Aus der Medizinischen Klinik mit Schwerpunkt Hämatologie und Onkologie am Campus Benjamin Franklin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Intragenetische *IKZF1*-Deletionen bei Erwachsenen mit *BCR-ABL*-negativer akuter lymphatischer Leukämie (ALL)

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Benjamin Michael Kobitzsch
aus Biberach an der Riß

Datum der Promotion: 01.03.2019

Inhaltsverzeichnis

Kurzzusammenfassung (deutsch)

Hintergrund: Mutationen des Transkriptionsfaktors *IKZF1* wurden in den letzten Jahren bei Patienten mit akuten Leukämien der B-Zell-Reihe (B-ALL) nachgewiesen. Neben komplettem Genverlust und Punktmutationen unterscheidet man zwei Typen von intragenetischen Deletionen: mono-allelischer Funktionsverlust (loss-of-function) und komplette Unterdrückung der Proteinfunktion (dominant-negativ). Für die große Patientengruppe von Erwachsenen mit *BCR-ABL*-negativer B-ALL gibt es nur begrenzte Daten zur Häufigkeit und der prognostischen Bedeutung von *IKZF1*-Alterationen.

Methodik: Wir untersuchten DNA-Proben von 482 Patienten mit *BCR-ABL*-negativer B-ALL, die im Rahmen der GMALL-Studienprotokolle 06/99 und 07/03 behandelt wurden, mittels PCR auf intragenetische Deletionen (Δ2-7, Δ2-8, Δ4-7, Δ4-8). Reverse-Transkriptase-PCRs (RT-PCR) wurden durchgeführt um Δ2-3 und andere seltene Deletionen zu erkennen.
Mittels quantitativer PCRs (Δ2-7, Δ4-7, Δ4-8) und Geldensitometrie wurde die relative Konzentration der Zellen mit *IKZF1*-Deletionen bestimmt. Es wurde zwischen Deletionen in einem Großteil der Zellen (“highdel”) und Deletionen in nur einem kleinen Teil der Zellen (“lowdel”) unterschieden. Der prognostische Effekt dieser beiden Gruppen wurde separat untersucht. Alle Deletionen wurden sequenziert und die DNA-Bruchpunkte analysiert.

Ergebnisse: 128 Patienten (27%) zeigten eine intragenetische *IKZF1*-Deletion, 37 davon wiesen mehr als eine Deletion auf (175 Deletionen insgesamt). 56 Patienten (12%) hatten nur loss-of-function Deletionen, 50 (10%) hatten nur dominant-negative Deletionen, während 22 Patienten beide Deletionstypen aufwiesen (5%). Mindestens eine highdel *IKZF1*-Deletion konnte bei 98 Patienten (20%) nachgewiesen werden.
Patienten mit einer loss-of-function *IKZF1*-Deletion zeigten ein signifikant reduziertes Gesamtüberleben (overall survival (OS) nach 5 Jahren 0.37 vs. 0.59, p=0.0012), während dominant-negative Deletionen keinen Effekt auf das Gesamtüberleben hatten (0.54 vs. 0.56, p=0.95).

In der Patientengruppe mit loss-of-function Deletionen waren nur highdel-Deletionen mit einem reduzierten Gesamtüberleben assoziiert (OS 0.28 vs. 0.59, p<0.0001), während Patienten mit einer lowdel-Deletion einen klinischen Verlauf ähnlich Patienten ohne loss-of-function Deletion aufwiesen. Der Effekt der highdel loss-of-function Deletionen war auch in der Standardrisiko-Subgruppe nach GMALL-Kriterien signifikant (0.37 vs. 0.68, p=0.0002).
In der Patientengruppe mit dominant-negativen Deletionen gab es keine Assoziation zwischen dem relativen Anteil an Zellen mit Deletionen und dem Gesamtüberleben (p=0.62).
Die Sequenzierung von 193 Deletionen ergab eine Häufung der Bruchpunkte innerhalb vier großer Bruchpunkt-Cluster. Bei 183 der 193 Sequenzen waren sowohl am proximalen als auch am distalen Bruchpunkt kryptische Rekombinations-Signal-Sequenzen (cRSS) nachweisbar.

Diskussion: In der Patientengruppe der Erwachsenen mit *BCR-ABL*-negativer B-ALL sind loss-of-function *IKZF1*-Deletionen mit einem schlechteren klinischen Verlauf assoziiert, wenn sie in einem großen Anteil der leukämischen Zellen auftreten. Diese Patienten sollten engmaschig auf Rezidive überwacht werden. Die unterschiedliche biologische Funktion der loss-of-function und dominant-negativen *IKZF1*-Deletionen sollte in weiteren Studien untersucht werden.

Abstract (english)

Background: Mutations of transcription factor *IKZF1* have recently been reported in B-cell precursor acute lymphoblastic leukemia (B-ALL). Besides deletions of the whole gene and point mutations, there are two types of intragenetic deletions (loss-of-function and dominant-negative). For the large subgroup of adult patients with *BCR-ABL*-negative B-ALL, there is only limited data on the frequency and the prognostic relevance of *IKZF1* alterations.

Methods: DNA samples from 482 patients with *BCR-ABL*-negative B-ALL treated within the GMALL study protocols 06/99 and 07/03 were analyzed by PCR for intragenetic deletions (Δ2-7, Δ2-8, Δ4-7, Δ4-8). RT-PCR was conducted to detect Δ2-3 and other rare deletions.
Quantitative PCRs (Δ2-7, Δ4-7, Δ4-8) and gel densitometry were used to quantify the relative concentration of *IKZF1*-deleted cells. Deletions were considered either present in the majority of cells (“highdel”) or in a small fraction of cells only (“lowdel”) and their prognostic effect was evaluated separately. All deletions were sequenced and breakpoint sequences were analyzed.

Results: Overall, 128 patients (27%) showed an intragenetic *IKZF1* deletions, 37 of them expressing more than one deletion (175 deletions in total). Fifty-six patients (12%) carried only loss-of-function deletions, 50 (10%) had only dominant-negative deletions while 22 patients exhibited both types of deletions (5%). At least one highdel *IKZF1* deletion could be found in 98 patients (20%).
Patients carrying a loss-of-function *IKZF1* deletion showed a significantly reduced overall survival (OS at 5 years 0.37 vs. 0.59, p=0.0012) while dominant-negative deletions had no effect on OS (0.54 vs. 0.56, p=0.95).
In the group of patients with loss-of-function deletions, only highdel deletions were linked to a reduced OS (0.28 vs. 0.59, p<0.0001) while patients with lowdel deletions showed a clinical course comparable to patients without loss-of-function deletions. This effect of highdel loss-of-function deletions was also significant in a subgroup of standard-risk patients according to GMALL criteria (0.37 vs. 0.68, p=0.0002).

There was no association between the relative amount of cells with dominant-negative deletions and overall survival (p=0.62).
Sequencing of 193 breakpoints revealed four major breakpoint clusters. In 183 of 193 cases, both proximal and distal breakpoints were linked to putative cryptic recombination signal sequences.

Discussion: In adult *BCR-ABL*-negative leukemia patients, loss-of-function *IKZF1* deletions that are present in a large fraction of leukemic cells are linked with an inferior clinical outcome. These patients should be monitored closely for relapses. Consecutive research is needed to further investigate the different biological function of non-functional and dominant-negative *IKZF1* deletions.

Eidesstattliche Versicherung

„Ich, Benjamin Michael Kobitzsch, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Intragenetische *IKZF1*-Deletionen bei Erwachsenen mit *BCR-ABL*-negativer akuter lymphatischer Leukämie (ALL)“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o.) und werden von mir verantwortet.

Mein Anteil an der ausgewählten Publikation entspricht dem, der in der untenstehenden gemeinsamen Erklärung mit dem Betreuer angegeben ist.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

Ausführliche Anteilserklärung

Publikation:

Kobitzsch B, Gökbuget N, Schwartz S, Reinhardt R, Brüggemann M, Viardot A, Wäsch R, Starck M, Thiel E, Hoelzer D, and Burmeister T. Loss-of-function but not dominant-negative intragenic IKZF1 deletions are associated with an adverse prognosis in adult BCR-ABL-negative acute lymphoblastic leukemia.
Haematologica. 2017; 102:xxx. doi:10.3324/haematol.2016.161273

Anteilserklärung wie in der Publikation angegeben:

"BK performed research, designed research and analyzed data, NG is the study physician of the GMALL study and analyzed data, RR organized sequencing, SS performed immunophenotyping, MB provided relapse samples, AV, RW, MS are major patient recruiters, ET supervised immunophenotyping, DH is the GMALL study head, TB is the principal investigator, designed research and analyzed data. All authors approved and made contributions to the manuscript."

Beitrag im Einzelnen:

Studiendesign: Die Idee der Studie stammt von Herrn PD Dr. Dr. Burmeister, der alle Arbeiten auch durchgehend inhaltlich begleitete und betreute. Von Beginn der Forschung an war Herr Kobitzsch an der Entwicklung des Forschungsdesigns beteiligt und machte eigene Beiträge dazu.
Herr Kobitzsch war an der Konzeption der genomischen PCRs beteiligt und etablierte das Verfahren inklusive Kontrollprimern und PCR-Bedingungen selbständig. Die RT-PCR und die quantitativen PCRs wurden von Herrn Kobitzsch gestaltet sowie deren Bedingungen im Labor etabliert. Das Verfahren der Quantifizierung mittels Geldensitometrie wurde von ihm entwickelt, ebenso wurden die einzelnen Primer für die Sequenzierung von Herrn Kobitzsch entworfen.

Datenerhebung: Herr Kobitzsch isolierte selbständig Teile des untersuchten Materials aus leukämischen Zellen oder aus archivierten Nukleinsäuren und stellte Teile der untersuchten cDNA her. Alle PCRs und RT-PCRs (insgesamt über 3000 PCRs) sowie alle quantitativen PCRs (über 500 qPCRs) wurden von ihm durchgeführt. Er bereitete

alle DNA-Proben für die Sequenzierung auf (über 190 Sequenzen) und isolierte die sequenzierten PCR-Banden aus dem Gel, die Sequenzierung selbst wurde durch das Max Planck Genomzentrum Köln vorgenommen.

Datenauswertung: Herr Kobitzsch wertete die konventionellen PCRs, die RT-PCRs und die quantitativen PCRs aus. Ebenso erfolgten durch ihn die Quantifizierung mittels Geldensitometrie und die Analyse der DNA-Sequenzen.
Die Fragestellungen für die statistische Auswertung wurden von Herrn Kobitzsch formuliert, die Auswertung der Daten erfolgte in der GMALL-Studienzentrale in Frankfurt am Main durch Frau Dr. Gökbuget.

Manuskript: Herr Kobitzsch formulierte große Teile des Manuskripts, insbesondere die Abschnitte zu Methoden und wesentliche Teile der Ergebnisse wurden von ihm erstellt. An der Formulierung der Abschnitte „Einleitung“ und „Diskussion“ hatte er große Anteile.
Alle Grafiken sowohl im Haupttext (Figure 1-5) als auch im Supplement (Supplementary Figures 1-4) wurden von Herrn Kobitzsch erstellt, ebenso alle Tabellen (Table 1, Supplementary Tables 1-12) bis auf eine Tabelle im Supplement („Putative cryptic recombination signal sequences near breakpoints“).

Datum	Unterschrift und Stempel des betreuenden Hochschullehrers	Unterschrift des Doktoranden

Auszug aus der Journal Summary List (ISI Web of Knowledge[SM])

Journal Data Filtered By: **Selected JCR Year: 2016** Selected Editions: SCIE,SSCI
Selected Categories: **"HEMATOLOGY"** Selected Category Scheme: WoS
Gesamtanzahl: 70 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	CIRCULATION RESEARCH	49,784	13.965	0.079890
2	BLOOD	161,962	13.164	0.313600
3	LEUKEMIA	23,538	11.702	0.059800
4	HAEMATOLOGICA	15,075	7.702	0.040460
5	Lancet Haematology	571	7.123	0.002680
6	ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY	32,950	6.607	0.051690
7	Journal of Hematology & Oncology	2,879	6.350	0.007920
8	BLOOD REVIEWS	2,380	6.342	0.005310
9	TRANSFUSION MEDICINE REVIEWS	1,254	5.745	0.002760
10	BRITISH JOURNAL OF HAEMATOLOGY	23,280	5.670	0.041040
11	THROMBOSIS AND HAEMOSTASIS	17,662	5.627	0.029740
12	STEM CELLS	20,822	5.599	0.038100
13	JOURNAL OF THROMBOSIS AND HAEMOSTASIS	18,059	5.287	0.041260
14	AMERICAN JOURNAL OF HEMATOLOGY	8,776	5.275	0.021330
15	JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM	16,998	5.081	0.029520
16	CRITICAL REVIEWS IN ONCOLOGY HEMATOLOGY	6,296	4.971	0.011240
17	BIOLOGY OF BLOOD AND MARROW TRANSPLANTATION	9,904	4.704	0.025270
18	SEMINARS IN HEMATOLOGY	2,157	4.042	0.003430
19	JOURNAL OF LEUKOCYTE BIOLOGY	17,441	4.018	0.023810
20	BONE MARROW TRANSPLANTATION	11,896	3.874	0.021220
21	SEMINARS IN THROMBOSIS AND HEMOSTASIS	4,054	3.629	0.007400
22	HAEMOPHILIA	6,137	3.569	0.012260
23	STEM CELLS AND DEVELOPMENT	7,446	3.562	0.018710
24	TRANSFUSION	12,469	3.386	0.021790
25	HEMATOLOGY-ONCOLOGY CLINICS OF NORTH AMERICA	2,120	3.226	0.004840
26	CYTOTHERAPY	4,952	3.203	0.008800

Druckexemplar der Publikation

Kobitzsch B, Gökbuget N, Schwartz S, Reinhardt R, Brüggemann M, Viardot A, Wäsch R, Starck M, Thiel E, Hoelzer D, and Burmeister T. Loss-of-function but not dominant-negative intragenic IKZF1 deletions are associated with an adverse prognosis in adult BCR-ABL-negative acute lymphoblastic leukemia.
Haematologica. 2017; 102:xxx. doi:10.3324/haematol.2016.161273

http://dx.doi.org/10.3324/haematol.2016.161273

Loss-of-function but not dominant-negative intragenic *IKZF1* deletions are associated with an adverse prognosis in adult *BCR-ABL*-negative acute lymphoblastic leukemia

Benjamin Kobitzsch,[1] Nicola Gökbuget,[2] Stefan Schwartz,[1] Richard Reinhardt,[3] Monika Brüggemann,[4] Andreas Viardot,[5] Ralph Wäsch,[6] Michael Starck,[7] Eckhard Thiel,[1] Dieter Hoelzer[2] and Thomas Burmeister[1]

[1]Department of Hematology, Oncology and Tumor Immunology, Charité Universitätsmedizin Berlin, Berlin; [2]Department of Medicine II, Hematology/Oncology, Goethe University, Frankfurt/Main; [3]Max Planck Genome Center, Köln; [4]Department of Hematology, University Hospital Schleswig-Holstein, Kiel; [5]Department of Medicine III (Hematology, Oncology), Ulm University, Ulm; [6]Department of Hematology, Oncology and Stem Cell Transplantation, University of Freiburg Medical Center, Freiburg and [7]Department of Hematology, Klinikum München-Schwabing, Munich, Germany

Haematologica 2017
Volume 102(10):xxxx-xxxx

Correspondence:
thomas.burmeister@charite.de

Received: January 12, 2017.
Accepted: July 18, 2017.
Pre-published: July 27, 2017.

doi:10.3324/haematol.2016.161273

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/102/10/xxx

ABSTRACT

Genetic alterations of the transcription factor IKZF1 ("IKAROS") are detected in around 15-30% of cases of *BCR-ABL*-negative B-cell precursor acute lymphoblastic leukemia. Different types of intragenic deletions have been observed, resulting in a functionally inactivated allele ("loss-of-function") or in "dominant-negative" isoforms. The prognostic impact of these alterations especially in adult acute lymphoblastic leukemia is not well defined. We analyzed 482 well-characterized cases of adult *BCR-ABL*-negative B-precursor acute lymphoblastic leukemia uniformly treated in the framework of the GMALL studies and detected *IKZF1* alterations in 128 cases (27%). In 20%, the *IKZF1* alteration was present in a large fraction of leukemic cells ("high deletion load") while in 7% it was detected only in small subclones ("low deletion load"). Some patients showed more than one *IKZF1* alteration (8%). Patients exhibiting a loss-of-function isoform with high deletion load had a shorter overall survival (OS at 5 years 28% *vs.* 59%; $P<0.0001$), also significant in a subgroup analysis of standard risk patients according to GMALL classification (OS at 5 years 37% *vs.* 68%; $P=0.0002$). Low deletion load or dominant-negative *IKZF1* alterations had no prognostic impact. The results thus suggest that there is a clear distinction between loss-of-function and dominant-negative *IKZF1* deletions. Affected patients should thus be monitored for minimal residual disease carefully to detect incipient relapses at an early stage and they are potential candidates for alternative or intensified treatment regimes. (*clinicaltrials.gov identifiers: 00199056 and 00198991*).

