blood (PB) or bone marrow (BM) samples of the 130 B-lineage leukemias, as well as from PB of some healthy adult controls by FicolI–Paque centrifugation (density: 1.077 g/cm³; Pharmacia, Uppsala, Sweden). All MNC samples were frozen and stored in liquid nitrogen. The cell samples were obtained according to the guidelines of the Medical Ethics Committee of the University Hospital Rotterdam/Erasmus University Rotterdam.

DNA was isolated from MNC samples as described previously.^{16,35}

PCR analysis

PCR amplification was performed as described previously. A mixture of 1.0 μ g DNA, 12.5 pmol of the upstream and downstream oligonucleotide primers (Table 1), and 1 unit of *Ampli-Taq* DNA polymerase (Perkin-Elmer, Norwalk, CT, USA) were used in each PCR of 100 μ l. The primers were designed according to known sequence data^{8,12,36–40} and carefully positioned, resulting in PCR products for optimal analysis of junctional regions of Kde rearrangements, by using either V κ family primers or the intron RSS primer (Ig κ RS) in combination with a Kde primer. The PCR reaction mixture was heated at 92°C for 3 min in a thermal cycler (Perkin-Elmer), followed by 35 cycles of denaturation, annealing and extension at 92°C for 45 s, at 60°C for 90 s, and at 72°C for 2 min, respectively. After the last cycle an additional extension step at 72°C for 10 min was performed.

Precursor-B-ALL (intron RSS-Kde)					Chronic B-cell leukemias (intron RSS-Kde)				
J -C intron	junctional region	Kde	case		J -C intron	junctional region	Kde	case	
<u>GCTTTCCTGATG</u>		<u>GGAGCCCTAGTG</u>			<u>GCTTTCCTGATG</u>		<u>GGAGCCCTAGTG</u>		
GCTTTCCTG	CCCCTGGGG	GCCCTAGTG	2308		GCTTTCCTGATG	A	AGCCCTAGTG	cK11	
GCTTTCCTGATG	cGG	GGAGCCCTAGTG	2678		GCTTTCCTGATG	cCC	GCCCTAGTG	cK13	
GCTTTCCTG	G	TAGTG	2789		GCTTTCCT	CCCCTGT	GTG	cK30	
GCTTTCCTGA	AA	GAGCCCTAGTG	3049		GCTTTCCTGATG	cT	CCCTAGTG	cK37	
GCTTTCCT	CAATCCTACT	GCCCTAGTG	3178		GCTTTCCTGATG		GAGCCCTAGTG	cK42	
GCTTTT	ACCC	GAGCCCTAGTG	3264		GCTTTCCT	CCCC	GGAGCCCTAGTG	cL04	
-16	CCCGGGA	-17	3869		GCTTTCCTGATG	AG	G	cL12	
GCTTTCC	GGGGGCT	CCTAGTG	3899		GCTTTCCTGATG		CCCTAGTG	cL18	
GCTTTCCTGATG	AG	GGAGCCCTAGTG	3899		GCTTTCCTG	CT	GAGCCCTAGTG	cL21	
GCTTTCCTGATG		GCCCTAGTG	4711		GCTTTCCTGATG	GA	AAGCCCTAGTG	cL27	
GCTTTCCTGATG	GTTGGG	CCTAGTG	5014		GCTTTCCTGA		GCCCTAGTG	cL30	
GCTTTCCTGATG	AAAAA	G	5162		GCTTTCC	TT	GAGCCCTAGTG	cL30	
GCTTTCCTGAT		CCCTAGTG	5236		GCTTTCCTG	TCC	GAGCCCTAGTG	cL34	
GCTTTCCTGAT		AGTG	5248		GCTTTCCT		GAGCCCTAGTG	cL42	
GCTTTCCTG	GTCA	AGCCCTAGTG	5248		-19	TTTGC	-15	cL44	

