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Fusion of *NUP214* to *ABL1* on amplified episomes in T-cell acute lymphoblastic leukemia

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In T-cell acute lymphoblastic leukemia (T-ALL), transcription factors are known to be deregulated by chromosomal translocations, but mutations in protein tyrosine kinases have only rarely been identified[1–3]. Here we describe the extrachromosomal (episomal)[4] amplification of *ABL1* in 5 of 90 (5.6%) individuals with T-ALL, an aberration that is not detectable by conventional cytogenetics. Molecular analyses delineated the amplicon as a 500-kb region from chromosome band 9q34, containing the oncogenes *ABL1* and *NUP214* (refs. 5,6). We identified a previously undescribed mechanism for activation of tyrosine kinases in cancer: the formation of episomes resulting in a fusion between *NUP214* and *ABL1*. We detected the *NUP214-ABL1* transcript in five individuals with the *ABL1* amplification, in 5 of 85 (5.8%) additional individuals with T-ALL and in 3 of 22 T-ALL cell lines. The constitutively phosphorylated tyrosine kinase NUP214-ABL1 is sensitive to the tyrosine kinase inhibitor imatinib[7,8]. The recurrent cryptic *NUP214-ABL1* rearrangement is associated with increased *HOX* expression[1] and deletion of *CDKN2A*[9], consistent with a multistep pathogenesis of T-ALL. *NUP214-ABL1* expression defines a new subgroup of individuals with T-ALL who could benefit from treatment with imatinib.

The Philadelphia translocation, resulting in the *BCR-ABL1* fusion gene, is typically found in chronic myeloid leukemia (CML) and precursor B-cell acute lymphoblastic leukemia (B-ALL) but is exceptionally rare in T-ALL[2,5,10]. To study the potential involvement of *ABL1* rearrangements in T-ALL, we screened 90 cases by fluorescence *in situ* hybridization (FISH), using *BCR* and *ABL1* probes. We observed no *BCR-ABL1* fusion signals, confirming the low frequency of this rearrangement in T-ALL. But we observed marked amplification (more than ten signals per nucleus) of *ABL1* in 5 of 90 individuals with T-ALL (**Fig. 1a** and **Tables 1** and **2**). Notably, the additional *ABL1* signals were extrachromosomal. Extrachromosomal amplification of oncogenes has been observed on double minute (dmin) chromosomes[11], visible by standard cytogenetics, or on cytogenetically invisible units, called episomes[4]. In the cases we studied, no dmin chromosomes were visible by G or R banding (**Supplementary Fig. 1** online), suggesting that *ABL1* amplification occurred on episomes.

Detailed FISH mapping of the episomes confirmed that they contained *ABL1*, *LAMC3* and *NUP214* (also called *CAN*), three genes localized within a 500-kb region on chromosome region 9q34 (**Fig. 1a,c**). Probes for *ASS*, *FUBP3* and *VAV2* did not hybridize to the episomes (**Fig. 1a,c**). The 5′ end of *ABL1* could not be detected on the episomes in four of six cases, delineating the proximal breakpoint in the first intron of *ABL1*. Cohybridization of the 3′ *ABL1* and *NUP214* probes on the episomes confirmed the presence of both genes on the same episomes (**Supplementary Fig. 1** online). Microarray-based comparative genomic hybridization (array CGH) confirmed the amplification of *ABL1* and *LAMC3* in the four cases that could be analyzed (**Fig. 1b,c**). BAC-57C19 sequences (5′ *ABL1*) were amplified only in individual 1, confirming the FISH findings (**Fig. 1b** and **Table 1**). BAC-5N16 sequences were not amplified in any of the four individuals, further delimiting the amplified region to a maximal size of 1 Mb (**Fig. 1b,c**). Array CGH also showed deletion of BAC-149I2, containing the tumor-suppressor gene *CDKN2A* (also called *p16*), in individuals 2–4, an observation that was confirmed by FISH (**Fig. 1a,b** and **Table 2**). In individuals 1–3, amplification of *ABL1* was confirmed by Southern blotting (**Supplementary Fig. 2** online).