Introduction

IKAROS family transcription factors have been identified as key players in lymphopoiesis.[1-5] Alterations of *IKZF1* in acute lymphoblastic leukemia (ALL) were first described in isolated cases in the early 1990s[6,7] but it took several years to recognize the important role of *IKZF1* in ALL development.[8,9] The crucial role of IKZF1 in ALL development has also recently been underlined by the finding that certain non-coding single nucleotide polymorphisms in *IKZF1* predispose to B lineage ALL development in later life.[10-12]

The first larger studies on the incidence and role of *IKZF1* alterations in ALL were exclusively conducted on pediatric patients and revealed a prevalence of 15-30% of *IKZF1* alterations in *BCR-ABL*-negative ALL[3,9] compared with a particularly large

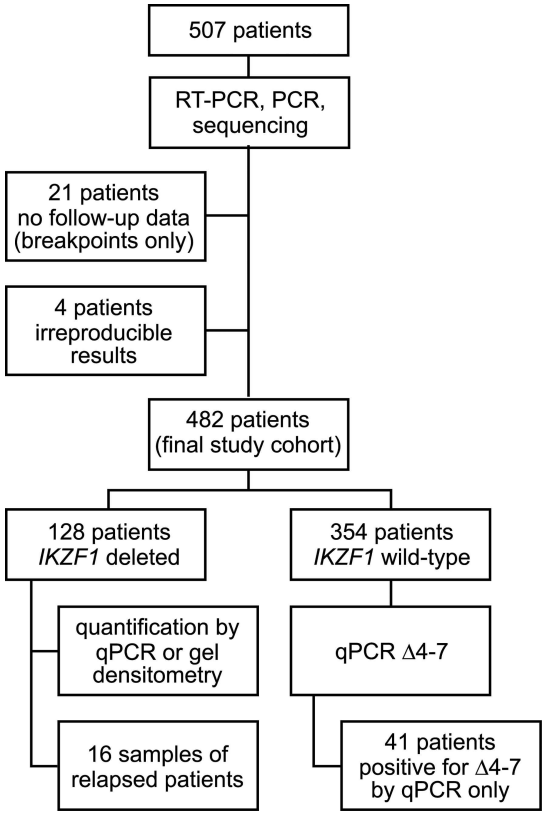


Figure 1. Flowchart of the analysis.

fraction in *BCR-ABL*-positive ALL (more than 60%).[8,13] *IKZF1*-altered *BCR-ABL*-negative pediatric ALL patients were reported to have an adverse prognosis[9,14-17] although this is still a subject of dispute.[18] The negative prognostic effect was even found within *BCR-ABL*-positive pediatric[19] and adult[13,20] patients.

In adult *BCR-ABL*-negative ALL patients, studies suggested a worse outcome for *IKZF1*-mutated patients, albeit there have been inconsistent results concerning the prognostic impact of different *IKZF1* alterations (*Online Supplementary Table S1*).[21-24] Furthermore, to the best of our knowledge, the effect of multiple *IKZF1* alterations or the impact of mutation load[25,26] has not been systematically studied in this population.

The *IKZF1* gene comprises eight exons, of which the first is non-coding. Its gene product is a 519 amino acid protein with six zinc finger domains.[4] The two carboxy-terminal zinc fingers (exon 8) are responsible for dimerization with other IKAROS family members.[27] The four amino-terminal zinc fingers (exons 4-6) mediate DNA binding. Besides point mutations and the loss of the complete *IKZF1* gene, various intragenic types of deletions have been experimentally observed. Loss of two or more amino-terminal zinc fingers encoded by exons 4-6 with deletion of the binding domain but retention of the dimerization domain results in dominant-negative isoforms, i.e. an isoform able to suppress the function of wild-type protein.[27] Loss of exon 2 with the ATG start codon abolishes gene transcription at all and loss of exon 8 removes the dimerization domain. The latter two have historically been called "haploinsufficient".[3] Since this term implies that the other allele is still functional, which could only be proven with certainty by single cell analysis, we will use the term "loss-of-function" for these alterations.

In this study, we present an in-depth analysis of 482 *BCR-ABL*-negative patients with B-precursor ALL with regard to their *IKZF1* status. Patients were treated uniformly in the framework of the German Multicenter ALL (GMALL) studies between 1999 and 2009. We present a detailed genetic analysis and an assessment of the prognostic impact of the various *IKZF1* alterations.

Methods

Patients' samples

Originally, 507 patients with *BCR-ABL*-negative B-cell precursor (BCP) ALL were studied (Figure 1). Four were excluded because of irreproducible results, and 21 for missing follow-up data (of these only breakpoint sequences are presented).

Of the remaining 482 patients who were treated within the GMALL protocols 06/99 (n=84; *clinicaltrials.gov identifier: 00199056*) or 07/03 (n=398; *clinicaltrials.gov identifier: 00198991*), we analyzed bone marrow (n=330) or peripheral blood with peripheral blasts (n=132; bone marrow or peripheral blood not specified in n=20) obtained at the time of diagnosis between 1999 and 2009 (for blast count see *Online Supplementary Tables S2* and *S3*). Matched samples from the time of relapse were available for 16 out of 482 patients

GMALL studies

Detailed information on treatment has been published previously.[28] The GMALL studies were approved by the ethics committee of the University of Frankfurt, Germany, and by local ethics committees of participating institutions, and were conducted according to the Declaration of Helsinki.

Immunophenotyping and molecular genetic analysis

At the time of diagnosis, immunophenotyping and molecular genetic analysis were performed at the GMALL central laboratory in Berlin, Germany. For all BCP-ALL patients, *BCR-ABL* status was determined by RT-PCR. Other molecular targets (*TCF3-PBX1, ETV6-RUNX1* and *MLL* fusion genes) were analyzed according to our diagnostic guidelines as outlined previously.[29,30]

Genomic PCR for Δ4-7, Δ2-7, Δ4-8, Δ2-8

For all patients, genomic PCR was performed using HotStarTaq Polymerase Mastermix (QIAGEN) with 40-200 ng DNA and 500 nM of each primer under the following conditions: 15 minutes (min) at 95°C, followed by 35 cycles of 30 seconds (sec) at 94°C, 30 sec at 65°C and 60 sec at 72°C. Primers were located in intron 1 (F2A ACTACAGAGACTTCAGCTCTATTCCATTTC, F2B TGATTTGGATGTGTGTGTTTCATGCGTGG), intron 3 (F4 CTTAGAAGTCTGGAGTCTGTGAAGGTC), intron 7 (R7 AGGGACTCTCTAGACAAAATGGCAGGA) and 3'UTR of IKZF1 (R8 CCTCCTGCTATTGCACGTCTCGGT). For primer combinations see *Online Supplementary Table S4*. In all PCRs, a fragment of intron 7 or 3'UTR was amplified as internal control with primer concentration of 100 nM (F7 ACCATCAAATACAGGTCAACAGGACTGA, product 1,257 bp) or 50 nM (F8 CCCACTGCACAGATGAACAGAGCA, product 1,229 bp). Primers were manufactured by metabion (Munich, Germany) or TIB Molbiol (Berlin, Germany) and HPLC-purified.

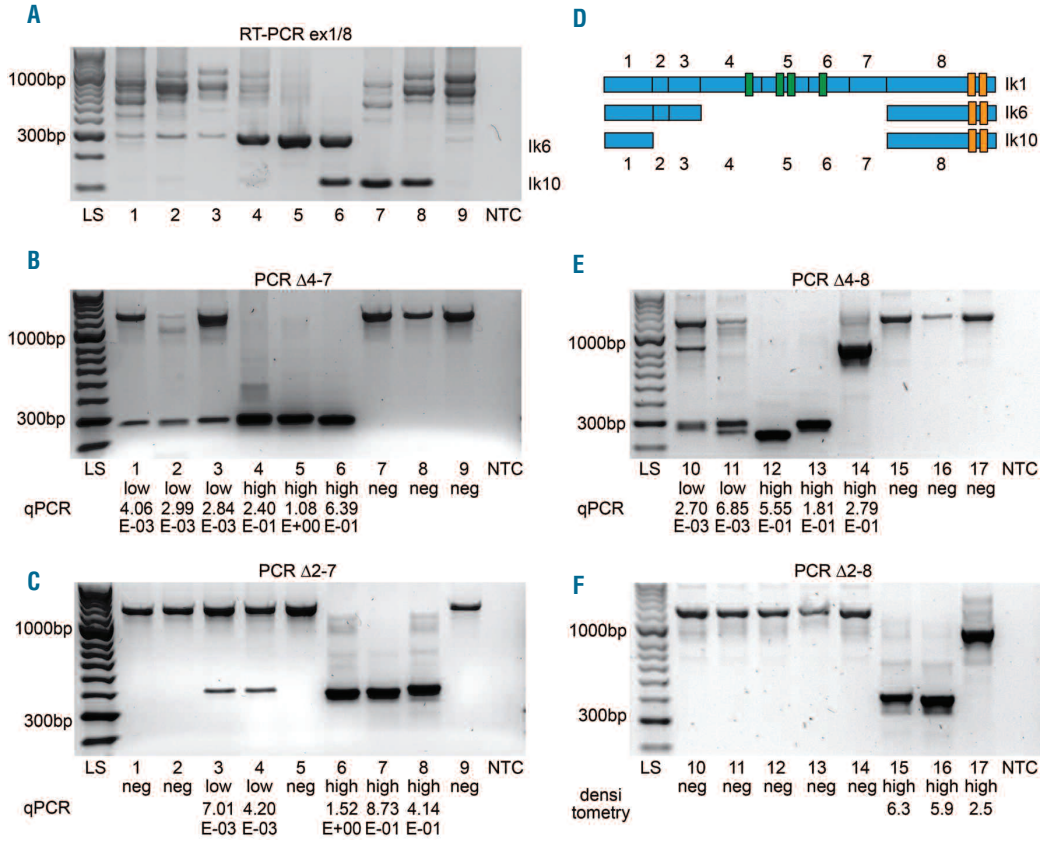


Figure 2. Detection of *IKZF1* deletions by RT-PCR and PCR screening. (A-C) RT-PCR ex1/8, PCR Δ4-7 and PCR Δ2-7 of the same 9 patients. (A) RT-PCR with primers in exon 1/8. Increased Ik6 expression in lanes 4-6 and increased Ik10 expression in lanes 6-8. Reduced full length isoform expression in lanes 1 and 7 is attributed to an additional deletion Δ2-3 in these 2 patients detected by another RT-PCR (see *Online Supplementary Figure S2*). (B) PCR Δ4-7. In lanes 1-3, Δ4-7 is present with a low deletion load; in lanes 4-6, the deletion is present with a high deletion load. Corresponding qPCR results are given below. Control band of 1257bp. (C) PCR Δ2-7 with low deletion load in lanes 3-4 and high deletion load in lanes 6-8. Control band of 1257bp. (D) Structure of the *IKZF1* transcript isoforms Ik1 (full-length), Ik6 (loss of exons 4-7) and Ik10 (loss of exons 2-7). (E-F) PCR Δ4-8 and PCR Δ2-8 of the identical patients in lanes 10-17. Control band of 1229 bp. (E) PCR Δ4-8. See double bands in lanes 10 and 11. (F) PCR Δ2-8. See variant breakpoint in lane 17.

Reverse transcriptase PCR

RT-PCR was performed with 2 µl cDNA, 500 nM of each primer and the HotStarTaq Polymerase Mastermix (QIAGEN) using the following conditions: 15 min at 95°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 64°C, and 60 sec at 72°C. Primers were located in exons 1 and 8 (RT-PCR ex1/8, primers ex1FA AAAGCGCGACGCACAAATCCA and ex8R CGTTGTTGATGGCTTGGTCCATCAC) or in exon 1 and exon 4 for detection of Δ2-3 (RT-PCR ex1/4, primers ex1FB CGAGGATCAGTCTTGGCCCCAA and ex4R GAATGCCTCCAACTCCCGACAAAG). Long *IKZF1* isoforms were used as internal control. Bands of unexpected sizes were excised from the gel and sequenced.

In cases where RNA was not available for RT-PCR, we used our own and the PCR described by Meyer *et al*. as genomic screening PCR.

Quantitative PCR for Δ4-7, Δ2-7, Δ4-8

Quantitative PCR was performed in duplicates either for all patients (Δ4-7) or for patients positive in genomic PCR (Δ2-7 and Δ4-8) using a Rotorgene 6000 cycler (Corbett, Concorde, Australia), the Thermo Scientific ABsolute QPCR Mix (Life Technologies, Darmstadt, Germany) with 200-250 ng DNA per PCR and the following conditions: 15 min at 95°C, followed by 55 cycles for 15 sec at 95°C, and 60 sec at 60°C.

As DNA standard, we used the cell-line BV-173 for Δ4-7 (DSMZ, Braunschweig, Germany)[31] or patient DNA (#100 for Δ2-7, #101 for Δ4-8). A PCR for the *HCK* gene served as internal control as described earlier.[32] Oligonucleotides are given in *Online Supplementary Table S4*. Deletions were considered to be present in a large fraction of leukemic cells ("high deletion load", "high-del") when the relative PCR signal was $>10^{-1}$, otherwise they were considered having a "low deletion load" ("lowdel"). The cut-off value was chosen *a priori* since this threshold appeared to separate samples with a high and low mutation load (*Online Supplementary Figure S1*). We used MLPA (SALSA MLPA P335 ALL-IKZF1 kit, MRC Holland, Amsterdam, the Netherlands) to correlelate the cut-off values of our quantitative PCRs with MLPA deletion values. We investigated a subset of patients with qPCR signals that we expected to yield a MLPA reduction of 0.3 or more (i.e. qPCR signal of 0.6 or higher). The chosen thresholds distinguishing high-del and lowdel corresponded to 5% deleted alleles in case of Δ2-7 and Δ4-7, and 10% in Δ4-8, but the latter could equally well have been placed at 5%, since there were no samples between 5% and 10%.

In cases negative for Δ4-7 by conventional PCR but positive by qPCR, qPCR measurements were repeated and were considered

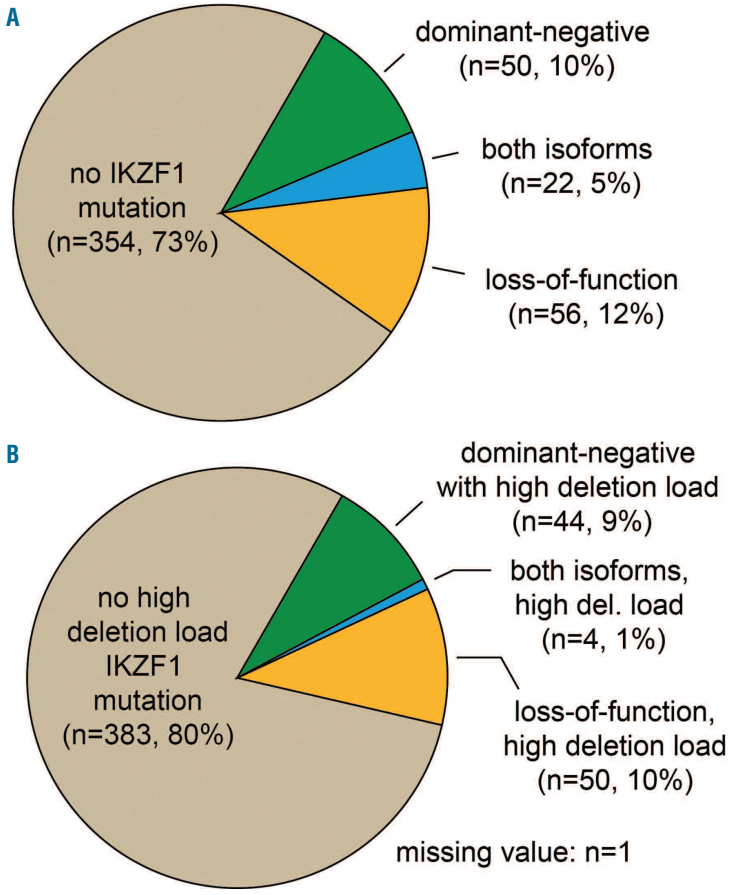


Figure 3. Prevalence of *IKZF1* deletions at the time of diagnosis. (A) Frequency of all deletions as detected by PCR (Δ2-7, Δ2-8, Δ4-7, Δ4-8) and RT-PCR (exon 1/4, exon 1/8). (B) Only deletions classified as high deletion load by quantitative PCR and densitometry.

positive when at least 3 out of 4 measurements were positive.

Gel densitometry

When no quantification by qPCR was possible (n=41), we assessed the relative amount of cells with *IKZF1* deletions (high *vs.* low deletion load) by gel band densitometry using the AlphaEaseFC v.4.0 software (Alpha Innotech, San Leandro, CA, USA). In deletions Δ2 (n=1) and Δ2-3 (n=17, missing values n=2), we compared deleted isoforms to full-length isoforms on RT-PCR images with a cut-off value of 0.60. In deletions Δ2-7 (n=5), Δ4-7 (n=3) and Δ5-7 (n=1) we compared deleted with long bands on RT-PCR images using a cut-off value of 1.20. In Δ2-8 (n=10) and Δ4-8 (n=2) we calculated the ratio of short PCR products to the long PCR control band with a cut-off value of 1.20.

Supplementary methods

Nucleic acid preparation, identification of rare genomic breakpoints (primer sequences specified in *Online Supplementary Table S5*),[33] DNA sequencing, bioinformatic analysis,[34] and statistical analysis are all described in the *Online Supplementary Methods*.