Precursor-B-ALL (V -Kde)					Chronic B-cell leukemias (V -Kde)				
V I	junctional region	Kde	case		V I	junctional region	Kde	case	
<u>GAATTTTCCTCC</u>		<u>GGAGCCCTAGTG</u>			<u>TAGTACTCCTCC</u>		<u>GGAGCCCTAGTG</u>		
B1(VII)	T	GAGCCCTAGTG	3178		A2(II)	ACAGCTTC	TCTCT	GAGCCCTAGTG cK07	
A17(II)	TCGCA	GAGCCCTAGTG	3264		A27/A11(III)	TAGCTCACCTCC	GTG	cK16	
L9(I)	A	GGAGCCCTAGTG	3510		A19/A3(II)	ACAAACTCC	GGAGCCCTAGTG	cK47	
A27(III)	GAGG	GTG	3725		L2(III)	TAAGTGCC	CCCGGA	CCTAGTG cL05	
A27(III)		GAGCCCTAGTG	3731		L12(I)	TAGTTATTGCT	CCTAGTG	cL07	
L4/L18A(I)		AGCCCTAGTG	3750		L1/L15(I)	TAGTTACCCTC	AGCCCTAGTG	cL13	
O18/O8(I)	ggGTGCA	GGAGCCCTAGTG	3797		O14/O4(I)	CAATGCCCTC	GAGCCCTAGTG	cL17	
A17(II)		GAGCCCTAGTG	3869		B3(IV)	TAGTACTCC	GAGCCCTAGTG	cL18	
L12(I)	T	TCCCTCTGG	-20	4533	L4/L18A(I)	TAGTTACCCTCC	CC	GAGCCCTAGTG cL20	
L9(I)	TAGTTACCC	GTGAACGG	-15	4686	A18(II)	ACGCCTTC	GAGCCCTAGTG	cL25	
L16/L2(III)	TAAGTGCC	CCCGAG	CCTAGTG	4842	A30/L14(I)	TAGTTACCC	GCCCTAGTG	cL32	
L2(III)	TAAGTGCC	TCCGGGACGGCTAACCA	GAGCCCTAGTG	4865	A27/A11(III)	TAGCTCACCT	AGCCCTAGTG	cL34	
A17(II)	ACACTGGCCTCC	A	CTAGTG	5014	B3(IV)	TAGTACTCCTCC	GAGCCCTAGTG	cL36	

Figure 2 Selected series of junctional regions of Kde rearrangements. Sequences of the junctional regions of Kde rearrangements are aligned with the known V_{κ} family germline sequences (N, any nucleotide), with the germline sequence of the J_{κ} - C_{κ} intron, and with the Kde germline sequence (double underlined). In the left column V_{κ} gene segment codes are indicated with the family number in brackets. In the right column the patient/case registration numbers are given. Lower case characters at the end of a junctional region represent P-region nucleotides. All other nucleotides at the junctional region represent N-region nucleotides. The used patient-specific oligonucleotide probes for hybridization analysis are indicated (underlined).

Table 2 Frequency of V_{κ} family usage in V_{κ} -Kde rearrangements in B-lineage leukemias

V_{κ} family (number of members) ^a	Precursor-B-ALL (66 alleles) ^b	Chronic B cell leukemias (38 alleles) ^c
$V_{\kappa}I$ (23)	23 (35%)	19 (50%)
$V_{\kappa}II$ (14)	19 (29%)	5 (13%)
$V_{\kappa}III$ (8)	17 (26%)	8 (21%)
$V_{\kappa}IV$ (1)	2 (3%)	4 (10%)
$V_{\kappa}V$ (1)	—	—
$V_{\kappa}VI$ (3)	—	1 (3%)
$V_{\kappa}VII$ (1)	5 (7%)	1 (3%)

^aNumber of V_{κ} gene segments belonging to a V_{κ} family which are potentially functional or have minor defects but still have the potential to become functional due to mutations.^{12,13}

^bThe data are based on 66 V_{κ} -Kde rearrangements in precursor-B-ALL, detected by Southern blot analysis¹¹ and confirmed by heteroduplex PCR analysis or sequencing.