The selective absence of the 5′ end of *ABL1* in the amplicon suggested that *ABL1* might be involved in the generation of a fusion gene. We therefore carried out RACE-PCR on *ABL1* transcripts from

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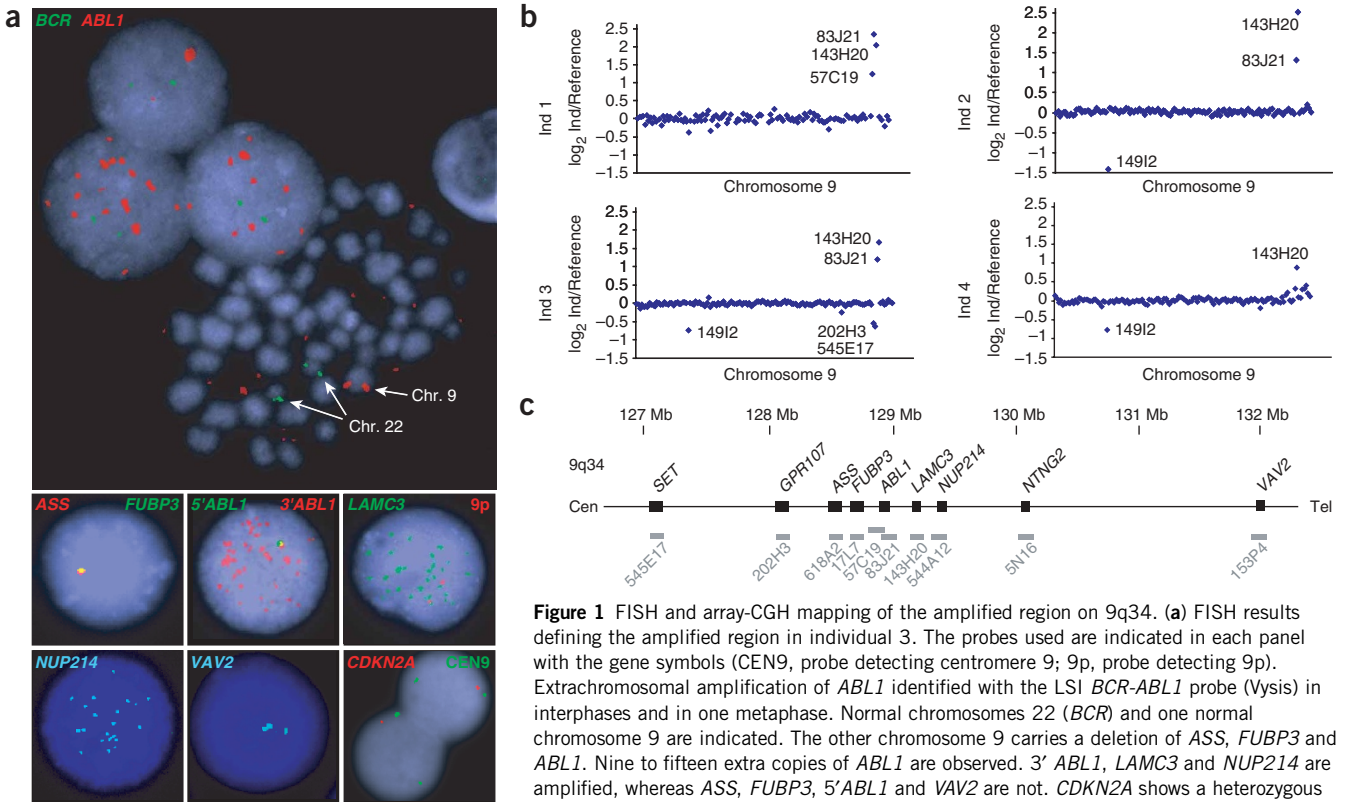

Figure 1 FISH and array-CGH mapping of the amplified region on 9q34. (**a**) FISH results defining the amplified region in individual 3. The probes used are indicated in each panel with the gene symbols (CEN9, probe detecting centromere 9; 9p, probe detecting 9p). Extrachromosomal amplification of *ABL1* identified with the LSI *BCR-ABL1* probe (Vysis) in interphases and in one metaphase. Normal chromosomes 22 (*BCR*) and one normal chromosome 9 are indicated. The other chromosome 9 carries a deletion of *ASS*, *FUBP3* and *ABL1*. Nine to fifteen extra copies of *ABL1* are observed. 3′ *ABL1*, *LAMC3* and *NUP214* are amplified, whereas *ASS*, *FUBP3*, 5′*ABL1* and *VAV2* are not. *CDKN2A* shows a heterozygous deletion. (**b**) Array-CGH results, showing hybridization signals for 138 clones from chromosome 9, confirm the amplification of *ABL1* and *LAMC3*. *NTNG2* is not amplified and 5′*ABL1* is amplified only in individual 1. The array does not contain a probe for the *NUP214* locus. Clone 149I2 contains *CDKN2A* and *CDKN2B*. (**c**) Locations of the probes used for FISH and array CGH along chromosome 9. Black boxes represent genes, gray boxes represent BACs. Cen, centromeric side; Tel, telomeric side.