Results

Patients' characteristics

All 482 patients were aged between 16 and 65 years at diagnosis (*Online Supplementary Table S6*). The median age was 32 years [interquartile range (IQR) 22-47]. Two hundred and eighty-five patients (59%) were male. The distribution of immunophenotypes was 111 pre-B ALL (cyIg$^+$; 23%), 314 common ALL (cyIg$^-$,CD10$^+$; 65%) and 57 pro-B ALL (CD10$^-$; 12%). Two hundred and fourteen patients (44%) were considered high risk, the remaining standard risk. All patients were *BCR-ABL*-negative and a *MLL* rearrangement was detected in 44 patients (39 *MLL-AF4, 4 MLL-ENL, 1 MLL-AF9*), a *TCF3-PBX1* fusion in 30, and an *ETV6-RUNX1* fusion in 3 cases.

Frequency of *IKZF1* deletions

Two RT-PCRs were used to detect short *IKZF1* isoforms (Figure 2A and *Online Supplementary Figure S2A-C*) and four separate PCRs to detect the Δ2-7, Δ2-8, Δ4-7 and Δ4-8 isoforms (Figure 2B-F). Deletions were then quantified using quantitative PCR or gel densitometry. Dominant-negative deletions (Δ4-7, Δ5-7) were compared to loss-of-function deletions (Δ2, Δ2-3, Δ2-7, Δ2-8, Δ4-8).

Overall, 128 of 482 (27%) patients carried an *IKZF1* deletion (Figure 3A). Among these patients, we detected 175 different *IKZF1* deletions. While 91 (19%) patients expressed only one deletion, in 37 (8%) patients more than one *IKZF1* deletion was detected: 2 (n=28), 3 (n=8) or 4 (n=1) deletions (*Online Supplementary Table S7*; for an example, see lanes 3, 4 and 6 in Figure 2).

Among the 175 *IKZF1* deletions, Δ4-7 was the most frequent (n=71). Δ2-7 was found in 47, Δ4-8 in 26, Δ2-3 in 19 and Δ2-8 in 10 patients. Rare deletions were Δ5-7 (n=1) and Δ2 (n=1). In summary, 56 patients (12%) carried only

Table 1. Effect of *IKZF1* deletions on overall survival.

Type of *IKZF1* deletion	Patient group	Cases pos/neg	Overall survival positive	Overall survival negative	*P*
Any mutation	all patients	128/354	0.46±0.05	0.59±0.03	ns (0.06)
Loss-of-function	all patients	78/404	0.37±0.06	0.59±0.02	0.0012
Dominant-negative	all patients	72/410	0.54±0.06	0.56±0.02	ns (0.95)
High deletion load loss of function	all patients	54/427	0.28±0.06	0.59±0.02	<0.0001
	SR	24/243	0.37±0.10	0.68±0.03	0.0002
	HR	30/184	0.26±0.08	0.46±0.04	ns (0.06)

ns: not significant; SR: standard risk according to GMALL; HR: high risk according to GMALL.

loss-of-function deletions, 50 (10%) had only dominant-negative deletions while 22 patients exhibited both types of deletions (5%).

We then quantified the amount of cells with *IKZF1* deletions, as a variable deletion load was apparent from gel images (Figure 2B and C). We avoided the simple terminology "clonal" and "subclonal" since we did not prove clonality in a strict sense and did not investigate clonal relationships. Instead, we adopted the terms "high deletion load" (highdel) and "low deletion load" (lowdel) for *IKZF1* aberrations present either in the vast majority of leukemic cells or only in a small fraction.

Out of 173 quantifiable deletions (n=2 not quantified), 106 (61%) were considered to have a high deletion load. At least one highdel *IKZF1* deletion could be found in 98 of 482 (20%) patients (Figure 3B). Among these, 50 had a highdel loss-of-function deletion only, 44 patients had a highdel dominant-negative deletion only, and there was a group of 4 patients expressing both deletions with a high deletion load level.

qPCR screening revealed 50 additional cases positive for Δ4-7 with a low deletion load not detectable by our conventional PCR. In 41 of these cases, the lowdel Δ4-7 was the only *IKZF1* deletion, while in 9 cases a loss-of-function deletion had been detected by conventional PCR. Patients with a lowdel Δ4-7 detected by qPCR only were considered *IKZF1* wild-type.

Prognostic impact of *IKZF1* deletions

Four hundred and twenty-eight (89%) patients reached a complete remission, 31 patients (6%) died during induction, and 23 patients (5%) had a treatment failure after induction. The overall survival was 55% at five years.

We first calculated the effect of any *IKZF1* deletion (n=128 *vs.* wild-type n=354) and then analyzed loss-of-function (n=78 *vs.* negative n=404) and dominant-negative deletions (n=72 *vs.* negative n=410) separately. We compared the effect of high to low deletion load and no deletion in the group of loss-of-function (n=54/23/404, missing value n=1) and dominant-negative deletions (n=48/24/410).

There was a non-significant trend towards inferior overall survival (OS) for patients with any *IKZF1* deletion (0.46 *vs.* 0.59; *P*=0.06) (*Online Supplementary Figure S3A*). Patients carrying a loss-of-function *IKZF1* deletion had a reduced OS (0.37 *vs.* 0.59; *P*=0.0012) (Figure 4A) while dominant-negative deletions had no effect on OS (0.54 *vs.* 0.56; *P*=0.95) (Figure 4B). Patients with both dominant-negative and loss-of-function deletions showed a clinical course comparable to loss-of-function deletions only (*Online Supplementary Figure S3B*). Analysis of the amount of *IKZF1*-deleted cells showed that the inferior survival in loss-of-function deletions was an effect of highdel loss-of-function deletions only (Figure 4C). Lowdel loss-of-function deletions did not influence the clinical course. In dominant-negative deletions, OS was not associated with the relative amount of *IKZF1*-deleted cells (Figure 4D).

Patients with highdel loss-of-function deletions showed a reduced OS (0.28 *vs.* 0.59; *P*<0.0001) (Table 1). In subgroups according to risk stratification, highdel loss-of-function *IKZF1* deletions conferred a negative prognostic effect on standard-risk patients (0.37 *vs.* 0.68; *P*=0.0002), while in high-risk patients, the trend towards inferior OS narrowly missed statistical significance (0.26 *vs.* 0.46; *P*=0.06).

Clinico-biological characteristics of patients with *IKZF1* deletions

Patients with *IKZF1* deletion showed a common immunophenotype significantly more often than patients without *IKZF1* deletions (98 in 128, 77%, *vs.* 216 in 354, 61%; *P*=0.0064). The former were also significantly more likely to be CD34-positive (112 in 127, 88%, *vs.* 209 of 353, 59%; *P*<0.0001; n=2 CD34 N/A). The occurence of *IKZF1* deletions was not associated with patients' age, gender, WBC or GMALL risk group, neither for all deletions (*Online Supplementary Table S8*) nor for different types of deletion (*Online Supplementary Table S9*).

TCF3-PBX1 and *IKZF1* deletions were mutually exclusive (0 of 30 *TCF3-PBX1*$^{+}$ *vs.* 64 of 250 *TCF3-PBX1*$^{-}$; *P*=0.0004). One in 3 *ETV6-RUNX1*-positive patients showed an *IKZF1* deletion. There was a trend towards a lower frequency of *IKZF1* deletions in *MLL*-positive patients (7 of 44 MLL+, 16% *vs.* 7 of 26 MLL-, 26%; *P*=0.3556).

Oligoclonality is more common in loss-of-function deletions

Some patients showed more than one *IKZF1* deletion (e.g. Δ2-7 and Δ4-7). Forty out of 175 deletions (23%) showed more than one chromosomal breakpoint resulting in the same type of RNA transcript. This oligoclonality may arise from multiple alterations in a single hyperdipoid clone or from alterations in different clones. This was evident either by gel electrophoresis (9 patients; see lanes 9-10 in Figure 2E and F) or by multiple sequences in chromatograms (2 breakpoints in 5 patients, Figure 5A; more than two breakpoints in 26 patients, Figure 5B). This kind of oligoclonal pattern occurred more often in loss-of-function deletions (31 of 103 deletions, 30%) compared with dominant-negative (9 of 72, 13%; *P*=0.0064).

Breakpoint sequences

Sequencing of 193 breakpoints revealed four clusters

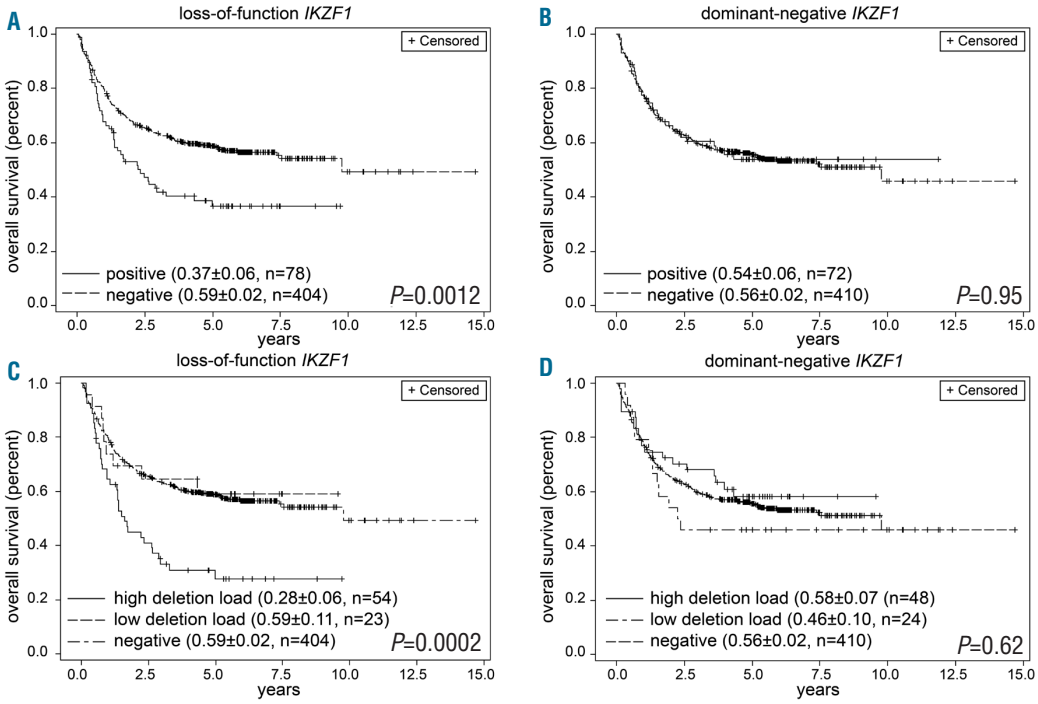


Figure 4. Overall survival (OS) depending on *IKZF1* deletions. (A) OS of patients with loss-of-function *IKZF1* deletions. (B) OS of patients with dominant-negative deletions. (C) OS of patients with high or low deletion load loss-of-function *IKZF1* deletions. (D) OS of patients with high or low deletion load dominant-negative *IKZF1* deletions.

(Figure 5C; for all breakpoints see *Online Supplementary Table S10*). In intron 1, 66 of 83 were located within 30bp. In intron 3, 106 of 108 proximal breakpoints were located within 40bp. All 132 distal breakpoints in intron 7 clustered within 43bp. Thirty-six of 42 breakpoints in the 3'UTR region were located in a 27bp region, and an additional 5 breakpoints clustered around 500bp proximally.

The remaining 17 breakpoints in intron 1 were more diverse, covering a region of 7kb. Distal (3') breakpoints in intron 3 (Δ2-3) were scattered all over the 40kb intron. In 183 of 193 (95%) molecularly characterized breakpoints, putative cryptic recombination signal sequences, either with 23bp or 12bp spacer, were identified at both breakpoint sites (5' and 3'). This was the case for the four major breakpoint clusters (Figure 5 and *Online Supplementary Table S11*) but also true for the majority of the atypical breakpoints in intron 1 and 3. In 10 of 25 atypical breakpoints, only one cRSS could be identified (8 only on the 3' site, 2 only on the 5' site) (*Online Supplementary Table S11*). There was no evidence of somatic hypermutation near the break sites.

Detection of deletions by RT-PCR

In 13 of 17 patients positive for Δ2-3 in RT-PCR ex1/4, a genomic breakpoint could be identified by Meyer's PCR (*Online Supplementary Figure S2A*).[33] In the remaining 4 patients, breakpoints were identified by a newly developed PCR (*Online Supplementary Figure S2B*). We also identified Δ2 once by RT-PCR ex1/4 and confirmed the genomic deletion. One patient expressed isoform Δ2-4 in RT-PCR ex1/8 but we could only find a deletion Δ2-3 on the genomic level and no deletion Δ2-4 or Δ4.

RT-PCR revealed 3 patients positive for Ik10 (lacking exons 2-7) but negative for Δ2-7 by genomic PCR due to a more proximal 5' breakpoint (*Online Supplementary Figure S4A*). In all 70 cases of RT-PCR positive for Ik6 (lacking exons 4-7) and negative for Ik6Δ (lacking exons 4-7 but with an additional 60 bp cryptic exon 3b),[7,35] genomic PCR was positive for deletion Δ4-7. In one patient with Ik6 and Ik6Δ we found two deletions Δ4-7, one with common breakpoints, one with a 5' breakpoint distal to the 60bp insert (*Online Supplementary Figure S4B*). The second patient with Ik6/Ik6Δ showed only a deletion Δ5-7 that was supposedly the reason for overexpression of Ik6 and Ik6Δ (*Online Supplementary Figure S4C*).

Comparison between diagnosis and relapse

DNA at the time of relapse was available from 16 patients carrying 20 *IKZF1* deletions. Four in 7 (57%) Δ4-7 and 9 in 13 (69%) loss-of-function deletions were conserved (P=0.65) (*Online Supplementary Table S12*). Eleven in 15 (73%) highdel and 1 in 4 lowdel deletions were conserved (P=0.12; 1 deletion not quantified). All genomic breakpoints were identical at the time of diagnosis and relapse. No newly acquired deletion Δ2-7, Δ2-7, Δ4-7 or Δ4-8 could be detected in relapse samples. We also investigated 5 relapse samples from patients who had shown a lowdel Δ4-7 *IKZF1* deletion at diagnosis, detectable only by quantitative PCR. None of these cases evolved into a major clone, i.e. with high deletion load at relapse.

Discussion

IKZF1 alterations have been recognized as recurrent aberrations in B precursor ALL but their prognostic impact

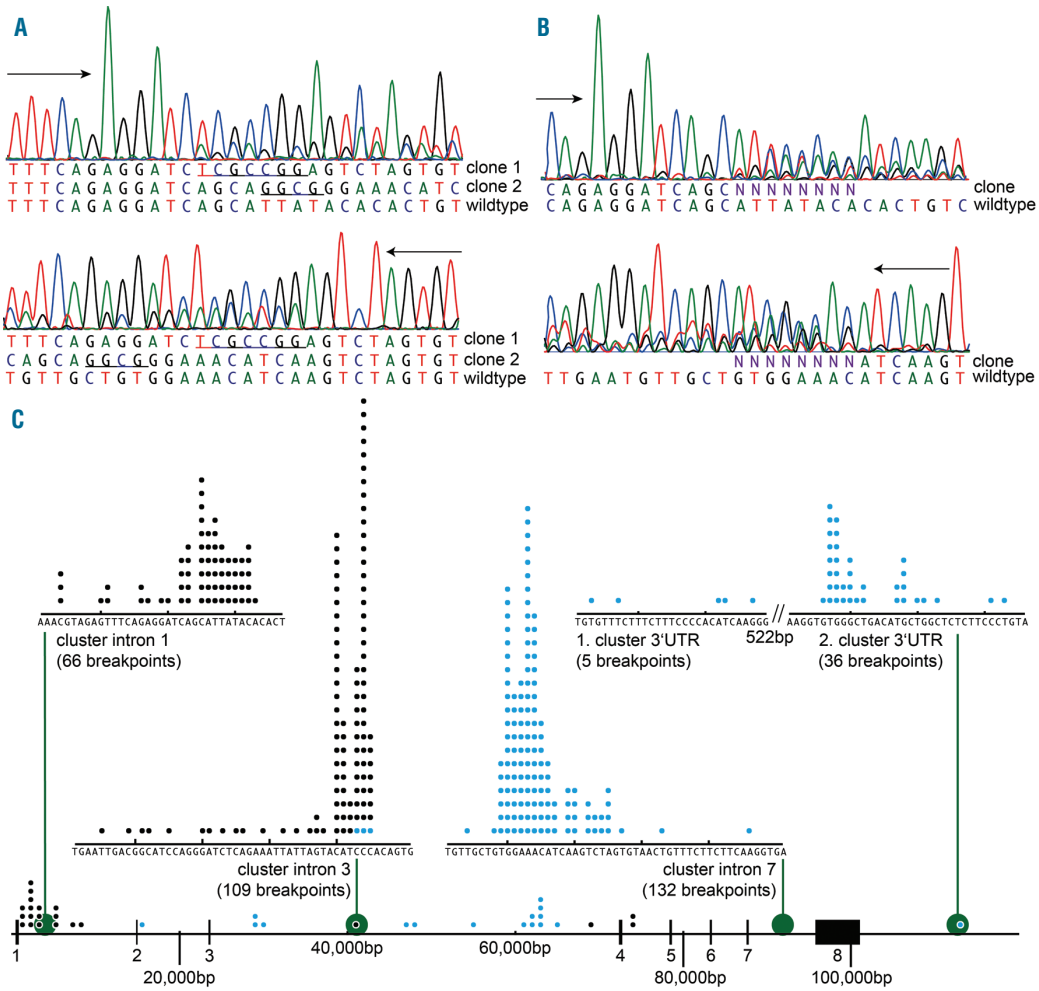


Figure 5. Distribution of *IKZF1* breakpoints and clonality of deletions. (A) Chromatogram of patient #189 showing two distinguishable clones (sequenced sense and antisense reverse complement). (B) Chromatogram of patient #395 showing oligoclonality at the breakpoint junction in both sequencing directions. (C) Distribution of breakpoints in the *IKZF1* gene locus. Proximal breakpoints are shown in black, distal breakpoints in blue. There are four major breakpoint clusters within intron 1, 3, 7 and 3'UTR of *IKZF1*.

in adult ALL is still not well defined. Two major studies involving more than 200 patients have focused on the prognostic impact in *BCR-ABL*-negative adult BCP ALL.