^cThe data are based on 38 V_{κ} -Kde rearrangements in chronic B cell leukemias, detected by Southern blot analysis¹¹ and confirmed by heteroduplex PCR analysis or sequencing.

Heteroduplex PCR analysis

PCR products were denatured for 5 min at 94°C. After denaturation, the samples were rapidly cooled down to 4°C and incubated for at least 60 min at this temperature. Size separation of the generated homoduplexes and heteroduplexes was obtained in a 6% non-denaturing polyacrylamide gel.⁴¹

Sequencing analysis

Junctional region sequences were either obtained with the T7-sequencing kit (Pharmacia) using ³⁵S radiolabeling (46 Kde rearrangements) or by direct cycle sequencing using fluorescently labeled dideoxynucleotides (Perkin-Elmer) (63 Kde rearrangements). The sequence reactions were performed using sequence primers (Table 1) following the manufacturer's instructions. The junctional region of each Kde rearrangement was sequenced twice, starting from two independent PCR reactions and from opposite sequence directions.

To obtain single-stranded PCR products for radioactive sequencing, asymmetric PCR reactions were performed using 12 pmol of the limiting primer and 600 pmol of the opposite primer followed by two consecutive 50% ethanol extractions. Subsequently, T7-sequencing was carried out using 50 pmol of the sequence primer and products were run in a denaturing 8% polyacrylamide gel.

For direct cycle sequencing excised homoduplex bands were used that were generated by heteroduplex analysis of the initial PCR product. DNA was eluted from gel fragments by incubation at 37°C for approximately 20 h in 0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA (pH 8.0), and 0.1% SDS and precipitated using 0.1 volumes 2 M sodium acetate pH 5.6, and 2 volumes 96% ethanol. Sequence reactions were carried out with 10 pmol primer and an annealing temperature of 50°C and analyzed using the ABI 373 sequencer (Perkin-Elmer, Applied Biosystems Division).

Specificity and sensitivity of Kde rearrangements as PCR targets

To determine the specificity of junctional regions, PCR-amplified Kde rearrangements were size-separated in 1.0% agarose gels, transferred to 0.45 μm Nytran-13N nylon membranes (Schleicher and Schuell, Dassel, Germany), and UV cross-linked as described.^{16,35} Subsequently, the nylon membranes were hybridized with γ -³²P-dATP 5' end-labeled junctional region oligonucleotide probes, designed according to the obtained sequence information. Hybridizations were performed at 50°C for 2 h in a hybridization buffer containing 3 \times SSC, 1% SDS, 10% Dextran sulphate, and 10 \times Denhardt's solution.

DNA dilution experiments were performed to determine the MRD-PCR sensitivity of Kde rearrangements. DNA from precursor-B-ALL patients containing either V κ -Kde or intron RSS-Kde rearrangements were diluted into control PB-MNC DNA from healthy adult individuals. The dilution steps were down to 10⁻⁷ of patient DNA into control DNA. After PCR analysis, 5 μl of the PCR products obtained was spotted in duplicate onto nylon membranes, UV cross-linked, and hybridized with junctional region probes as described above.

Results

PCR, heteroduplex and sequencing analysis of Kde rearrangements

In all 130 selected B-lineage leukemia cases, the Southern blot data could be confirmed by PCR analysis of the two types of Kde rearrangements with the designed oligonucleotide primers (Table 1). A total of 189 Kde rearrangements were identified: 87 in 63 precursor-B-ALL and 102 in 67 chronic B cell leukemias. Heteroduplex analysis appeared to be a powerful tool in discrimination between monoallelic and biallelic rearrangements as well as between monoclonal and polyclonal rearrangements.⁴¹

A total of 109 Kde rearrangements were analyzed for their sequence: 49 Kde rearrangements in 36 precursor-B-ALL and 60 Kde rearrangements in 45 chronic B cell leukemias. A selected series of the obtained sequences is shown in Figure 2.