individual 4. Sequencing of the PCR products identified an in-frame fusion between exon 31 of *NUP214* and exon 2 of *ABL1*. Subsequent RT-PCR confirmed the presence of *NUP214-ABL1* fusion transcripts in five individuals with *ABL1* amplification from whom cDNA was available (**Fig. 2**). These results are compatible with a model in which the genomic region from *ABL1* to *NUP214* is circularized, generating a *NUP214-ABL1* fusion gene (**Fig. 3a**), and the copy number of the episome is increased owing to unequal segregation during cell division. Consistent with this model, we observed a variable number of episomes (5–50) in different cells from a single individual. In individual 3, episome formation may have originated from a deletion, as one chromosome 9 carries a deletion of a region slightly larger than the amplified region (**Fig. 1a,b** and **Table 1**). Similar deletions associated with amplification of *MYC* on dmin chromosomes or episomes have been reported[12].

To confirm these findings, we screened an additional 85 individuals with T-ALL by RT-PCR. We detected the *NUP214-ABL1* fusion transcript in 5 of these individuals (**Fig. 2a** and **Table 2**). We next

Table 1 FISH results in six individuals with abnormal *ABL1* hybridization patterns

	LSI *BCR-ABL1*		Number of signals observed									
Individual	Nuclei[a]	Metaphases[b]	373J8 (*NIBL*)	202H3 (*GPR107*)	618A2 (*ASS*)	17L7 (*FUBP3*)	57C19 (5′ *ABL1*)	83J21 (3′ *ABL1*)	143H20 (*LAMC3*)	544A12 (*NUP214*)	153P4 (*VAV2*)	83N9 (*LHX3*)
1 (BM)	M (82%)	3/9	2	2	2	2	M	M	M	M	2	2
2 (PB)	M (75%)	12/19	2	2	2	2	2	M	M	M	2	2
3 (BM)	M (76%)	14/20	2	1	1	1	1	M	M	M	2	2
4 (BM)	M (23%)	16/40	2	2	2	2	2	M	M	M	3 (20%)	2
5 (BM)	M (6%)	13/30	2	2	2	2	M	M	M	M	2	2
6 (PE)	M (96%)	9/10	NA	NA	2	2	2	M	NA	M	2	NA
6 (BM)	M (16%)	1/1										

[a]The percentage of cells showing multiple (>10) signals. [b]The number of abnormal metaphases examined.
All BAC probes are derived from the RPCI-11 library; the locations of most of the BACs and genes are shown in **Figure 1c**. BM, on bone marrow; M, multiple (more than ten); NA, not analyzed; PB, on peripheral blood; PE, on pleural effusion.