Moorman *et al.*[21] investigated 304 patients and found *IKZF1* deleted patients (29%) to have a lower OS, but this was only seen in a univariate analysis. The authors stated cautiously that "there was evidence to suggest that the poor outcome was not linked to the expression of the IK6 isoform but rather to other types of *IKZF1* deletions".[21] Beldjord *et al.*[22] investigated 216 younger adults and observed a significantly higher cumulative incidence of relapse in patients with focal *IKZF1* alterations (25%) but not with whole gene deletion. No statistically significant difference between patients with different focal alterations was observed.

Our present study included 482 homogenously treated patients and revealed *IKZF1* alterations in 128 cases. The incidence of focal deletions (27%) was comparable to both studies mentioned above. Our study is the first to systematically address the issue of *IKZF1* mutation load and its implications for prognosis on a larger scale. This is of diagnostic interest if *IKZF1* alterations are to be used as molecular markers for risk stratification and/or for detecting minimal residual disease.[15,26] Ninety-eight patients revealed a high deletion load *IKZF1* aberration while 29 patients showed low deletion load *IKZF1* alterations only (n=1 not quantified). Regarding clinical implications, only high deletion load loss-of-function *IKZF1* alterations were of prognostic relevance and conferred an adverse prognosis while low deletion load *IKZF1* alterations or dominant-negative *IKZF1* alterations did not have a prognostic effect.

In animal studies, double *IKZF1* knock-out mice show a total absence of B cells.[36] Mice with only *IKZF1* deletions did not develop BCP ALL, but haploinsufficiency of *IKZF1* in *BCR-ABL*-transgenic mice significantly accelerated the development of BCP ALL.[37] Current evidence suggests that *IKZF1* alterations alone are not sufficient to cause leukemia in humans but are an important co-factor or secondary event in the development and acceleration of ALL

disease.

It may seem unexpected that the loss of one *IKZF1* allele without apparent functional alteration of the other allele should have such a significant prognostic effect. However, this is supported by the above mentioned mouse model of Virely *et al*.[37] The observation that loss-of-function *IKZF1* deletions frequently occur in a small fraction of cells, but only seem to have an impact on prognosis if they are found in a large fraction, requires some explanation. A hypothetical explanation is the assumption that RAG-mediated *IKZF1* deletions occur sporadically during all stages of B-cell maturation because of the ongoing process of VDJ recombination.[38,39] However, only those *IKZF1* aberrations occurring at a very early maturation stage are thought to result in a cell phenotype with the full capacity of self-renewal, i.e. a "leukemia stem cell phenotype".[40] *IKZF1* alterations occurring at later stages of B-cell maturation should result in low deletion load aberrations.

The extremely narrow clustering of breakpoints in regions comprising only a few nucleotides strongly argues in favor of a specific mechanism. The analysis of the breakpoint junctions revealed four breakpoint clusters in the vicinity of recombination signal sequences suggestive of a break mechanism involving the immunoglobulin VDJ recombination enzyme complex. RAG1 and RAG2 and other genes involved in VDJ rearrangement are not expressed at a very early stage of differentiation but only after lymphoid committment,[41] which would be in line with the assumption that *IKZF1* deletions are a later event in the path towards the malignant phenotype. The fact that cRSS could not be identified in 10 out of 193 breakpoints may be explained by limitations of the RSSsite software, since some of these breaks occurred in near vicinity, suggesting a specific mechanism.

The PCR method used in this study has the advantage that it can also detect *IKZF1* alterations in a small fraction of leukemic cells, which is not possible when using MLPA.[26] Since we analyzed the final *IKZF1* cDNA transcript, we were in principle also able to detect deletions or aberrant splice isoforms arising from alterations involving only a few nucleotides that would escape detection by MLPA. However, MLPA has the advantage of also detecting whole gene deletions that are not detectable with our PCR-based approach. As long as there are no reliable PCR-based detection methods for the former, and given the fact that low deletion load alterations are prognostically irrelevant, we consider MLPA to be a suitable detection method.

To summarize, we detected partial *IKZF1* gene deletions in approximately 27% of cases of adult *BCR-ABL*-negative adult ALL. Only high deletion load loss-of-function *IKZF1* alterations, but not dominant-negative *IKZF1* alterations, had negative prognostic implications and should thus be monitored closely, while those that were found in a small fraction of cells did not influence prognosis. We report extensive molecular data on these alterations which should help to establish suitable diagnostic methods for their detection and which shed additional light on the molecular pathogenesis.

Acknowledgments

The authors are grateful for the excellent technical work of D. Gröger, R. Lippoldt and colleagues and the members of the MPI sequencing team in Cologne. They thank all involved patients and physicians for participating in the GMALL studies. TB was supported by DFG grant BU 2453/1-1.

References

1. Georgopoulos K, Bigby M, Wang JH, et al. The Ikaros gene is required for the development of all lymphoid lineages. Cell. 1994;79(1):143-156.
2. Georgopoulos K. Haematopoietic cell-fate decisions, chromatin regulation and ikaros. Nat Rev Immunol. 2002;2(3):162-174.
3. Kastner P, Dupuis A, Gaub MP, Herbrecht R, Lutz P, Chan S. Function of Ikaros as a tumor suppressor in B cell acute lymphoblastic leukemia. Am J Blood Res. 2013;3(1):1-13.
4. Olsson L, Johansson B. Ikaros and leukaemia. Br J Haematol. 2015;169(4):479-491.
5. John LB, Ward AC. The Ikaros gene family: Transcriptional regulators of hematopoiesis and immunity. Mol Immunol. 2011;48(9-10):1272-1278.
6. Sun L, Heerema N, Crotty L, et al. Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. Proc Natl Acad Sci USA. 1999;96(2):680-685.
7. Sun L, Crotty ML, Sensel M, et al. Expression of dominant-negative Ikaros isoforms in T-cell acute lymphoblastic leukemia. Clin Cancer Res. 1999;5(8):2112-2120.
8. Mullighan CG, Miller CB, Radtke I, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008;453(7191):110-114.
9. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470-480.
10. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. Nat Genet. 2009;41(9):1006-1010.
11. Treviño LR, Yang W, French D, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat Genet. 2009;41(9):1001-1005.
12. Burmeister T, Bartels G, Gröger D, et al. Germline variants in IKZF1, ARID5B, and CEBPE as risk factors for adult-onset acute lymphoblastic leukemia: an analysis from the GMALL study group. Haematologica. 2014;99(2):e23-5.
13. Martinelli G, Iacobucci I, Storlazzi CT, et al. IKZF1 (Ikaros) deletions in BCR-ABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. J Clin Oncol. 2009;27(31):5202-5207.
14. Kuiper RP, Waanders E, van der Velden VH, et al. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. Leukemia. 2010;24(7):1258-1264.
15. Waanders E, van der Velden VH, van der Schoot CE, et al. Integrated use of minimal residual disease classification and IKZF1 alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. Leukemia. 2011;25(2):254-258.
16. Dörge P, Meissner B, Zimmermann M, et al. IKZF1 deletion is an independent predictor of outcome in pediatric acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol. Haematologica. 2013;98(3):428-432.
17. Clappier E, Grardel N, Bakkus M, et al. IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's Leukemia Group study 58951. Leukemia. 2015;29(11):2154-2161.
18. Palmi C, Valsecchi MG, Longinotti G, et al. What is the relevance of Ikaros gene deletions as a prognostic marker in pediatric Philadelphia-negative B-cell precursor acute lymphoblastic leukemia. Haematologica. 2013;98(8):1226-1231.
19. van der Veer A, Zaliova M, Mottadelli F, et al. IKZF1 status as a prognostic feature in BCR-ABL1-positive childhood ALL. Blood. 2014;123(11):1691-1698.
20. DeBoer R, Koval G, Mulkey F, et al. Clinical impact of ABL1 kinase domain mutations and IKZF1 deletion in adults under age 60 with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL): molecular analysis of CALGB (Alliance) 10001 and 9665. Leuk Lymphoma. 2016;57(10):2298-2306.
21. Moorman AV, Schwab C, Ensor HM, et al.

IGH@ translocations, CRLF2 deregulation, and microdeletions in adolescents and adults with acute lymphoblastic leukemia. J Clin Oncol. 2012;30(25):3100-3108.
22. Beldjord K, Chevret S, Asnafi V, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood. 2014;123(24):3739-3749.
23. Mi JQ, Wang X, Yao Y, et al. Newly diagnosed acute lymphoblastic leukemia in China (II): prognosis related to genetic abnormalities in a series of 1091 cases. Leukemia. 2012;26(7):1507-1516.
24. Dhédin N, Huynh A, Maury S, et al. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. Blood. 2015; 125(16):2486-2496.
25. Dupuis A, Gaub MP, Legrain M, et al. Biclonal and biallelic deletions occur in 20% of B-ALL cases with IKZF1 mutations. Leukemia. 2013;27(2):503-507.
26. Caye A, Beldjord K, Mass-Malo K, et al. Breakpoint-specific multiplex polymerase chain reaction allows the detection of IKZF1 intragenic deletions and minimal residual disease monitoring in B-cell precursor acute lymphoblastic leukemia. Haematologica. 2013;98(4):597-601.
27. Sun L, Liu A, Georgopoulos K. Zinc finger-mediated protein interactions modulate Ikaros activity, a molecular control of lymphocyte development. EMBO J. 1996;15(19):5358-5369.
28. Brüggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood. 2006;107(3):1116-1123.
29. Burmeister T, Meyer C, Schwartz S, et al. The MLL recombinome of adult CD10-negative B-cell precursor acute lymphoblastic leukemia: results from the GMALL study group. Blood. 2009;113(17):4011-4015.
30. Burmeister T, Gökbuget N, Schwartz S, et al. Clinical features and prognostic implications of TCF3-PBX1 and ETV6-RUNX1 in adult acute lymphoblastic leukemia. Haematologica. 2010;95(2):241-246.
31. Nakayama M, Suzuki H, Yamamoto-Nagamatsu N, et al. HDAC2 controls IgM H- and L-chain gene expressions via EBF1, Pax5, Ikaros, Aiolos and E2A gene expressions. Genes Cells. 2007;12(3):359-373.
32. Burmeister T, Marschalek R, Schneider B, et al. Monitoring minimal residual disease by quantification of genomic chromosomal breakpoint sequences in acute leukemias with MLL aberrations. Leukemia. 2006;20(3):451-457.
33. Meyer C, zur Stadt U, Escherich G, et al. Refinement of IKZF1 recombination hotspots in pediatric BCP-ALL patients. Am J Blood Res. 2013;3(2):165-173.
34. Merelli I, Guffanti A, Fabbri M, et al. RSSsite: a reference database and prediction tool for the identification of cryptic Recombination Signal Sequences in human and murine genomes. Nucleic Acids Res. 2010;38 (Web Server Issue):W262-267.
35. Payne KJ, Dovat S. Ikaros and tumor suppression in acute lymphoblastic leukemia. Crit Rev Oncog. 2011;16(1-2):3-12.
36. Wang JH, Nichogiannopoulou A, Wu L, et al. Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. Immunity. 1996;5(6):537-549.
37. Virely C, Moulin S, Cobaleda C, et al. Haploinsufficiency of the IKZF1 (IKAROS) tumor suppressor gene cooperates with BCR-ABL in a transgenic model of acute lymphoblastic leukemia.[letter]. Leukemia. 2010;24(6):1200-1204.
38. Iacobucci I, Storlazzi CT, Cilloni D, et al. Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). Blood. 2009;114(10):2159-2167.
39. Yu W, Nagaoka H, Jankovic M, et al. Continued RAG expression in late stages of B cell development and no apparent re-induction after immunization. Nature. 1999;400(6745):682-687.
40. Warner JK, Wang JC, Hope KJ, Jin L, Dick JE. Concepts of human leukemic development. Oncogene. 2004;23(43):7164-7177.
41. Nagaoka H, Yu W, Nussenzweig MC. Regulation of RAG expression in developing lymphocytes. Curr Opin Immunol. 2000;12(2):187-190.

Supplementary Methods

Supplementary Tables

Supplementary Figures

Supplementary Methods

Nucleic acid preparation

DNA and RNA were prepared by TRIzol (Life Technologies, Darmstadt, Germany) or by AllPrep DNA/RNA (QIAGEN, Hilden, Germany). TRIzol DNA was purified using DNA Clean & Concentrate (Zymo Research, Freiburg, Germany). Reverse transcription was performed using between 150ng-1µg RNA, either by Ready-To-Go You-Prime First-Strand Beads (GE Healthcare Europe, Freiburg, Germany) or by Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany).

Identification of rare genomic breakpoints

To identify genomic breakpoints in patients positive for Δ2-3 in RT-PCR ex1/4 we used the multiplex PCR by Meyer et al.[1] with all 16 primers at 150 nM and the FastStart High Fidelity PCR System kit (Roche) under the following conditions: 2 min at 94°C, 10 cycles of 10 sec at 94°C, 30 sec at 64°C, 5 min at 68°C followed by 25 cycles with additional 20 sec elongation for each cycle. Cases negative in this PCR were further investigated with a different PCR Δ2-3B (forward primer by Meyer and reverse primers I3-R1A GTCCTTTGCACTGATGACTTATTCCCATG, I3-R1B CATCTGGGTTTGGATATGTTCATGCTGAC, I3-R1C CTACCCTGTAAATACCATCCCCTAGTCC, I3-R13B CACTGACAGACAAGAAGTTAGCTGAGG, with 250 nM of each primer).

In cases with atypical RT-PCR products, breakpoints were identified using primers as specified in Supplemental Methods (Tables S4-5). For Δ2 (primer concentration 150 nM) and Δ5-7 (primer concentration 300 nM) the FastStart High Fidelity PCR System (Roche) was used as described above. PCRs Δ2-7B and Δ4-7B were used with the HotStarTaq kit (QIAGEN) at 500 nM primer concentration and the following conditions: 15 min at 95°C, followed by 35 cycles of 30 sec at 94°C, 60 sec min at 65°C and 2.5 min at 72°C.

Sequencing and bioinformatic analysis

All PCR products were purified using the GenUP PCR Cleanup Kit (Biotechrabbit, Hennigsdorf, Germany). Multiple bands were excised from agarose gel and purified using the Thermoscientific GeneJET Gel Extraction Kit (Life Technologies, Darmstadt, Germany). Products were analyzed by Sanger sequencing using routine methods at the Max Planck Genome Center, Cologne, Germany. All sequences were submitted to the EMBL nucleotide sequence database (accession numbers LN875583-LN875775) and were analyzed using RSSsite for the presence of cryptic recombination signal sequences (cRSS) near the two breakpoint locations.[2]

[1] Meyer C, zur Stadt U, Escherich G, Hofmann J, Binato R, da Conceição Barbosa T et al. Refinement of IKZF1 recombination hotspots in pediatric BCP-ALL patients. Am J Blood Res 2013; 3: 165-173.

[2] Merelli I, Guffanti A, Fabbri M, Cocito A, Furia L, Grazini U et al. RSSsite: a reference database and prediction tool for the identification of cryptic Recombination Signal Sequences in human and murine genomes. Nucleic Acids Res 2010; 38 Suppl: W262-7.

Statistical analysis

Survival analyses were performed according to the Kaplan-Meier method. Overall survival was calculated from date of diagnosis until death or last follow-up. Disease free survival was calculated from date of first complete remission to relapse or death from any cause. Survival rates are given as probabilities of survival at 5 years, with a 95% confidence interval. The log-rank test was used to compare survival curves. Differences between 2 groups were compared by the two-tailed Fisher's test, differences between 3 or more groups by Pearson's chi square. For all analyses, $p \leq 0.05$ was considered statistically significant. Statistics were calculated using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and IBM SPSS Statistics v22 (IBM Germany, Ehningen, Germany).