V κ gene segment usage in B-lineage leukemias

V κ -Kde rearrangements represented approximately 75% of Kde rearrangements in precursor-B-ALL and approximately 45% in chronic B cell leukemias.¹¹ The usage of the different V κ families determined by PCR analysis is summarized in Table 2. V κ I family members are more frequently used (approximately 50%) in V κ -Kde rearrangements in chronic B cell leukemias, whereas the V κ -Kde rearrangements in precursor-B-ALL utilized the V κ I, V κ II, and V κ III family members in comparable frequencies (35, 29 and 26%, respectively). Kde rearrangements to the V κ V and V κ VI families were rare. Interestingly, the single member V κ VII family was used in 5/66 (7%) precursor-B-ALL (Table 2).

All V κ gene segment sequences obtained by (direct) sequence analysis of V κ -Kde rearrangements, had significant homology to known germline sequences.^{12,13} When examined, proximal V κ gene segments were used in the majority of V κ -Kde rearrangements in precursor-B-ALL and chronic B cell leukemias. Privileged V κ family members in precursor-B-

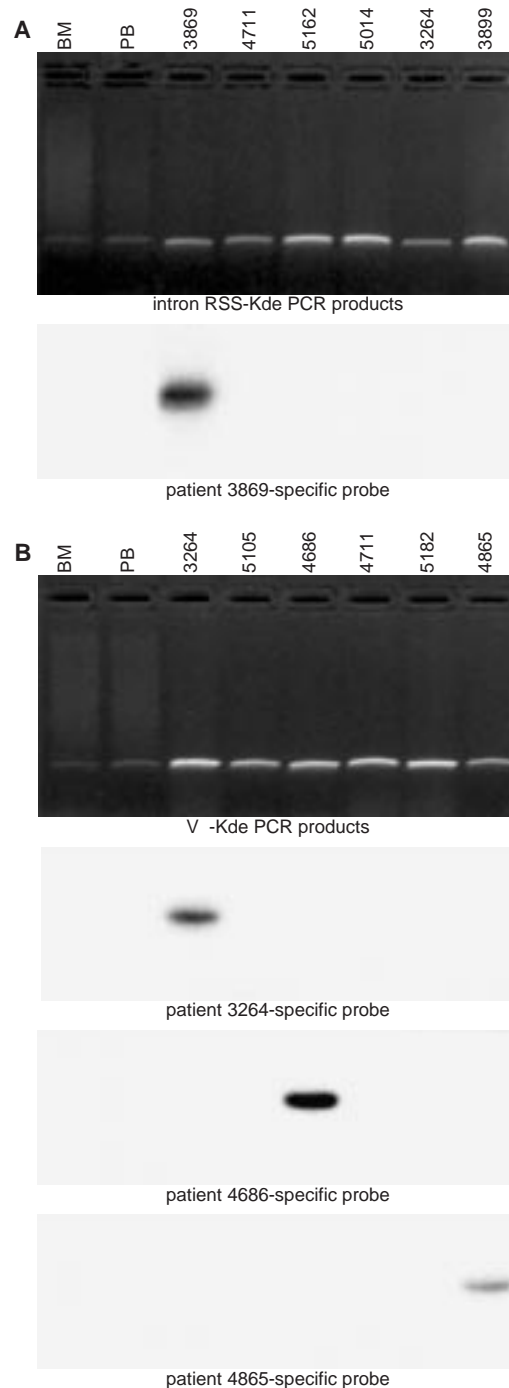


Figure 3 The diversity of the Kde rearrangement junctional regions allows patient-specific hybridization. PCR products of Kde rearrangements to either intron RSS (A) or to V κ gene segments (B) from several precursor-B-ALL patients and normal BM and PB were run in an agarose gel and subsequently blotted. The filters were successively hybridized with four patient-specific oligonucleotide probes (underlined in Figure 2), which exclusively recognized the corresponding PCR products.