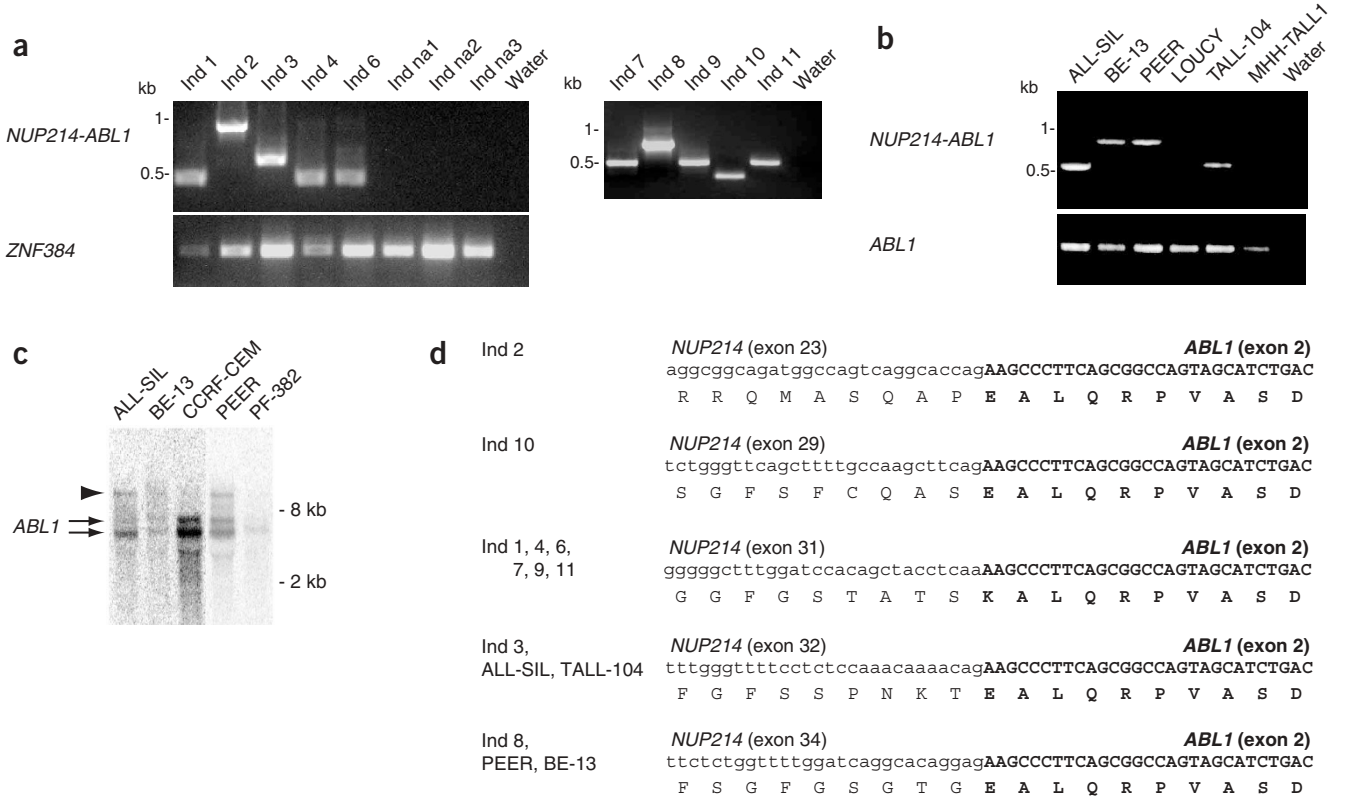

Figure 2 Detection of the *NUP214-ABL1* fusion transcript by RT-PCR and northern blotting. (**a**) Detection of different *NUP214-ABL1* fusion transcripts in five individuals with T-ALL with *ABL1* amplification (individuals 1–4 and 6), and absence of this fusion in individuals with T-ALL lacking *ABL1* amplification (individuals na1–na3). *NUP214-ABL1* fusion transcripts were also detected in 5 of 85 additional individuals with T-ALL screened by RT-PCR (individuals 7–11). (**b**) Detection of different *NUP214-ABL1* fusion transcripts in four T-ALL cell lines by RT-PCR. (**c**) Detection of an aberrant *ABL1* transcript (arrowhead) in the cell lines PEER, ALL-SIL and BE-13 by northern blotting. (**d**) Sequence of the different variant *NUP214-ABL1* transcripts detected. All fusions are in-frame (sequence translated with the one-letter amino acid abbreviations).

tested human T-ALL cell lines for the presence of the fusion. We detected the *NUP214-ABL1* transcript in 3 of 22 independent T-ALL cell lines: ALL-SIL, PEER and TALL-104, and in BE-13, a tetraploid subline of PEER (**Fig. 2b** and **Supplementary Table 1** online). The presence of an aberrant *ABL1* transcript in the cell lines was also confirmed by northern blotting (**Fig. 2c**). Taken together, these data indicate that ~6% of individuals with T-ALL carry a cryptic *NUP214-ABL1* fusion gene.

Among T-ALL cases, we observed variants of the *NUP214-ABL1* fusion gene due to different breakpoints in *NUP214* (ranging from intron 23 to intron 34; **Fig. 2d**). All breakpoints in *ABL1* occurred in intron 1, and so exon 2 of *ABL1* is present in all fusion variants; this coincides with the *ABL1* breakpoint observed in Philadelphia-positive CML and B-ALL. The *NUP214-ABL1* fusion mRNAs are predicted to encode proteins of 2,210–3,175 amino acids with molecular weights of ~239–333 kDa (**Fig. 3b**).

NUP214 is a component of the nuclear pore complex, which mediates nucleocytoplasmic transport[13]. *NUP214* is widely expressed and is involved in the pathogenesis of acute myeloid leukemia associated with the t(6;9)(p23;q34) *DEK-NUP214* fusion[6]. In the DEK-NUP214 fusion protein, however, the C-terminal region of NUP214 (encoded by exons 18–36) is present, whereas the predicted NUP214-ABL1 fusions retain the N-terminal region of NUP214, which includes the predicted coiled-coil domains that may serve as oligomerization motifs (**Fig. 3b**).