Supplementary Tables

Supplementary Table 1: Results of previous studies on the prognostic effect of *IKZF1* deletions in BCR-ABL-negative adult patients (Abbreviations: pts = patients; CIR = cumulative incidence of relapse; EFS = event-free survival; RFS = relapse-free survival; OS = overall survival; HR = hazard ratio; n.s. = not significant)

study	pts	*IKZF1* deletion	value	statistic	results
Beldjord 2014	216	focal vs. wildtype	CIR	multivariate Cox model	**HR 2.65 (1.48-4.73), p=0.001**
	324	focal vs. wildtype		univariate Cox model	**HR 2.24 (1.39-3.62), p=0.001**
		complete vs. wildtype		univariate Cox model	HR 1.01 (0.91-1.11), n.s. (p=0.85)
		Δ4-7 vs. Δ2-7/Δ4-8 vs. other		Kaplan Meyer	n.s. (no p-value given)
Moorman 2012	304	any deletion vs. wildtype	EFS RFS OS	multivariate Cox model	1.26 (0.89-1.78), n.s. (p=0.196) 1.23 (0.78-1.93) , n.s. (p=0.375) 1.23 (0.86-1.76) , n.s. (p=0.263)
		any deletion vs. wildtype	EFS RFS OS	univariate Cox model	**1.54 (1.12-2.12), p=0.008** 1.48 (0.98-2.24), n.s. (p=0.63) **1.55 (1.11-2.16), p=0.010**
		other deletions vs. Ik6	EFS	univariate Cox model	**HR 2.17 (1.21-3.89), p=0.009**
Mi 2012	134	Ik6 vs. wildtype	RFS	Log-rank test	n.s. (p=0.114)
Dupuis 2012	113	any deletion vs. wildtype	PFS	Log-rank test	**0.004**
		haploinsufficient and null-mutations vs. wildtype	OS PFS	Log-rank test	**0.01** **0.003**

Supplementary Table 2: Blast count of all 482 patient samples, percentage by samples type

material	<50% blasts	50-75% blasts	>75% blasts	total
bone marrow	14 (4,3%)	36 (10,9%)	280 (84,8%)	330
peripheral blood	22 (16,7%)	34 (25,8%)	76 (57,5%)	132
bone marrow or peripheral blood	3 (15,0%)	4 (20%)	13 (65,0%)	20
total	39 (8,0%)	74 (15,4%)	369 (76,6%)	482

Supplementary Table 3: Blast count of 127 patient samples that were *IKZF1* deleted and where *IKZF1* deletions were quantified

material	<50% blasts	50-75% blasts	>75% blasts	total
bone marrow	2 (2,3%)	7 (7,9%)	79 (89,8%)	88
peripheral blood	2 (5,9%)	11 (32,3%)	21 (61,8%)	34
bone marrow or peripheral blood	2 (40%)	1 (20%)	2 (40%)	5
total	6 (4,7%)	19 (15,0%)	102 (80,3%)	127

Supplementary Table 4: Oligonucleotides used in experiments

Experiment	Name	Oligonucleotide sequence (5'-3')
PCR Δ2-7	IKZF1-F2A	ACTACAGAGACTTCAGCTCTATTCCATTTC
	IKZF1-F2B	TGATTTGGATGTGTGTGTTTCATGCGTGG
	IKZF1-F7	ACCATCAAATACAGGTCAACAGGACTGA
	IKZF1-R7	AGGGACTCTCTAGACAAAATGGCAGGA
PCR Δ2-8	IKZF1-F2A	ACTACAGAGACTTCAGCTCTATTCCATTTC
	IKZF1-F2B	TGATTTGGATGTGTGTGTTTCATGCGTGG
	IKZF1-F8	CCCACTGCACAGATGAACAGAGCA
	IKZF1-R8	CCTCCTGCTATTGCACGTCTCGGT
PCR Δ4-7	IKZF1-F4	CTTAGAAGTCTGGAGTCTGTGAAGGTC
	IKZF1-F7	ACCATCAAATACAGGTCAACAGGACTGA
	IKZF1-R7	AGGGACTCTCTAGACAAAATGGCAGGA
PCR Δ4-8	IKZF1-F4	CTTAGAAGTCTGGAGTCTGTGAAGGTC
	IKZF1-F8	CCCACTGCACAGATGAACAGAGCA
	IKZF1-R8	CCTCCTGCTATTGCACGTCTCGGT
PCR Δ2-3 (Meyer 2013)	IKZF1.I1.F1B	AGTTCACTTCTGTCAAGCGTCTGTTGCTCT
	IKZF1.I1.F2	TGGATGTGTGTGTTTCATGCGTGGTTAATA
	IKZF1.I1.F3	TCATGTGGACCATGGCTTTCTTGTATTTCT
	IKZF1.I1.F4	TGGCTGAAAATGGGTCCTAATTAGTGGAAA
	IKZF1.I3.R2	GATGGCACTGGCAGTCATTTCTCTATGTCT
	IKZF1.I3.R4	TCTAGGAAGGACTTGGGCACATTGAAGAAT
	IKZF1.I3.R5	CTGTTACTGCCTGCAGGATAGACTTCTGGA
	IKZF1.I3.R6	TCTCGGCACTTACACACACTCTCTTTAGGC
	IKZF1.I3.R7	GGTACCCCAACCCATCCTTATACATGACAC
	IKZF1.I3.R8	CTGGCACTTCTGTCAAAACCTCACATCTCT
	IKZF1.I3.R9	CTTCCGGGTCCAGGATCTCCATATAACAAT
	IKZF1.I3.R10	TTTCATATAAAATGCTGCGAACACCTTGGA
	IKZF1.I3.R11	TATTCTCTTTCACAGGACAGTTTCCCAGCA
	IKZF1.I3.R12	AATGTACACTGTTAGTCCCCACCTGACCAA
	IKZF1.I3.R13	TGACTGAGACATAATGGACAAGAGCCCAAT
	IKZF1.I3.R14	CAAGGACTCTATGACTCGGTACCACTTGGA
PCR Δ2-3B	IKZF1.I1.F1B	AGTTCACTTCTGTCAAGCGTCTGTTGCTCT
	IKZF1.I1.F2	TGGATGTGTGTGTTTCATGCGTGGTTAATA
	IKZF1.I1.F3	TCATGTGGACCATGGCTTTCTTGTATTTCT
	IKZF1.I1.F4	TGGCTGAAAATGGGTCCTAATTAGTGGAAA
	IKZF1-I3-R1A	GTCCTTTGCACTGATGACTTATTCCCATG
	IKZF1-I3-R1B	CATCTGGGTTTGGATATGTTCATGCTGAC
	IKZF1-I3-R1C	CTACCCTGTAAATACCATCCCCTAGTCC
	IKZF1-I3-R13B	CACTGACAGACAAGAAGTTAGCTGAGG
RT-PCR ex1/8	IKZF1-ex1FA	AAAGCGCGACGCACAAATCCA
	IKZF1-ex8R	CGTTGTTGATGGCTTGGTCCATCAC
RT-PCR ex1/4	IKZF1-ex1FB	CGAGGATCAGTCTTGGCCCCAA
	IKZF1-ex4R	GAATGCCTCCAACTCCCGACAAAG

qPCR Δ2-7	IKZF1-q27-F1	CATGTACATTTTTGATCTAGGTCTTAG
	IKZF1-q27-R1	GTTAAATAAAGAACCCTCAGGCAT
	IKZF1-q27-P1	FAM-TCAGGAATAAAATGCAAATCACCTTGAAGA-BBQ
qPCR Δ4-7	IKZF1-q47-F1	CAGCCCATAGGGTATAAATAATCTG
	IKZF1-q47-R1	TTAAATAAAGAACCCTCAGGCATTC
	IKZF1-q47-P1	FAM-AATTGACGGCATCCAGGGATCTCAG-BBQ1
qPCR Δ4-8	IKZF1-q48-F1	AAAATATTCTTAGAAGTCTGGAGTCTG
	IKZF1-q48-R1	CCAAGCATGTCTCGGCATAC
	IKZF1-q48-R2	GAAAAGCACTATTCCACGTAGAC
	IKZF1-q48-P1	Cy5-TGAAGGTCACACCCTCTGGTCTT-BBQ
hck internal control	hck-f	TATTAGCACCATCCATAGGAGGCTT
	hck-r	GTTAGGGAAAGTGGAGCGGAAG
	hck-p	HEX-TAACGCGTCCACCAAGGATGCGAA-BHQ1

Supplementary Table 5: Oligonucleotides used on single patients only

Experiment	**Patient**	**Name**	**Oligonucleotide sequence (5'-3')**
PCR Δ2	#119	IKZF1.I1.F1B	AGTTCACTTCTGTCAAGCGTCTGTTGCTCT
		IKZF1.I1.F2	TGGATGTGTGTGTTTCATGCGTGGTTAATA
		IKZF1.I1.F3	TCATGTGGACCATGGCTTTCTTGTATTTCT
		IKZF1.I1.F4	TGGCTGAAAATGGGTCCTAATTAGTGGAAA
		IKZF1-R2A	CCCCAGCTACCCTATCCTTTGAACAG
		IKZF1-R2B	CCAATGAAGAAATGTCGTACTTTCCGC
		IKZF1-R2C	CTTGCATCCCTTCATCACTGTCTTGG
PCR Δ2-7B	#85, #199, #291	IKZF1.I1.F1B	AGTTCACTTCTGTCAAGCGTCTGTTGCTCT
		IKZF1.I1.F2	TGGATGTGTGTGTTTCATGCGTGGTTAATA
		IKZF1.I1.F3	TCATGTGGACCATGGCTTTCTTGTATTTCT
		IKZF1.I1.F4	TGGCTGAAAATGGGTCCTAATTAGTGGAAA
		IKZF1-R7	AGGGACTCTCTAGACAAAATGGCAGGA
PCR Δ4-7B	#338	IKZF1-F4B	ACTCTGACTATACTCTCTCCTGGTATCACA
		IKZF1-F4C	CAAACTGTTCTGGGCCAATATCACCAC
		IKZF1-F4D	TTCCCAACCTCCTCCTTCATTAGTGG
		IKZF1-F4E	TTTGGTTCTGTTACAGCTCTCAGTGAC
		IKZF1-F4F	TGCAGCTAAGATTCCAGACCAGGTAT
		IKZF1-R7	AGGGACTCTCTAGACAAAATGGCAGGA
PCR Δ5-7	#424 (and #225)	IKZF1-F5A	GAGTGGCCTCCTGTATTGTTTCTTTCAGC
		IKZF1-F5B	GATTGTCTGTGCCTATCTAGTTCCCATCTG
		IKZF1-R7	AGGGACTCTCTAGACAAAATGGCAGGA

Supplementary Table 6: Characteristics of all patients

Sex	
Male	285 (59.1%)
Female	197 (40.9%)
Age	
15-25	172 (35.7%)
26-35	97 (20.1%)
36-45	78 (16.2%)
46-55	87 (18.0%)
56-65	48 (10.0%)
Immunophenotype	
pre B ALL	111 (23.0%)
common ALL	314 (65.2%)
pro B ALL	57 (11.8%)
Leukocyte	
≤30/nL	308 (64.8%)
>30/nL	167 (35.2%)
no data	7
Risk group	
Standard risk	268 (55.6%)
High risk	214 (44.4%)
CNS involvement	
No	372 (94.4%)
Yes	22 (5.6%)
No data	88
Clinical course	
CR	428 (88.8%)
ED	31 (6.4%)
Failure	23 (4.8%)
Total	482 (100%)

Supplementary Table 7: Characteristics of patients with multiple *IKZF1* mutations (high deletion load mutations are shown in dark blue, low deletion load mutations in light blue, unquantified mutations in grey)

patient	Δ4-7	Δ2-7	Δ4-8	Δ2-8	Δ2-3	number of mutations
#29	Δ4-7	Δ2-7				2
#36		Δ2-7			Δ2-3	2
#46			Δ4-8		Δ2-3	2
#58	Δ4-7				Δ2-3	2
#100	Δ4-7	Δ2-7				2
#126	Δ4-7	Δ2-7				2
#127	Δ4-7		Δ4-8			2
#133	Δ4-7	Δ2-7				2
#143			Δ4-8		Δ2-3	2
#154		Δ2-7	Δ4-8			2
#157		Δ2-7			Δ2-3	2
#160			Δ4-8	Δ2-8		2
#174	Δ4-7				Δ2-3	2
#189		Δ2-7	Δ4-8			2
#198	Δ4-7	Δ2-7				2
#199		Δ2-7			Δ2-3	2
#204		Δ2-7			Δ2-3	2
#210		Δ2-7	Δ4-8			2
#215		Δ2-7			Δ2-3	2
#243		Δ2-7	Δ4-8			2
#256		Δ2-7			Δ2-3	2
#257	Δ4-7				Δ2-3	2
#266		Δ2-7			Δ2-3	2
#276	Δ4-7	Δ2-7				2
#335	Δ4-7	Δ2-7				2
#360		Δ2-7	Δ4-8			2
#400	Δ4-7		Δ4-8			2
#414	Δ4-7	Δ2-7				2
#108	Δ4-7	Δ2-7	Δ4-8			3
#111	Δ4-7	Δ2-7	Δ4-8			3
#113	Δ4-7	Δ2-7			Δ2-3	3
#175	Δ4-7	Δ2-7	Δ4-8			3
#285	Δ4-7	Δ2-7			Δ2-3	3
#365	Δ4-7	Δ2-7	Δ4-8			3
#395	Δ4-7	Δ2-7	Δ4-8			3
#461	Δ4-7	Δ2-7		Δ2-8		3
#470	Δ4-7	Δ2-7	Δ4-8	Δ2-8		4

Supplementary Table 8: Characteristics of patients according to *IKZF1* status

	mutation	wild-type	P
Sex			
Male	72 (56.3%)	213 (60.2%)	0.4636 (Fisher)
Female	56 (43.7%)	141 (39.8%)	
Age			
15-25	49 (38.3%)	123 (34.7%)	0.3843 (X^2)
26-35	26 (20.3%)	71 (20.1%)	
36-45	17 (13.3%)	61 (17.2%)	
46-55	19 (14.8%)	68 (19.2%)	
56-65	17 (13.3%)	31 (8.8%)	
Immunophenotype			
pre B ALL	19 (14.8%)	92 (26.0%)	0.0064 (X^2)
common ALL	98 (76.6%)	216 (61.0%)	
pro B ALL	11 (8.6%)	46 (13.0%)	
WBC			
<30/nl	79 (62.7%)	229 (65.6%)	0.5869 (Fisher)
>30/nl	47 (37.3%)	120 (34.4%)	
Missing values	7		
Risk group			
Standard Risk	67 (52.3%)	201 (56.8%)	0.4074 (Fisher)
High Risk	61 (47.7%)	153 (43.2%)	
CNS involvement			
No	100 (94.4%)	272 (94.4%)	1.0000 (Fisher)
Yes	6 (5.6%)	16 (5.6%)	
Missing values	88		
Clinical course			
CR	114 (89.1%)	314 (88.7%)	0.9936 (X^2)
ED	8 (6.2%)	23 (6.5%)	
Failure	6 (4.7%)	17 (4.8%)	

Supplementary Table 9: Characteristics of patients according to different *IKZF1* deletion types

	dominant-negative only	both forms of deletion	loss-of-function only	wild-type	P (X^2)
Sex					
Male	30 (60.0%)	16 (72.7%)	26 (46.43%)	213 (60.2%)	0.1330
Female	20 (40.0%)	6 (27.3%)	30 (53.57%)	141 (39.8%)	
Age					
15-25	22 (44.0%)	7 (31.8%)	20 (35.7%)	123 (34.7%)	0.5485
26-35	8 (16.0%)	7 (31.8%)	11 (19.6%)	71 (20.1%)	
36-45	6 (12.0%)	2 (9.1%)	9 (16.1%)	61 (17.2%)	
46-55	6 (12.0%)	2 (9.1%)	11 (19.6%)	68 (19.2%)	
56-65	8 (16.0%)	4 (18.2%)	5 (8.9%)	31 (8.8%)	
Immunophenotype					
pre-B	6 (12,0%)	5 (22.7%)	8 (14.3%)	92 (26.0%)	0.0781
Common	39 (78,0%)	15 (68.2%)	44 (78.6%)	216 (61.0%)	
pro-B	5 (10,0%)	2 (9.1%)	4 (7.1%)	46 (13.0%)	
WBC					
<30/nl	34 (68.0%)	14 (63.6%)	31 (57.4%)	229 (65.6%)	0.6518
>30/nl	16 (32.0%)	8 (36.4%)	23 (42.6%)	120 (34.4%)	
Missing values	n=7				
Risk group					
Standard Risk	29 (58.0%)	12 (54.5%)	26 (46.4%)	201 (56.8%)	0.5252
High Risk	21 (42.0%)	10 (45.5%)	30 (53.6%)	153 (43.2%)	
CNS involvement					
No	41 (95.4%)	16 (100%)	43 (91.5%)	272 (94.4%)	0.6190
Yes	2 (4.6%)	0	4 (8.5%)	16 (5.6%)	
Missing values	n=88				
Clinical course					
CR	45 (90.0%)	20 (90.9%)	49 (87.5%)	314 (88.7%)	0.9042