ALL were L1, L9 and A30 (V κ I), A17 (V κ II), A27 (V κ III), and B1 (V κ VII). In chronic B cell leukemias no family member was used preferentially.

Table 3 Characteristics of the junctional region sequences of Kde rearrangements in B-lineage leukemias

B-lineage leukemias	V κ segments ^a				Kde rearrangements to intron RSS ^b			
	junctional nucleotides	N-region nucleotides	P-region nucleotides	deleted nucleotides	junctional nucleotides	N-region nucleotides	P-region nucleotides	deleted nucleotides
precursor-B-ALL	5.0 (0–30)	4.9 (0–30)	0.1 (0–1)	10.3 (0–49)	4.1 (0–10)	4.0 (0–10)	0.1 (0–1)	7.7 (0–33)
chronic B cell leukemias	1.3 (0–9)	1.2 (0–8)	0.1 (0–1)	4.3 (0–10)	2.2 (0–8)	2.0 (0–8)	0.2 (0–1)	6.8 (0–34)

Average number of nucleotides per rearranged allele is given. The range is given between brackets.

^aBased on 19 sequences in chronic B cell leukemias and 34 sequences in precursor-B-ALL.

^bBased on 41 sequences in chronic B cell leukemias and 15 sequences in precursor-B-ALL.

Junctional regions of Kde rearrangements

Table 3 summarizes the characteristics of the junctional region sequences of Kde rearrangements in B-lineage leukemias. The sizes of the junctional regions differed from 0 to maximally 30 nucleotides with an average of 4.7 nucleotides in the precursor-B-ALL group. In the chronic B cell leukemia group the sizes of the junctional regions differed from 0 to maximally nine nucleotides with an average of 1.9 nucleotides. In a large part of V κ -Kde rearrangements, no randomly inserted N-region nucleotides were found in chronic B cell leukemias (Figure 2), but more inserted nucleotides (mean, 2.2) and trimming of nucleotides (mean, 6.8) were found in the junctional regions of intron RSS-Kde rearrangements (Table 3).

Specificity of patient-specific oligonucleotide probes and primers

To determine the specificity of the patient-specific oligonucleotide probes, the Kde rearrangement PCR products of several patient DNA samples were size-separated, blotted and successively hybridized with four patient-specific oligonucleotide probes (Figure 3). In all cases, the patient-specific oligonucleotide probes detected the corresponding patient-derived PCR products exclusively. In addition, positive hybridization signals were not detected in either normal BM or normal PB (Figure 3).

Kde rearrangements as PCR target for MRD detection

To establish the sensitivity of MRD-PCR analysis of Kde rearrangements, DNA of 14 precursor-B-ALL patients with V κ -Kde rearrangements (10 patients) or intron RSS-Kde rearrangements (four patients) was diluted in DNA from PB-MNC of a healthy individual. PCR analysis and subsequent hybridization of the spotted PCR products with the patient-specific oligonucleotide probes revealed hybridization signals down to 10⁻³ or 10⁻⁶; three PCR targets reached sensitivities of 10⁻³, six targets reached 10⁻⁴, three targets reached 10⁻⁵, and two targets reached 10⁻⁶. No clear relationship was found between the number of deleted and inserted nucleotides and the sensitivity of the PCR targets. For example, a V κ II-Kde rearrangement with 25 deleted and two inserted nucleotides had a sensitivity of 10⁻³, whereas a V κ II-Kde rearrangement with five deleted and three inserted nucleotides had a sensitivity of 10⁻⁶ (Figure 4).