ABL1 is an ubiquitously expressed cytoplasmic tyrosine kinase that is fused to BCR in CML and precursor B-ALL cases with the t(9;22)(q34;q11) translocation[5,10] and to ETV6 in leukemias with the t(9;12)(q34;p13) translocation[14]. Like BCR-ABL1 and ETV6-ABL1 fusion proteins, the NUP214-ABL1 fusions contain the SH3, SH2 and kinase domains of ABL1 (**Fig. 3b**), suggesting that NUP214-ABL1 acts as a constitutively activated tyrosine kinase. We assessed this possibility by analyzing tyrosine phosphorylation of NUP214-ABL1 and CRKL, a direct target of the ABL1 kinase[15]. We detected the NUP214-ABL1 fusion protein in the cell lines PEER, BE-13 and ALL-SIL, using antibodies directed against ABL1 or NUP214 (**Fig. 4a**). NUP214-ABL1 and CRKL were phosphorylated in the cell lines expressing NUP214-ABL1, whereas CRKL was not phosphorylated in the cell line LOUCY, which does not express NUP214-ABL1 (**Fig. 4a**). Addition of imatinib, a selective inhibitor of ABL1 kinase activity[7], decreased phosphorylation of both NUP214-ABL1 and CRKL (**Fig. 4b**) and inhibited the proliferation of ALL-SIL (**Fig. 4c**). The proliferation of PEER and BE-13 was not affected by imatinib, although the phosphorylation of NUP214-ABL1 was inhibited (**Supplementary Fig. 3** online). This is probably due to the fact that the growth of these cell lines became independent of NUP214-ABL1 activity because they acquired additional mutations. Similar findings were previously reported in cell lines expressing BCR-ABL1 (ref. 16). Phosphorylation of CRKL was also inhibited by imatinib in primary bone marrow cells from individual 4 (**Fig. 4d**). These results

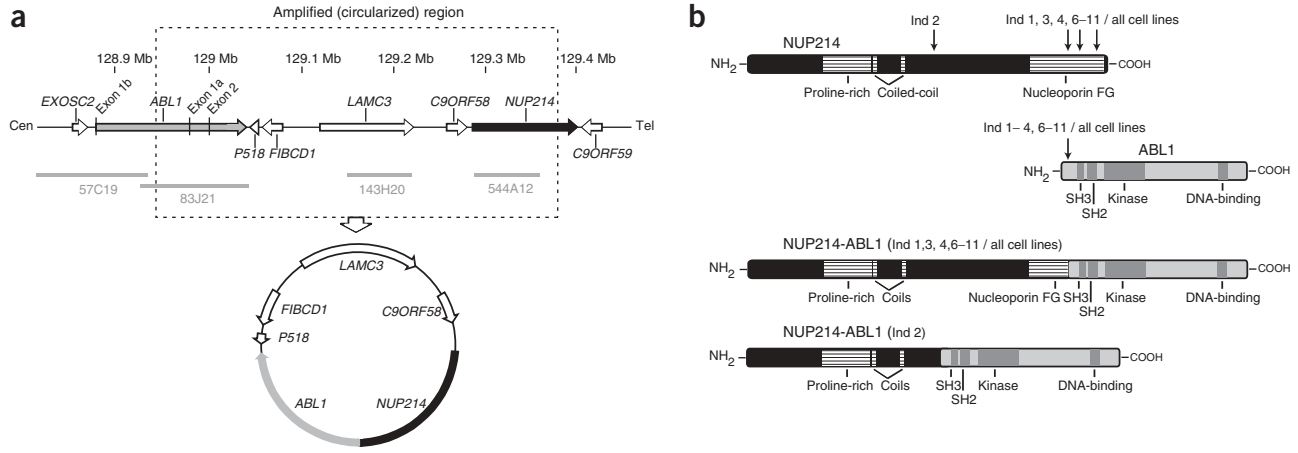

Figure 3 Schematic representation of the amplified region and the NUP214-ABL1 fusion protein. (**a**) Detailed scheme of the amplified (circularized) region, and the proposed structure of the episome. Genes (arrows), BAC clones (gray bars), and their positions along chromosome 9 are indicated. Cen, centromeric side; Tel, telomeric side. (**b**) Schematic representation of the NUP214 and ABL1 proteins. Two main NUP214-ABL1 fusion proteins are generated: a shorter fusion (239 kDa) present in only one individual, and longer fusions (310–333 kDa) present in most individuals with T-ALL with *ABL1* amplification. All fusions contain the predicted coiled-coil domains of NUP214 and the SH3, SH2 and tyrosine kinase domains of ABL1.

indicate that NUP214-ABL1 is a constitutively activated tyrosine kinase that activates similar pathways as BCR-ABL1 and is sensitive to inhibition with imatinib.