Supplementary Table 10: Sequence of all breakpoints with accession numbers

del	accession number	patient	proximal breakpoint	proximal sequence	insert	distal sequence	distal breakpoint
Δ2	LN875583	#119	50.312.112	GCACAGCTCCTGACCATGCATGAAGGTCCTCTGAAATCGGTAAG		CTGAACANAAAGCCTCCAAGATGAAATTAGTTTTACTGTTAAACTTCA	50.319.280
Δ2-3	LN875584	#36	50.305.636	GGCACAGCTTTCAAATGCAGCTTCCCTCTCTCTAGGGACTGCAG	GGGGGA	CATTTGCACATGTACATACACATGTACACACGTGCACACGTGGTCACT	50.359.627
Δ2-3	LN875585	#46	50.306.999	GAAATAAATTCCATGTGCATATGCACATATGCACACAGAGCGTG	AGAAGG	TATTGGGAGTAGATTTAACCATTATGTAAATTGGATTTTTTAATTTTA	50.351.221
Δ2-3	LN875586	#58	50.307.772	AAGGGCACATGTACATTTTTGATCTAGGTCTTAGAAACGTAGAG	CCCC	GAATTCGGTGTTTCAAGGCTTACACTTTGATGCCCAAGACTGCACAAG	50.366.344
Δ2-3	LN875587	#113	50.307.794	TCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATAC	GGG	CGTCACTTTAACAGTCACTGAGCTGTGACTCTTGGGGGAAAGATTGTG	50.367.267
Δ2-3	LN875588	#118	50.305.920	TAAGGCCAGGTTCAATTTGGTTATGAGTCGAGGGGTGGGGGGGA	GGG	ACAGTGTGGTGTTCAGAGGCATAGGCTCTAGGCTCCCTGGCAGCACTG	50.332.955
Δ2-3	LN875589	#143	50.308.978	TTGCATGGATTGGAATAGCCATTGTGTTCTTCCGTCTTCCCTGC	CTTATGG	GGTGTTCAGAGGCATAGGCTCTAGGCTCCCTGGCAGCACTGAGAGATA	50.332.962
Δ2-3	LN875590	#157	50.308.981	CATGGATTGGAATAGCCATTGTGTTCTTCCGTCTTCCCTGCTGG	CTCCAAAG	GGTATTGGTGTCTCTCTTTCTCTCCCACTCTCCCCAGTGTTGGAATTG	50.369.485
Δ2-3	LN875591	#174	50.310.862	TAATTGTTACCAAGCCATTGATGCTTCTATTCTTCCCTTTGCCC	ACCCTGGG	GAGCTAATAGCTGTACCCTAAATGATCCTGGCTTTGAATTCTCTTATC	50.352.225
Δ2-3	LN875592	#199	50.306.409	TGAACTAAATGGTCATGTTTTCTTCCCCTTTTGTTTCACGGTGA	A	CCCACAGTGAATTACCACCTTACTAAAATATTCATGGGTATATACTAT	50.345.195
Δ2-3	LN875593	#204	50.306.408	TTGAACTAAATGGTCATGTTTTCTTCCCCTTTTGTTTCACGGTG	GGGG	CCACAGTGAATTACCACCTTACTAAAATATTCATGGGTATATACTATG	50.345.196
Δ2-3	LN875594	#215	50.307.794	TCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATAC	GA	AAGTGGGAAGTGTCTTGACAGAATTCGGTGTTTCAAGGCTTACACTTT	50.366.324
Δ2-3	LN875595	#256	50.307.793	ATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATA	GG	CGTCACTTTAACAGTCACTGAGCTGTGACTCTTGGGGGAAAGATTGTG	50.367.267
Δ2-3	LN875596	#257	50.307.788	TTTTGATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCA	CCCCCCCG	GTCACTGAGCTGTGACTCTTGGGGGAAAGATTGTGCGTGTGTGTGTGT	50.367.280
Δ2-3	LN875597	#266	50.305.919	TTAAGGCCAGGTTCAATTTGGTTATGAGTCGAGGGGTGGGGGGG	CCTTA	GCTATATCAGATAACATCTTGTACTAGGTTTTGGAATAGACCGGTGAG	50.334.210
Δ2-3	LN875598	#304	50.306.927	TCTCTATATATTAATGTACTTATACACACACTTC	CACC	GCTGGCTCCTAGAGTGCGAGGAGCTCTCAGTGACTGCTGTGCATACTC	50.364.747
Δ2-3	LN875599	#327	50.306.408	TTGAACTAAATGGTCATGTTTTCTTCCCCTTTTGTTTCACGGTG		CACAGTGAATTACCACCTTACTAAAATATTCATGGGTATATACTATGG	50.345.197
Δ2-3	LN875600	#351	50.307.787	TTTTTGATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	CCCTTCCAA	CACTGAGCTGTGACTCTTGGGGGAAAGATTGTGCGTGTGTGTGTGTGT	50.367.282
Δ2-3	LN875601	#443	50.308.968	TTTCTTTGTGTGCTTGCATGGATTGGAATAGCCATTGTGTTCTTCCG	CCTTCTCCC	CAAGGACAGAGTACTGCTTTCAGCCACCATTTGTCCAATGAGTGGCTG	50.373.025
Δ2-7	LN875602	#29 clone 1	50.307.794	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATAC	C	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875603	#29 clone 2	50.307.785	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCA	CCC	CAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.939
Δ2-7	LN875604	#36	50.307.792	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTAT	TCC	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ2-7	LN875605	#50	50.307.790	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATT	CCTGGGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875606	#85	50.305.725	CCACTCACAATTTCCCACTGCGCCGCAGGCAGTATATTTCAGCT	GGGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875607	#100	50.307.773	GATCTAGGTCTTAGAAACGTAGAGT	CAGAG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875608	#108	50.307.792	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTAT	TAC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875609	#111 clone 1	50.307.778	GATCTAGGTCTTAGAAACGTAGAGTTTCAG	GGG	GGT	50.395.968
Δ2-7	LN875610	#111 clone 2	50.307.791	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTA	CCCCT	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875611	#112	50.307.773	GATCTAGGTCTTAGAAACGTAGAGT	GGGGAG	GTTTCTTCTTCAAGGT	50.395.955

Δ2-7	LN875612	#113	50.307.791	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTA	NNNNNNNNNNNN	GTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.942
Δ2-7	LN875613	#126	50.307.794	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATAC	CCCTAGG	GTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.942
Δ2-7	LN875614	#130	50.307.781	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGG	CTCGAGGGG	AGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.941
Δ2-7	LN875615	#133	50.307.791	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTA	NNNNNNNNNNN	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ2-7	LN875616	#147	50.307.785	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCA	CCCCGAG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875617	#154	50.307.788	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCA	CCCCTGGGATCA	CTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.944
Δ2-7	LN875618	#157	50.307.785	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCA	GCCGT	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875619	#175	50.307.792	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTAT	TTAA	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875620	#178	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	TTCCC	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875621	#189 clone 1	50.307.784	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATC	TCGCCGG	AGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.941
Δ2-7	LN875622	#189 clone 2	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	AGGCG	GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.931
Δ2-7	LN875623	#198	50.307.788	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCA	CCT	TAGTGTAACTGTTTCTTCTTCAAGGT	50.395.945
Δ2-7	LN875624	#199	50.306.409	TGAACTAAATGGTCATGTTTTCTTCCCCTTTTGTTTCACGGTGA	NNNNNNNNNNN	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875625	#204	50.307.784	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATC	CGGGGG	CTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.944
Δ2-7	LN875626	#210	50.307.782	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGA	NNNNNNNNNNNNNNG	CTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.944
Δ2-7	LN875627	#215 clone 1	50.307.784	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATC	GATTCTC	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ2-7	LN875628	#215 clone 2	50.307.785	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCA	CCATAG	GTGTAACTGTTTCTTCTTCAAGGT	50.395.947
Δ2-7	LN875629	#217	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	CCC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875630	#221	50.307.361	ATGTTGGTTCTTGTCATATTCTAAGGGAGATTGATGTAAGTGGC	CCTAGGGGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875631	#226	50.307.766	GATCTAGGTCTTAGAAAC	TCGCGG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875632	#243	50.307.790	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATT	CTCCCC	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ2-7	LN875633	#256	50.307.793	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATA	ATCCCCAC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875634	#266	50.307.790	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATT	CCACAGGG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875635	#276	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	CCCCATN	TCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.938
Δ2-7	LN875636	#285	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	NNNNNNNNNN	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875637	#291	50.305.733	AATTTCCCACTGCGCCGCAGGCAGTATATTTCAGCTTTGAGATA	TCGGCCGGGGACG	AGTGTAACTGTTTCTTCTTCAAGGT	50.395.946
Δ2-7	LN875638	#307	50.307.788	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCA	CCCT	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ2-7	LN875639	#316	50.307.779	GATCTAGGTCTTAGAAACGTAGAGTTTCAGA	AGCATTCCTCGCGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875640	#329	50.307.788	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCA	NNNNNNNNNNNN	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875641	#335	50.307.791	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTA	A	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875642	#337	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	CTTTCTTC	TCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.938
Δ2-7	LN875643	#340	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	NNNNNNNNNNNNN	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875644	#349	50.307.790	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATT	CGCCTTT	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933

Δ2-7	LN875645	#360	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	CACGGGG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ2-7	LN875646	#365	50.307.788	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCA	CCCCAAG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875647	#395	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	NNNNNNNN	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ2-7	LN875648	#410	50.307.793	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATA		GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.931
Δ2-7	LN875649	#414	50.307.792	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTAT	NN	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875650	#432	50.307.794	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATAC	CTGAGG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ2-7	LN875651	#450	50.307.795	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATACA	GG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ2-7	LN875652	#454	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	CTCCCCC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ2-7	LN875653	#461	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	NNNNNNNNNNNNN	AGTGTAACTGTTTCTTCTTCAAGGT	50.395.946
Δ2-7	LN875654	#470 clone 1	50.307.785	GATCTAGGTCTTAGAAACGTA**T**AGTTTCAAAGGATCA	NCCTCCC	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ2-7	LN875655	#470 clone 2	50.307.766	GATCTAGGTCTTAGAAAC	CCATGG	GTGTAACTGTTTCTTCTTCAAGGT	50.395.947
Δ2-8	LN875656	#1	50.307.793	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTATA	GAA	GGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ2-8	LN875657	#12	50.307.766	GATCTAGGTCTTAGAAAC	AGGGCGCTGCGACAT	TGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.764
Δ2-8	LN875658	#99	50.307.778	GATCTAGGTCTTAGAAACGTAGAGTTTCAG	CCTCCCG	GGGTCTACGTGGAATAGTGCTTTTCCACAGAGTAGCTACTAGCCACAC	50.416.223
Δ2-8	LN875659	#104	50.307.790	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATT	CCCA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ2-8	LN875660	#160	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	CCCGGGGA	TGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.767
Δ2-8	LN875661	#458	50.307.784	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATC	CCTTCCCGGGGG	GCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.765
Δ2-8	LN875662	#461 clone 1	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	CCCTGGGG	TGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.764
Δ2-8	LN875663	#461 clone 2	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	CCTA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ2-8	LN875664	#464	50.307.789	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCAT	CATGG	GGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ2-8	LN875665	#482	50.307.787	GATCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGC	CGGGGA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ4-7	LN875666	#7	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	CCCT	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875667	#17	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	NNNNNNNNNN	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875668	#20	50.345.194	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACAT	NNNNCNAGGN	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875669	#25	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGGGG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875670	#29	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	CCCCTAG	GTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.942
Δ4-7	LN875671	#40	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	AGG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875672	#58	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC		GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875673	#80	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GAT	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936

Δ4-7	LN875674	#87	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCCTTCGTCCCGGC CCGTTTTGTGTGGG TTGAATCGTAGCCAC TATATCCACCACAGT CTGACATCGCCTGAC AATAACCACACCTGG AACTGGAGGCGGGG CTGTCAGGAGGAG CTTCCCAGGGAACA GGGAGGGTCCAGAC AGCTGAGCCAGGGG CCCCCAGGACTGGG GACGTGGGGGGCT GCTTAGGTACCAGAC ATGGCCTCCCATCGG	GTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.942
Δ4-7	LN875675	#88	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	TCTTCCC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875676	#100	50.345.186	GAATTGACGGCATCCAGGGATCTCAGAAATTAT	AGGG	TCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.938
Δ4-7	LN875677	#103	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	GGG	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875678	#108	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	GGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875679	#110	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	GAGGGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875680	#111	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	NNNN	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875681	#113	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	CCCA	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875682	#116	50.345.167	GAATTGACGGCATC	AGTTGCGGGAGC	CAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.939
Δ4-7	LN875683	#121	50.345.190	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGT	TCTCTTC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875684	#126	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC		TCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.938
Δ4-7	LN875685	#127	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC		AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ4-7	LN875686	#133	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	GACCTTCCCTAGT GCCCAGCGGGGGT GGGAAAGGT	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875687	#138	50.345.175	GAATTGACGGCATCCAGGGATC	CCTGTCCAA	GCTGTGGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.926
Δ4-7	LN875688	#142	50.345.196	GAATTGATGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	TGCGGGTGGGCC GGGA	TCGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.930
Δ4-7	LN875689	#146 clone 1	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC		ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875690	#146 clone 2	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	CC	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875691	#148	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GAGTGGG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875692	#170	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	GCC	GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.931
Δ4-7	LN875693	#174	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	G	GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.931
Δ4-7	LN875694	#175	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC		AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ4-7	LN875695	#179	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	CC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875696	#186	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GCCGCCCGCTG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875697	#197	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GAC	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875698	#198	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	AAACAGG	CAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.939

Δ4-7	LN875699	#203	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	AAAC	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875700	#205	50.345.194	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACAT	AGGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875701	#207	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGG	TCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.938
Δ4-7	LN875702	#217	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	T	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875703	#233	50.345.181	GAATTGACGGCATCCAGGGATCTCAGAA	CCCCGAACG	AGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.941
Δ4-7	LN875704	#236	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC		GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875705	#257	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	TTT	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875706	#267	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGGAGGG	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875707	#276	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	C	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875708	#285	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCTCCCCCACCAGAG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875709	#295	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCAGAGCCGGG	GTGTAACTGTTTCTTCTTCAAGGT	50.395.947
Δ4-7	LN875710	#320	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	TCGG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875711	#335	50.345.189	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAG	NNNNNNN	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ4-7	LN875712	#338 clone 1	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	TGGTTCTC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875713	#338 clone 2	50.373.284	TCCAATGCTTCCCTGCTGTTCTGCTCCCCACCTGCAAGCGCCCA	T	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875714	#342	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GA	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875715	#343	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCCC	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875716	#345	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	G	GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.931
Δ4-7	LN875717	#355	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	NNNN	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875718	#356	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	G	GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.931
Δ4-7	LN875719	#361	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGGG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875720	#362	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	GAAAGTCTGGGC	GTAACTGTTTCTTCTTCAAGGT	50.395.949
Δ4-7	LN875721	#365	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	CCCCAAGGGGG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875722	#370	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	CGGGGCG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875723	#376	50.345.188	GAATTGACGGCATCCAGGGATCTCAGAAATTATTA		AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875724	#395	50.345.192	GAATTGATGGCATCCAGGGATCTCAGAAATTATTAGTAC	NN	TAGTGTAACTGTTTCTTCTTCAAGGT	50.395.945
Δ4-7	LN875728	#400	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	TT	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ4-7	LN875729	#403	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	GGGGACGA	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875727	#405	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCGTCAGG	GTGTAACTGTTTCTTCTTCAAGGT	50.395.947
Δ4-7	LN875730	#407	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	CCCC	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933
Δ4-7	LN875731	#414	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGCT	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875732	#417	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875733	#425	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	CAG	ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ4-7	LN875734	#434	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	T	AAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.933

Δ4-7	LN875725	#437	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC		GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875735	#441	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	TTTTAAGGG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875736	#452	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GA	GAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.932
Δ4-7	LN875737	#461	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	NNNNNNNNNNNNN	AGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.941
Δ4-7	LN875726	#470	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GN	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-7	LN875738	#479	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCCGGGG	ATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.937
Δ4-7	LN875739	#483	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	TT	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875740	#500	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGAG	CATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.936
Δ4-7	LN875741	#509	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCTTG	AACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.934
Δ4-8	LN875742	#46	50.345.122	AGAAGTCTGGAGTCTGTGAAGGTCACACCCTCTGGT	GGGGGATAATCTGG	CCTGTATGCCGAGACATGCTTGGG	50.416.778
Δ4-8	LN875743	#49	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	GGA	CTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.758
Δ4-8	LN875744	#59	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	CA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ4-8	LN875745	#97	50.345.164	GAATTGACGGC	GTCG	GGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.768
Δ4-8	LN875746	#101 clone 1	50.345.157	GAAT	CCGACGGCGG	GTGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.753
Δ4-8	LN875747	#101 clone 2	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	TCCCAA	ATCAAGGGTCTACGTGGAATAGTGCTTTTCCACAGAGTAGCTACTAGC	50.416.218
Δ4-8	LN875748	#108	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	ATA	GGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.756
Δ4-8	LN875749	#111 clone 1	50.345.177	GAATTGACGGCATCCAGGGATCTC	NNNNNNNNNNN	TGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.759
Δ4-8	LN875750	#111 clone 2	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	AGGGTATTA	TGTTTCTTTCTTTCCCCACATCAAGGGTCTACGTGGAATAGTGCTTTT	50.416.199
Δ4-8	LN875751	#123	50.345.184	GAATTGACGGCATCCAGGGATCTCAGAAATT	CTCCTTGCGA	GCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.757
Δ4-8	LN875752	#127	50.345.197	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCCC	C	GGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.756
Δ4-8	LN875753	#139	50.345.161	GAATTGAC	CCAGGGA	GTTTCTGTGACTTCCAGTCCCCTCCTGCCAGTAGCAACCTACAAAACA	50.416.848
Δ4-8	LN875754	#143	50.345.178	GAATTGACGGCATCCAGGGATCTCA	CTTC	CTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.770
Δ4-8	LN875755	#154	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CC	GCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.757
Δ4-8	LN875756	#160	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	NNNNNNN	GCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.757
Δ4-8	LN875757	#175	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	NNNNNNNNNNNNNNN	TGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.759
Δ4-8	LN875758	#189	50.345.194	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACAT	NNN	GGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ4-8	LN875759	#191	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	GGAGGG	GGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ4-8	LN875760	#210	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	TCCCA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ4-8	LN875761	#243	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ4-8	LN875762	#289	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCCCAAA	GCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.757
Δ4-8	LN875763	#360 clone 1	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTA**T**TACATCC	GGGGACA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754