Discussion

Until recently, only a few studies about V κ gene usage in *IGK* gene rearrangements in normal and malignant (precursor) B cells were reported.^{42–46} But with the discovery of the PCR technique the analysis of antigen specific receptor gene rearrangements as well as the analysis of *IGK* gene rearrangements is moving rapidly.^{47–56} However, reports about V κ gene usage in Kde rearrangements are not available. V κ family

Table 4 V κ family usage in V κ -J κ rearrangements in normal and malignant lymphoid cells as compared to V κ family usage in V κ -Kde rearrangements in B-lineage leukemias

V κ family	V κ -J κ rearrangements (%)		V κ -Kde rearrangements (%)	
	Normal B cells ^a	Malignant lymphoid cells ^b (106 alleles)	Chronic B cell leukemias ^c (36 alleles)	Precursor-B-ALL ^c (61 alleles)
V κ I	50	58	53	38
V κ II	8	13	14	31
V κ III	25	19	22	28
V κ IV	14	10	11	3

^aOnly percentages of V κ family usage in functional V κ -J κ rearrangements are reported in the literature; data are obtained from Refs 42, 45 and 46.

^bThe malignant lymphoid cells concern precursor-B-ALL ($n = 6$), non-Hodgkin lymphoma ($n = 20$), CLL ($n = 52$), PLL ($n = 6$), HCL ($n = 5$), Waldenstrom macroglobulinemia ($n = 7$), and multiple myeloma ($n = 10$) and are obtained from Refs 53, 54 and 55.

^cData derived from this study.

usage ($V_{\kappa I-IV}$) in our Kde rearrangements was compared with V_{κ} family usage in $V_{\kappa}-J_{\kappa}$ rearrangements as reported in the literature and summarized in Table 4.^{42,45,46,54,55} This comparison revealed that V_{κ} family usage in $V_{\kappa}-J_{\kappa}$ gene rearrangements and V_{κ} -Kde rearrangements is comparable except for a higher tendency of $V_{\kappa II}$ family usage in V_{κ} -Kde gene rearrangements of precursor-B-ALL. The most frequently used V_{κ} gene segments of each V_{κ} family found in $V_{\kappa}-J_{\kappa}$ gene rearrangements are also frequently used in V_{κ} -Kde gene rearrangements.^{48-50,54,55} Distal V_{κ} gene segments are rarely used in V_{κ} -Kde gene rearrangements. Apparently, the usage of V_{κ} gene segments is not clearly dependent on the type of rearrangement ($V_{\kappa}-J_{\kappa}$ or V_{κ} -Kde), but particular V_{κ} gene segments (eg $V_{\kappa I}$ family, L1, L9, and A30; $V_{\kappa II}$ family, A17; and $V_{\kappa III}$ family, A27) are probably more prone to rearrange than other V_{κ} gene segments.

Sequence analysis of the Kde rearrangements revealed a striking difference in junctional region diversity between precursor-B-ALL and chronic B cell leukemias (Figure 2 and Table 2). Two to three times more insertion of N-region nucleotides and deletion of nucleotides was found in the junctional regions of Kde rearrangements in precursor-B-ALL as compared to chronic B cell leukemias, but trimming of nucleotides in intron RSS-Kde rearrangements was comparable. Apparently, TdT is still active in precursor-B-ALL at the time Kde rearrangements take place, whereas in chronic B cell leukemias TdT activity is decreased or absent at the time of Kde rearrangements. The latter is in line with the small junctional regions of $V_{\kappa}-J_{\kappa}$ rearrangements (average insertion: 1–2 nucleotides) in normal B-lymphocytes.^{34,47-52} This is confirmed by the finding that normal precursor-B cells rearrange $Ig\kappa$ genes at the same time as they undergo V_H to D-J rearrangements, which do not contain much N-region diversity (average <1 nucleotides).³⁴ We hypothesize that the continuous recombinase and TdT activity in precursor-B-ALL cells,⁵⁶⁻⁵⁸ cause the frequently occurring continuing gene rearrangements,²⁷⁻³³ including the high frequency of IgK gene rearrangements and deletions,¹¹ and the relatively large N-regions in Kde rearrangements (Figure 2).