Constitutively activated tyrosine kinases are sufficient to induce myeloproliferative disease, but they require the cooperative effect of other mutations to induce acute leukemia[17]. In agreement with this, we identified additional mutations in cells expressing the *NUP214-ABL1* fusion. We detected deletion of the tumor-suppressor genes *CDKN2A* and *CDKN2B* (also called *p15*) in seven of the nine cases we were able to evaluate. Screening for oncogenes known to be involved in T-ALL identified the mutually exclusive overexpression of *TLX1* (also called *HOX11*) and *TLX3* (also called *HOX11L2*) in four and five individuals, respectively (**Table 2**). Similar observations were made in the T-ALL cell lines, where expression of *TLX1* and *NKX2-5* was reported for ALL-SIL and PEER, respectively (**Supplementary Table 1** online)[18]. These data provide genetic support for a multi-step

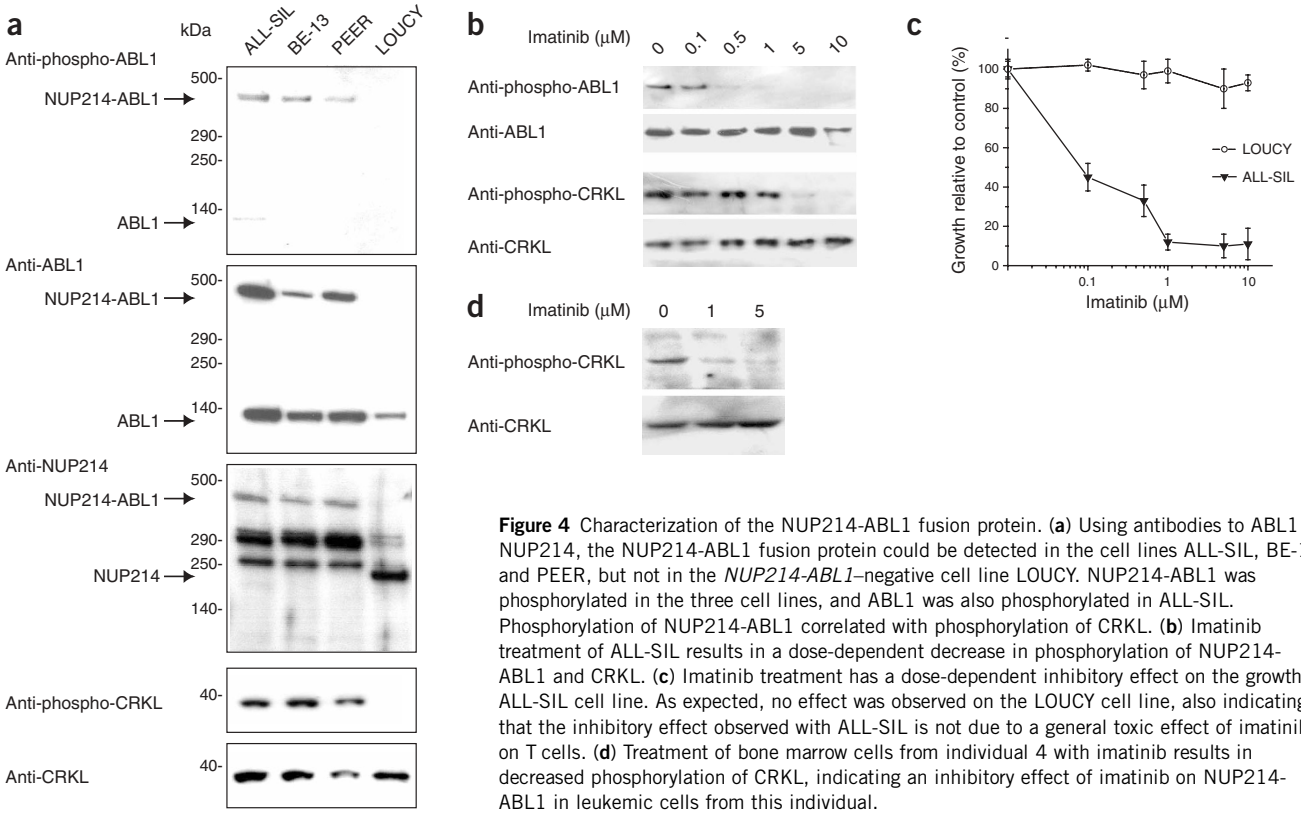

Figure 4 Characterization of the NUP214-ABL1 fusion protein. (**a**) Using antibodies to ABL1 or NUP214, the NUP214-ABL1 fusion protein could be detected in the cell lines ALL-SIL, BE-13 and PEER, but not in the *NUP214-ABL1*–negative cell line LOUCY. NUP214-ABL1 was phosphorylated in the three cell lines, and ABL1 was also phosphorylated in ALL-SIL. Phosphorylation of NUP214-ABL1 correlated with phosphorylation of CRKL. (**b**) Imatinib treatment of ALL-SIL results in a dose-dependent decrease in phosphorylation of NUP214-ABL1 and CRKL. (**c**) Imatinib treatment has a dose-dependent inhibitory effect on the growth of ALL-SIL cell line. As expected, no effect was observed on the LOUCY cell line, also indicating that the inhibitory effect observed with ALL-SIL is not due to a general toxic effect of imatinib on T cells. (**d**) Treatment of bone marrow cells from individual 4 with imatinib results in decreased phosphorylation of CRKL, indicating an inhibitory effect of imatinib on NUP214-ABL1 in leukemic cells from this individual.