Δ4-8	LN875764	#360 clone 2	50.345.173	GAATTGACGGCATCCAGGGA	AGG	GCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.765
Δ4-8	LN875765	#365	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCAG	GGGCTGAC**G**TGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ4-8	LN875766	#395	50.345.196	GAATTGANGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	NN	GGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ4-8	LN875767	#400	50.345.193	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACA	N	GGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.755
Δ4-8	LN875768	#406	50.345.192	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTAC	CCCCA	TGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.754
Δ4-8	LN875769	#469	50.345.195	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATC	TCCTCCCCC CCCGCCG	TGTATGCCGAGACATGCTTGGG	50.416.780
Δ4-8	LN875770	#470 clone 1	50.345.196	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAGTACATCC	TCCAGAG	TCAAGGGTCTACGTGGAATAGTGCTTTTCCACAGAGTAGCTACTAGCC	50.416.219
Δ4-8	LN875771	#470 clone 2	50.345.172	GAATTGACGGCATCCAGGG	NNNNNNN	GCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.765
Δ4-8	LN875772	#470 clone 3	50.345.163	GAATTGACGG	CCCTCGG	GCTGGCTCTCTTCCCTGTATGCCGAGACATGCTTGGG	50.416.765
Δ4-8	LN875773	#495	50.345.189	GAATTGACGGCATCCAGGGATCTCAGAAATTATTAG	CCCCCGG	TCTTTCTTTCCCCACATCAAGGGTCTACGTGGAATAGTGCTTTTCCAC	50.416.203
Δ5-7	LN875774	#225	50.378.449	AAGGTAGGCTACCCTGTGATAGACACTTAACAGGATACTCGGGG		ACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.935
Δ5-7	LN875775	#424	50.378.410	TCCCAGCCTCGCCTTTGTAATGAGGTGGAAATTAACATGAAGGT	TTCCGGGGC	TCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGT	50.395.938

Supplementary Table 11: Putative cryptic recombination signal sequences near breakpoints

1. four major breakpoint clusters

breakpoint cluster	breakpoint region (cluster unterlined)	RSS (5'-3')	strand	type
intron 1	TCTAGGTCTTAGAAACGTAGAGTTTCAGAGGATCAGCATTAT ACACACTGTCACACACACACACACTTAAAATTCAGATGAGGA	CACTGTCACACACACACACA CTTAAAAT	+	RSS12
intron 3	TAATCTGAATTGACGGCATCCAGGGATCTCAGAAATTATTAG TACATCCCACAGTGAATTACCACCTTACTAAAATATTC	CACAGTGAATTACCACCTTAC TAAAATA	+	RSS12
intron 7	TTTTAGATTTTGCTGATGGCATTGCTTGTTGAATGTTGCTGT GGAAACATCAAGTCTAGTGTAACTGTTTCTTCTTCAAGGTGA TTTG	CACAGCAACATTCAACAAGC AATGCCATCAGCAAAATCT	-	RSS23
3'UTR	CATGTGCTTTTTCTCAAGCAGGCACACTGGTCCCTTTCAAGG TGTGGGCTGACATGCTGGCTCTCTTCCCTGTATGCCGA	CACACCTTGAAAGGGACCAG TGTGCCTGCTTGAGAAAAA	-	RSS23

2. atypical breakpoints outside clusters

nr	patient	Δel	RSS (5'-3')	strand	location	type
1	#36	Δ2-3	CAGAGTGAGGAGGAGCTGATCTGACATT	+	intron 3	RSS12
2			CACTCTGATCTTTACCATCACCAGACTC	+	intron 3	RSS12
3	#46	Δ2-3	CACCCCCACTCCCCATATTATAAAAACT	-	intron 3	RSS12
4			CACAGTAACTCTTAATTGTTTAATTCAGTTCGTGTGTTA	+	intron 3	RSS23
5	#58	Δ2-3	CACAGCCAGGACAGGAGCTGCAGCAACT	-	intron 1	RSS12
6			CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12
7			CATAGAGACACCAGAGAGAGAACAATGTTCACAGCCAGG	-	intron 1	RSS23
8	#85	Δ2-7	CAACATCCTCAAAAACAATACAATGATA	-	intron 7	RSS12
9			CACAGCAACATTCAACAAGCAATGCCATCAGCAAAATCT	-	intron 7	RSS23
10	#113	Δ2-3	CACTGAGCTGTGACTCTTGGGGGAAAGA	+	intron 3	RSS12
11			CATGCTGGGAAACTGTCCTGTGAAAGAGAATAGAAACCT	+	intron 3	RSS23
12			CACATTGGGTGGGGGAAAAATTCCTGTTTTCCCCAACCA	-	intron 3	RSS23
13			CAATGTGCTGCATTTTCTAATTTTCTATGAACACTTCCT	+	intron 3	RSS23
14			CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12
15	#118	Δ2-3	CACTGTGAGATGCAAGCTGAAATAAACC	-	intron 3	RSS12
16			CACAGTGTGGTGTTCAGAGGCATAGGCTCTAGGCTCCCT	+	intron 3	RSS23
17			CACACTCAATCATTTGTTCTGGAGTCCAGAGGGAAAATA	-	intron 3	RSS23
18	#119	Δ2	CACTGTGACTTCCGGCCCCAGGGAAGCT	-	intron 2	RSS12
19			CACAGTCATGACTGTTTGTTCATTAAGC	+	intron 2	RSS12
20			CACAGTGCTTGGTATGCTCATGGGGGAGGAATAGGGGCT	+	intron 2	RSS23
21	#143	Δ2-3	CACTGTGAGATGCAAGCTGAAATAAACC	-	intron 3	RSS12
22			CACAGTGTGGTGTTCAGAGGCATAGGCTCTAGGCTCCCT	+	intron 3	RSS23
23			CACACTCAATCATTTGTTCTGGAGTCCAGAGGGAAAATA	-	intron 3	RSS23
24			CACAGTGGGTGGCCTGAGCCCAGAGCAGCTCCCCATATC	+	intron 1	RSS23
25			CACAGGGATATGGGGAGCTGCTCTGGGCTCAGGCCACCC	-	intron 1	RSS23
26	#157	Δ2-3	CACATTTGCATAAATATAGACAGAAAGC	-	intron 3	RSS12
27			CACAGTGGGTGGCCTGAGCCCAGAGCAGCTCCCCATATC	+	intron 1	RSS23
28			CACAGGGATATGGGGAGCTGCTCTGGGCTCAGGCCACCC	-	intron 1	RSS23
29	#174	Δ2-3	CACTCTCTTTAGGCACAGTTGTAAAAAT	-	intron 3	RSS12
30			CACAGTATATGGAATTTGATTCAAAAAT	-	intron 1	RSS12
31			CACAGTATATGGAATTTGATTCAAAAATCAGGTTCCTTA	-	intron 1	RSS23
32	#199	Δ2-3	CACAGTGAATTACCACCTTACTAAAATA	+	intron 3	RSS12
33			CATATTACTCAGAATCATATTGTCTCCAAAGCACAAACT	+	intron 3	RSS23
34			CACCGTGAAACAAAAGGGGAAGAAAACA	-	intron 1	RSS12
35			CACAGTCAATCAGAGCTGGTGACCAGAACATTTTATTGA	+	intron 1	RSS23
36	#199	Δ2-7	CAACATCCTCAAAAACAATACAATGATA	-	intron 7	RSS12

37			CACAGCAACATTCAACAAGCAATGCCATCAGCAAAATCT	-	intron 7	RSS23
38			CACCGTGAAACAAAAGGGGAAGAAAACA	-	intron 1	RSS12
39			CACAGTCAATCAGAGCTGGTGACCAGAACATTTTATTGA	+	intron 1	RSS23
40	#204	Δ2-3	CACAGTGAATTACCACCTTACTAAAATA	+	intron 3	RSS12
41			CATATTACTCAGAATCATATTGTCTCCAAAGCACAAACT	+	intron 3	RSS23
42			CACCGTGAAACAAAAGGGGAAGAAAACA	-	intron 1	RSS12
43			CACAGTCAATCAGAGCTGGTGACCAGAACATTTTATTGA	+	intron 1	RSS23
44	#215	Δ2-3	CACAGCCAGGACAGGAGCTGCAGCAACT	-	intron 1	RSS12
45			CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12
46			CATAGAGACACCAGAGAGAGAACAATGTTCACAGCCAGG	-	intron 1	RSS23
47	#221	Δ2-7	CAACATCCTCAAAAACAATACAATGATA	-	intron 7	RSS12
48			CACAGCAACATTCAACAAGCAATGCCATCAGCAAAATCT	-	intron 7	RSS23
49			CAATCTCCCTTAGAATATGACAAGAACC	-	intron 1	RSS12
50			CACAGCCAGGACAGGAGCTGCAGCAACT	-	intron 1	RSS12
51			CATAGAGACACCAGAGAGAGAACAATGTTCACAGCCAGG	-	intron 1	RSS23
52	#225	Δ5-7	CAACATCCTCAAAAACAATACAATGATA	-	intron 7	RSS12
53			CACAGCAACATTCAACAAGCAATGCCATCAGCAAAATCT	-	intron 7	RSS23
54			CACTGTACAGTCAGGCTTTAAATGAATT	-	intron 4	RSS12
55			CACACTCAGCCCTAAGTGAAGCAAGCGTGCATGAGAGTA	+	intron 4	RSS23
56	#256	Δ2-3	CACTGAGCTGTGACTCTTGGGGGAAAGA	+	intron 3	RSS12
57			CATGCTGGGAAACTGTCCTGTGAAAGAGAATAGAAACCT	+	intron 3	RSS23
58			CACATTGGGTGGGGGAAAAATTCCTGTTTTCCCCAACCA	-	intron 3	RSS23
59			CAATGTGCTGCATTTTCTAATTTTCTATGAACACTTCCT	+	intron 3	RSS23
60			CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12
61	#257	Δ2-3	CACTGAGCTGTGACTCTTGGGGGAAAGA	+	intron 3	RSS12
62			CATGCTGGGAAACTGTCCTGTGAAAGAGAATAGAAACCT	+	intron 3	RSS23
63			CACATTGGGTGGGGGAAAAATTCCTGTTTTCCCCAACCA	-	intron 3	RSS23
64			CAATGTGCTGCATTTTCTAATTTTCTATGAACACTTCCT	+	intron 3	RSS23
65			CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12
66	#266	Δ2-3	CACAACACATGTACCACATGCACATATA	-	intron 3	RSS12
67			CACCACATATACCCCCCACATATATACA	-	intron 3	RSS12
68			CACATACATGCACACACAAACATATGAC	-	intron 3	RSS12
69			CACACACATACATGCACACACAAACATA	-	intron 3	RSS12
70			CACAGAACTTCATGACAGTTTTGATTTTAGATTAAAGTA	+	intron 3	RSS23
71			CACATACATATACATACATCACACACCACATATACCCCC	-	intron 3	RSS23
72			CACATACATGCACACACAAACATATGACACACACAACAT	-	intron 3	RSS23
73			CACACACATACATGCACACACAAACATATGACACACACA	-	intron 3	RSS23
74			CACATACACACACACACCACACACATACATGCACACACA	-	intron 3	RSS23
75	#291	Δ2-7	CAACATCCTCAAAAACAATACAATGATA	-	intron 7	RSS12
76			CACAGCAACATTCAACAAGCAATGCCATCAGCAAAATCT	-	intron 7	RSS23
77	#304	Δ2-3	CATCCAGGGTAGGGACTGAACAAAGTCA	-	intron 3	RSS12
78	#327	Δ2-3	CACAGTGAATTACCACCTTACTAAAATA	+	intron 3	RSS12
79			CATATTACTCAGAATCATATTGTCTCCAAAGCACAAACT	+	intron 3	RSS23
80			CACCGTGAAACAAAAGGGGAAGAAAACA	-	intron 1	RSS12
81			CACAGTCAATCAGAGCTGGTGACCAGAACATTTTATTGA	+	intron 1	RSS23
82	#351	Δ2-3	CACTGAGCTGTGACTCTTGGGGGAAAGA	+	intron 3	RSS12
83			CATGCTGGGAAACTGTCCTGTGAAAGAGAATAGAAACCT	+	intron 3	RSS23
84			CACATTGGGTGGGGGAAAAATTCCTGTTTTCCCCAACCA	-	intron 3	RSS23
85			CAATGTGCTGCATTTTCTAATTTTCTATGAACACTTCCT	+	intron 3	RSS23
86			CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12

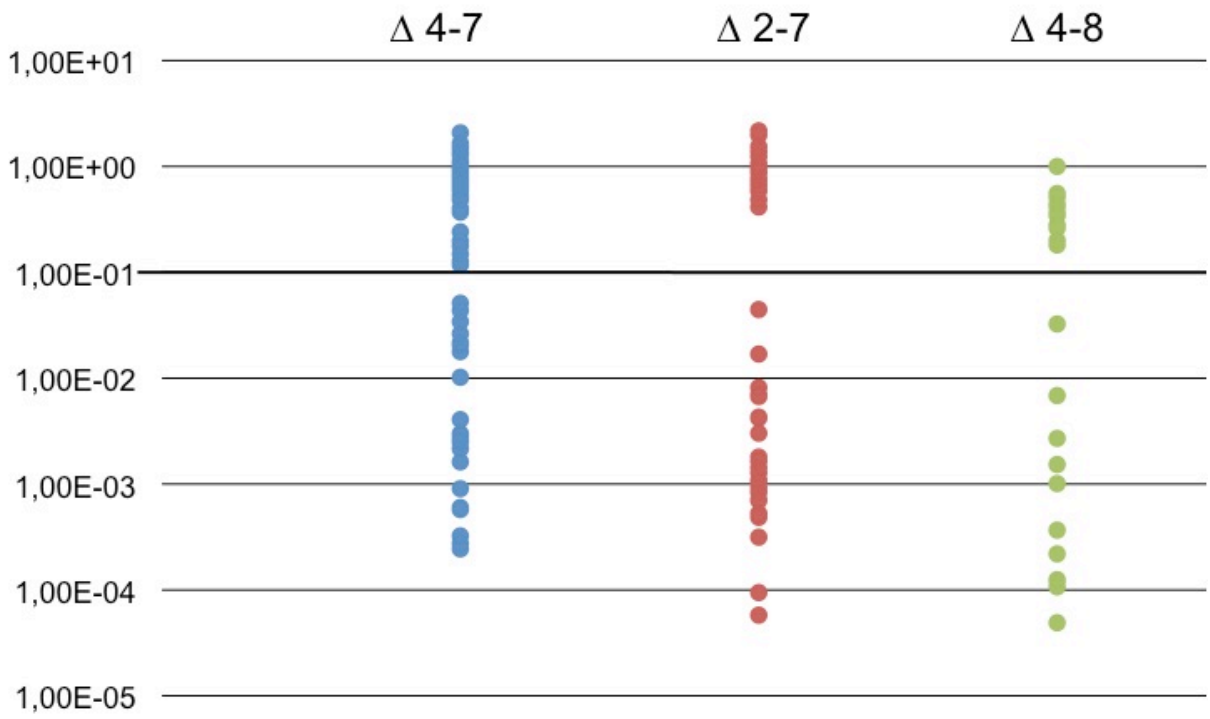
87	#424	Δ5-7	CAACATCCTCAAAAACAATACAATGATA	-	intron 7	RSS12
88			CACAGCAACATTCAACAAGCAATGCCATCAGCAAAATCT	-	intron 7	RSS23
89			CACTGTACAGTCAGGCTTTAAATGAATT	-	intron 4	RSS12
90			CACACTCAGCCCTAAGTGAAGCAAGCGTGCATGAGAGTA	+	intron 4	RSS23
91	#443	Δ2-3	CACTGAGAGCTGTAACAGAACCAAAAGA	-	intron 3	RSS12
92			CACTGTCACTGAGAGCTGTAACAGAACC	-	intron 3	RSS12
93			CACAATGGATGCTGCCTTAGATATCACA	-	intron 3	RSS12
94			CACATTGACCTCAGGACAGTATGTGATAGGCTCTTGTGC	+	intron 3	RSS23
95			CACTCTGGCTCAGGCCCACCCTGGGCTCTTTCACTGACT	-	intron 3	RSS23
96			CACTGTCACTGAGAGCTGTAACAGAACCAAAAGAGAACT	-	intron 3	RSS23
97			CACAGTGGGTGGCCTGAGCCCAGAGCAGCTCCCCATATC	+	intron 1	RSS23
98			CACAGGGATATGGGGAGCTGCTCTGGGCTCAGGCCACCC	-	intron 1	RSS23
99	#101 #111 #495	Δ4-8	CACAGTGAATTACCACCTTACTAAAATA	+	intron 3	RSS12
100			CATATTACTCAGAATCATATTGTCTCCAAAGCACAAACT	+	intron 3	RSS23
101			CACTGTGCTGCAGGTTCTGGCGTCATGATGTTCCTTCCA	-	3'UTR	RSS23
102			CACAGTGTGTTTCTTTCTTTCCCCACATCAAGGGTCTAC	+	3'UTR	RSS23
103	#139	Δ4-8	CACAGTGAATTACCACCTTACTAAAATA	+	intron 3	RSS12
104			CATATTACTCAGAATCATATTGTCTCCAAAGCACAAACT	+	intron 3	RSS23
105			CACACCTTGAAAGGGACCAGTGTGCCTGCTTGAGAAAAA	-	3'UTR	RSS23
106	#470	Δ4-8	CACAGTGAATTACCACCTTACTAAAATA	+	intron 3	RSS12
107			CATATTACTCAGAATCATATTGTCTCCAAAGCACAAACT	+	intron 3	RSS23
108			CACTGTGCTGCAGGTTCTGGCGTCATGATGTTCCTTCCA	-	3'UTR	RSS23
109			CACAGTGTGTTTCTTTCTTTCCCCACATCAAGGGTCTAC	+	3'UTR	RSS23
110			CACTGTGCTAGACCTTGGGGAGCTCCAGGGAGCAAGGCA	-	3'UTR	RSS23
111			CACAGTGCCTGGCACAAGGTGAGGGGGGTGCCCAGAAAA	+	3'UTR	RSS23
112			CACAAGGTGAGGGGGGTGCCCAGAAAAGATTCAATTCCC	+	3'UTR	RSS23
113	#99	Δ2-8	CACTGTCACACACACACACACTTAAAAT	+	intron 1	RSS12
114			CACTGTGCTGCAGGTTCTGGCGTCATGATGTTCCTTCCA	-	3'UTR	RSS23
115			CACAGTGTGTTTCTTTCTTTCCCCACATCAAGGGTCTAC	+	3'UTR	RSS23
116			CACTGTGCTAGACCTTGGGGAGCTCCAGGGAGCAAGGCA	-	3'UTR	RSS23
117			CACAGTGCCTGGCACAAGGTGAGGGGGGTGCCCAGAAAA	+	3'UTR	RSS23
118			CACAAGGTGAGGGGGGTGCCCAGAAAAGATTCAATTCCC	+	3'UTR	RSS23