Detection of MRD by use of the PCR technique is a powerful tool for monitoring leukemic cells during and after treatment, because these techniques are potentially able to detect low frequencies of leukemic cells down to 10^{-4} – 10^{-6} .²⁶ The applicability of the MRD-PCR technique is dependent on the presence of suitable targets and on the detection limit of the technique, which is influenced by the occurrence of the involved MRD-PCR target in normal cells. IgH , $TCRG$, and $TCRD$ gene rearrangements are the most commonly used MRD-PCR targets in ALL patients.²²⁻²⁶ However, these three types of MRD-PCR targets might not be stable during the disease process due to continuing rearrangements or secondary rearrangements,^{4,27-33} which will lead to false negative results. In this study, we investigated the applicability of Kde rearrangements as new MRD-PCR target in precursor-B-ALL.

Kde rearrangements occur in half of the precursor-B-ALL cases, in 28% on one allele and in 23% on both alleles.¹¹ Comparably to other Ig and TCR gene rearrangements, the Kde rearrangements also allowed the design of patient-specific junctional region probes for detection of leukemic cells. The sensitivity of this new MRD-PCR target was determined by use of dilution experiments, in which DNA of 14 precursor-B-ALL with either V_{κ} -Kde rearrangements or intron RSS-Kde rearrangements was diluted in DNA of PB-MNC from healthy individuals. These dilution experiments revealed sensitivities of $\leq 10^{-4}$ in approximately 80% (11/14) of the tested Kde

rearrangements. No clear relationship was found between the number of deleted and inserted nucleotides and the sensitivity of the PCR targets (Figure 4). Furthermore, it should be noted that Kde rearrangements are 'end-stage' rearrangements, which cannot undergo further rearrangements.^{7,10,11} Therefore, Kde rearrangements are likely to remain stable during the disease course, which is in contrast to IgH , $TCRG$ and $TCRD$ gene rearrangements in ALL.²⁷⁻³³ This implies that the chance of false-negative MRD-PCR results will probably be very low.

In conclusion, our data indicate that Kde rearrangements represent valuable patient-specific PCR targets, which can be used for detection of MRD in approximately 50% of precursor-B-ALL patients. In the meantime, this PCR target has been included in the ongoing collaborative MRD study in childhood ALL of the International BFM Study Group.

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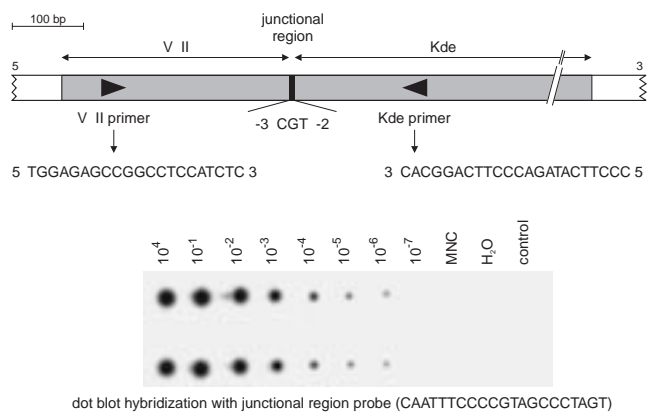


Figure 4 Dilution experiment in which DNA from a precursor-B-ALL patient with a $V_{\kappa II}$ -Kde rearrangement was diluted in PB-MNC DNA from a healthy individual. PCR products obtained after amplification with the $V_{\kappa II}$ -5' and Kde-3' primers were directly spotted in duplicate to a nylon membrane, which was hybridized with the ³²P-labeled patient-specific junctional region probe. Leukemia-derived PCR products were detectable down to the approximately 10^{-6} dilution mixture despite the short junctional region.

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