Table 2 Characteristics of 11 individuals with T-ALL with *ABL1* amplification or *NUP214-ABL1* fusion

							FISH results		Expression data			
Individual	Sex	Age (y)	WBC[a]	Percent blast[b]	Immune phenotype[c]	Karyotype[d]	*CDKN2A*[f]	*ABL1*	*NUP214-ABL1*	Other[g]	Response to therapy, outcome	OS[e]
1	M	52	134	96	Cortical	46,XY,del(12)(p13) [4]/ 46,XY [1]	+/+	Amp	+	*TLX1*	CR, early relapse	7
2	M	3	162	95	Cortical	46,XY [12]	–/–	Amp	+	*TLX1*	CR	57+
3	M	23	81	92	Cortical	48,XY,t(3;11)(p12;p15), t(7;10)(q35;q24), t(8;10)(q21;q21),+11,+12 [5] / 46,XY [4]	–/+	Amp	+	*TLX1*	CR, alloBMT, toxic death	10
4	M	7	196	88	Mature	46, XY [33]	–/+	Amp	+	*TLX3*	CR	40+
5	M	6	52	82	Pre-T	47,XY,del(6)(q21),+8 [12] / 46,XY [4]	+/+	Amp	NA	NA	CR, early relapse	14
6	M	25	8	40[h]	Mature[i]	46,Y,add(X)(p22), t(8;22)(p22;q12), del(13)(q14q22) [10]	–/+	Amp	+	*TLX3*	Early toxic death	0.5
7	F	Ped	NA	96	Cortical	Near tetraploid with del(11)(q23)	–/–[j]	NA	+	*TLX3*	CR	194+
8	F	Ped	NA	86	Mature[i]	NA	–/–[j]	NA	+		CR, early relapse	7
9	M	9	NA	NA	NA	NA	NA	NA	+	*TLX3*	CR	14+
10	M	4	NA	NA	NA	NA	–/–[j]	NA	+	*TLX3*	CR	176+
11	F	31	NA	75	Pre-T	46,XX [20]	NA	NA	+	*TLX1*	Early toxic death	1

[a]White blood cells ($\times 10^9$ per l). [b]In bone marrow. [c]Following EGIL classification[27]. [d]Karyotype obtained from bone marrow culture at diagnosis, except for individual 2, for whom peripheral blood was analyzed. [e]Overall survival (mo). [f]+/+, no deletion; –/+, hemizygous deletion; –/–, homozygous deletion. [g]Molecular screening for *BCR-ABL1*, *SIL-TAL1*, *ETV6-RUNX1*, *TLX1* and *TLX3* transcripts and *MLL* rearrangement (Southern blot). [h]40% blasts in bone marrow and 77% in pleural effusion. [i]Mature T phenotype with aberrant expression of CD13. [j]Results obtained by quantitative PCR (not by FISH).
+, alive at last analysis; Amp, amplified; CR, complete remission; NA, not available; Ped, pediatric.

pathogenesis of T-ALL: deletion of a tumor-suppressor gene (*CDKN2A*, *CDKN2B*), deregulated expression of a transcription factor (*TLX1*, *TLX3*) and expression of a constitutively activated tyrosine kinase (*NUP214-ABL1*).

Our results identify the episomal fusion of *NUP214* to *ABL1* as a new mechanism for the generation of a fusion gene. This gene rearrangement is cryptic by conventional cytogenetics but readily detected by FISH using a commercially available *ABL1* probe. Amplification of *ABL1* in a subset of individuals with T-ALL was recently reported, although the molecular and physiologic consequences of amplification were not elucidated[19]. FISH with the *ABL1* probes is distinctive and seems to be pathognomonic for the presence of the *NUP214-ABL1* fusion in T-ALL. We observed hybridization of *ABL1* on 9q34, as expected, as well as at multiple extrachromosomal sites in metaphases and multiple signals in most interphase cells. It will be interesting to investigate whether this fusion also occurs in T-ALL as a result of t(9;9)(q34;q34) or in other hematological malignancies, particularly *BCR-ABL1*–negative myeloproliferative diseases.