Supplementary Table 12: Comparison between diagnosis and relapse of 20 mutations in 16 patients with *IKZF1* mutations at the time of diagnosis.

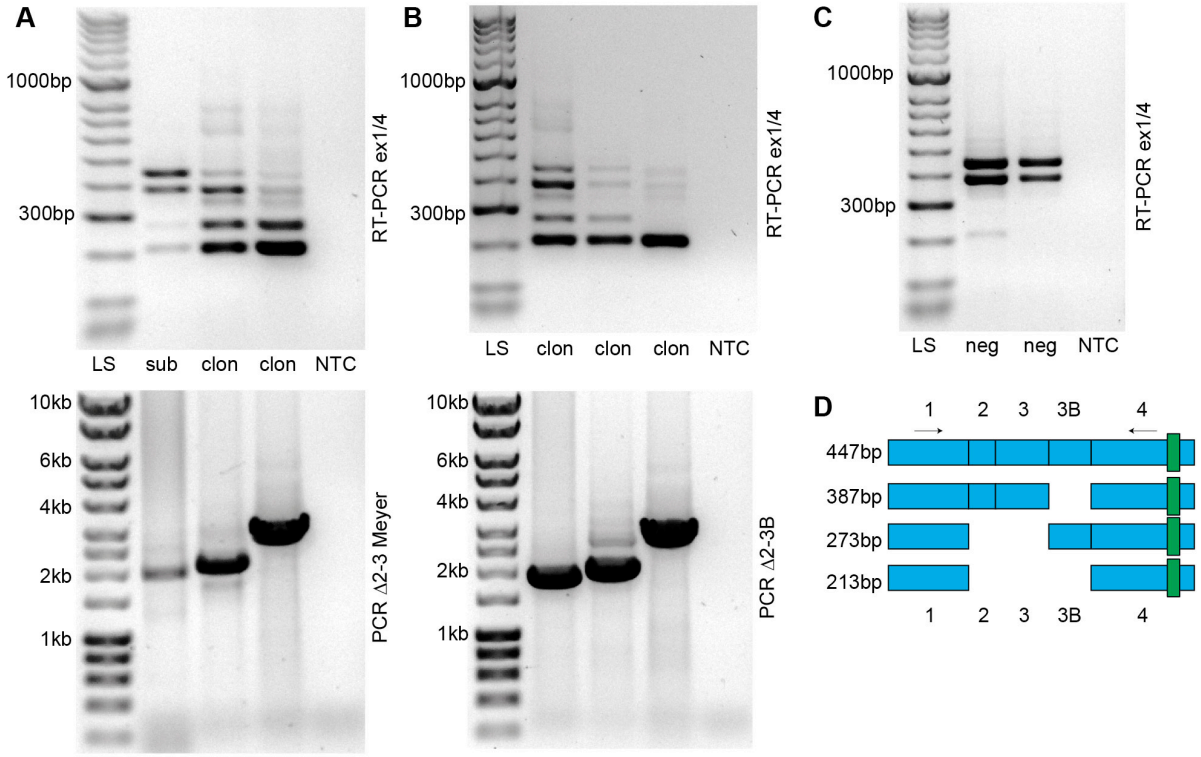
patient	deletion	deletion load	relapse
#110	Δ4-7	high deletion load	conserved
#112	Δ2-7	high deletion load	conserved
#119	Δ2	high deletion load	lost
#121	Δ4-7	high deletion load	conserved
#130	Δ2-7	high deletion load	conserved
#179	Δ4-7	low deletion load	lost
#198	Δ2-7	low deletion load	lost
	Δ4-7	high deletion load	lost
#199	Δ2-3	N/A	conserved
	Δ2-7	low deletion load	conserved
#204	Δ2-3	high deletion load	lost
	Δ2-7	high deletion load	conserved
#243	Δ2-7	high deletion load	conserved
	Δ4-8	high deletion load	conserved
#289	Δ4-8	high deletion load	lost
#479	Δ4-7	high deletion load	conserved
#482	Δ2-8	high deletion load	conserved
#483	Δ4-7	high deletion load	conserved
#495	Δ4-8	high deletion load	conserved
#500	Δ4-7	low deletion load	lost

Supplementary Figures

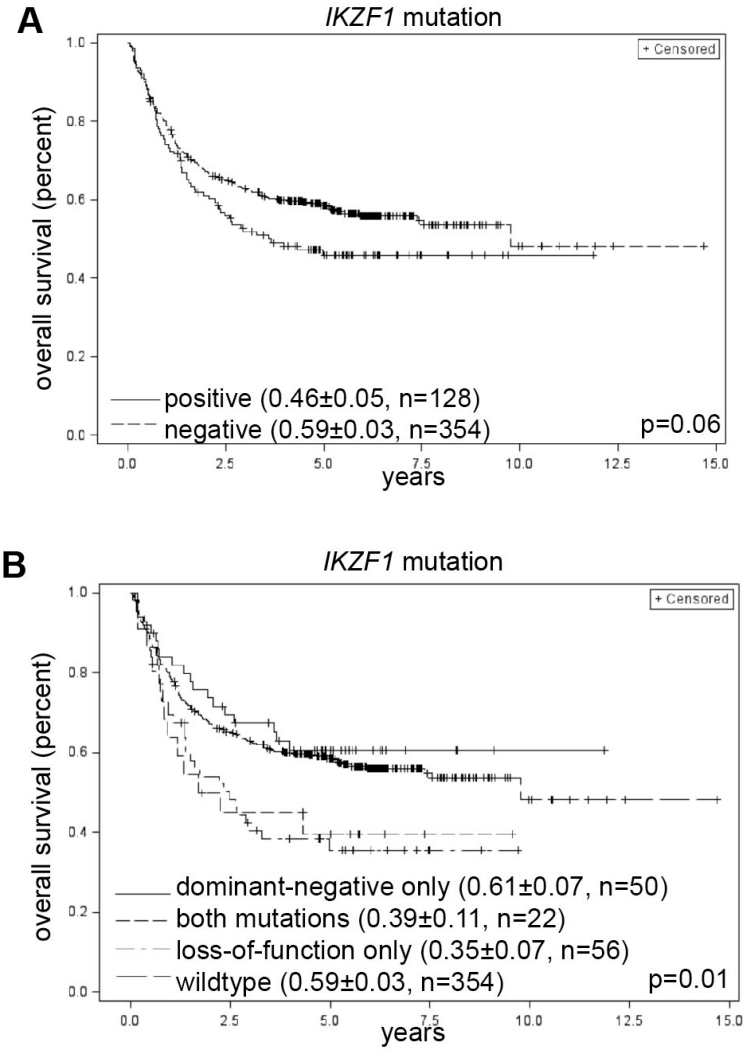
Supplementary Figure 1: Quantification of deletions Δ4-7, Δ2-7 and Δ4-8 by quantitative PCR. Relative concentration of deleted cells was calculated in relation to a standard curve by cell line BV-173 (Δ4-7) or patient DNA (#100 for Δ2-7, #101 for Δ4-8). Deletions with a relative concentration >1,00E-01 are considered „high deletion load“, all other deletions are considered „low deletion load“.



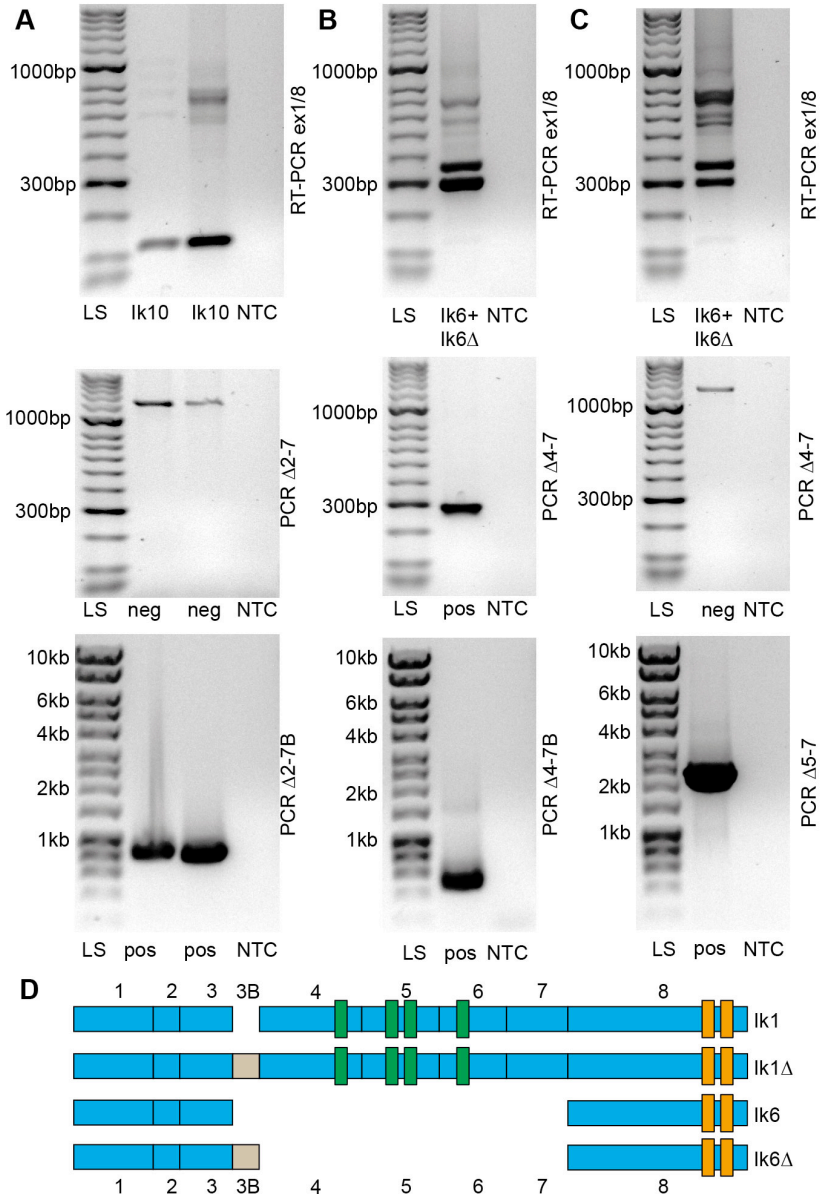
Supplementary Figure 2: Detection of Δ2-3 by RT-PCR. (A) Patients positive for Δ2-3 on RT-PCR (above) show a corresponding lesion detectable by the PCR described by Meyer (below). (B) In this subgroup of patients positive for Δ2-3 in RT-PCR ex1/4, a genomic breakpoint could only by identified by a novel PCR Δ2-3 B. (C) Patients negative for Δ2-3 on RT-PCR. (D) Structure of the 4 PCR products detectable by RT-PCR ex1/4.



Supplementary Figure 3: Additional evaluation of the prognostic effect of IKZF1 mutations. (A) Overall survival of patients with and without any *IKZF1* mutation. (B) Overall survival of patients with *IKZF1* loss-of-function mutations only, *IKZF1* dominant-negative mutations only or both forms of *IKZF1* mutations.



Supplementary Figure 4: Detection of rare breakpoints by RT-PCR. (A) Patients #85 and #291 show Ik10 expression on RT-PCR (above), no breakpoint by PCR Δ2-7 (middle) and a breakpoint by PCR Δ2-7 variant (below). (B) Patient #338 exhibits Ik6 and Ik6Δ on RT-PCR (above), a breakpoint by PCR Δ4-7 (middle) and a second breakpoint distal to exon 3b by PCR Δ4-7 variant (below). (C) Patient #424 shows Ik6 and Ik6Δ expression by RT-PCR (above), no PCR Δ4-7 (middle) and a band by a PCR Δ5-7 (below). (D) Structure of isoforms Ik6 and Ik6Δ.



Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Vollständige Publikationsliste

Paper

Kobitzsch B, Gökbuget N, Schwartz S, Reinhardt R, Brüggemann M, Viardot A, Wäsch R, Starck M, Thiel E, Hoelzer D, and Burmeister T. Loss-of-function but not dominant-negative intragenic IKZF1 deletions are associated with an adverse prognosis in adult BCR-ABL-negative acute lymphoblastic leukemia.
Haematologica. 2017; 102:xxx. doi:10.3324/haematol.2016.161273
Online abrufbar unter: http://dx.doi.org/10.3324/haematol.2016.161273
Impact factor 2016: 7.702

Posterpräsentation

Kobitzsch B, Gökbuget N, Schwartz S, Reinhardt R, Brueggemann M, Viardot A, Wäsch R, Starck M, Thiel E, Hoelzer D, Burmeister T 2015: Non-Functional ("haploinsufficient"), but Not Dominant Negative Clonal IKZF1 Deletions Confer an Adverse Prognosis in Adult BCR-ABL-Negative Acute Lymphoblastic Leukemia. Poster auf dem 57th Annual Meeting & Exposition der American Society of Hematology. Orlanda, FL, USA, 05.-08.12.2015.
Online abrufbar unter: http://www.bloodjournal.org/content/126/23/2617

Kongressbeitrag

Wulff I, Kobitzsch B, Hesselbarth U, Peters H 2015: Studentisch generierte POL-Lernziele im Vergleich zu den Modul-Lernzielen der Fakultät. Kurzvortrag auf der Gemeinsamen Jahrestagung der Gesellschaft für Medizinische Ausbildung (GMA) und des Arbeitskreises zur Weiterentwicklung der Lehre in der Zahnmedizin (AKWLZ). Leipzig, 30.09.-03.10.2015.
Online abrufbar unter: http://dx.doi.org/10.3205/15gma106

Danksagung

Mein Dank gilt an erster Stelle Herrn PD Dr. med. Dr. rer. nat. Thomas Burmeister, dem Betreuer dieser Arbeit. Ich danke ihm für die Überlassung des Themas, sein Vertrauen in meine Arbeit und die Finanzierung der Studie. Seiner kontinuierlichen und zuverlässigen Betreuung über mehrere Jahre hinweg ist es zu verdanken, dass meine Dissertation in dieser Form erfolgreich abgeschlossen werden konnte.

Zudem danke ich Daniela Gröger für die Einarbeitung in die unterschiedlichen Forschungsmethoden und die gemeinsame Arbeit im Labor.

Abschließend danke ich allen Mitgliedern der GMALL-Studiengruppe, besonders Frau Dr. Nicola Gökbuget und Herrn Prof. Dr. Dieter Hoelzer, sowie allen Patientinnen und Patienten und den behandelnden Ärztinnen und Ärzten, die diese Studie ermöglicht haben.