Survival data for the eleven individuals that we studied are indicative of a rather aggressive course of disease in the four adults with the *NUP214-ABL1* fusion (two early relapses and two toxic deaths; **Table 2**). The finding that NUP214-ABL1 is sensitive to the tyrosine kinase inhibitor imatinib suggests that new therapeutic approaches in T-ALL with the *NUP214-ABL1* fusion may improve outcome or decrease treatment-related morbidity. This study demonstrates the power of high-resolution array CGH for the detection of acquired genetic unbalances in cancer cells. We show that cryptic (episomal) amplifications may generate new fusion genes, in addition to cryptic deletions[20]. Taken together, these findings suggest that genome-wide screens for deletions and amplifications of tyrosine kinases in a broad spectrum of hematological malignancies and solid tumors may identify more of these aberrations.

METHODS

Affected individuals. We retrospectively selected 90 individuals with T-ALL from the database of the Department of Human Genetics (Leuven) for the initial FISH screening. We later added an additional individual (individual 6, from the Erasme Hospital, Brussels) to the study. We screened a set of 85 individuals with T-ALL from the database of the Dana-Farber Cancer Institute (Boston) for the presence of the *NUP214-ABL1* fusion by RT-PCR. We reviewed the clinical diagnosis, morphology and immunophenotypic data. This study was approved by the Ethical Committee of the Medical Faculty of the University of Leuven and informed consent was obtained from all subjects.

Cytogenetics and FISH. We carried out cytogenetic studies on bone marrow or blood cells using direct or short-term cultures without mitogens and R banding. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature[21]. In cases where bone marrow was not available, we karyotyped lymph node or pleural effusions. We carried out FISH on stored fixed cell suspension originally used for karyotyping, as described[22]. We were able to hybridize successfully the same metaphases up to three times. Initial screening was done using the LSI BCR-ABL ES (Vysis) translocation probe. On average, we scored ten metaphases and 200 nuclei. We investigated cases showing aberrant hybridization signals using a panel of BAC probes mapping at 9q34 (**Fig. 1c** and **Table 1**). We obtained BACs from the RPCI11 library.

Array CGH. We carried out array CGH using Code Linked Slides (AP Biotech) containing the 3,527 BAC clones from the Wellcome Trust Sanger Institute 1 Mb Clone Set[23], a gift from N. P. Carter (The Wellcome Trust Sanger Institute, UK). We amplified BAC DNA by degenerate oligonucleotide–primed PCR[23]. We spotted aminolinked PCR products at a concentration of 200 ng μl^{-1} on the slides using a Molecular Dynamics Generation III printer (AP Biotech). We printed the clones in two replicates at different positions on the array. We labeled test and reference genomic DNA using the Bioprime DNA Labeling System (Invitrogen) using Cy3- and Cy5-labeled dCTPs (AP Biotech). We prepared probes and preblocked the slide performed as described[23]. We hybridized the slides for 48 h under coverslips in a humid chamber saturated with 20% formamide and 2× saline sodium citrate. We carried out post-hybridization washes and image and data analysis as described[24]. Spot intensities were corrected for the local background. Only spots with signal intensities of Cy5 and Cy3 twofold above background signal intensities were retained. For each clone, we calculated a ratio of Cy5 over Cy3 fluorescent intensity. We normalized the data by dividing the fluorescent intensity ratio at each spot by the mean of all ratios of the autosomes. Two values of the duplicate clones were averaged and a $\log_2$ value was calculated. If the variation among the two intensity ratios was larger than 10%, the data point was eliminated from the analysis. Log_2 ratios between –0.2 and 0.2 were accepted to be normal. Ratios

below or above this normal range were interpreted to be due to a clone deletion or duplication, respectively. If the $\log_2$ ratio was above 1, the clone was considered to be amplified.

Cell culture and western blotting. We incubated bone marrow cells in RPMI-1640 medium supplemented with 10% fetal calf serum for 2 h in the presence of different concentrations of imatinib. We lysed cells in 1.5× sample buffer, separated them using SDS-PAGE and transferred them to PVDF membranes. We cultured T-ALL cell lines in RPMI-1640 medium supplemented with 20% fetal calf serum. For dose-response curves, we grew 3×10^5 cells per ml in 24-well plates with different concentrations of imatinib and determined the number of viable cells initially and after 48 h of incubation. We calculated the percentage of viable cells relative to the control (no imatinib) at each time point for three independent wells.

For western blotting, we cultured the cell lines in the presence of different concentrations of imatinib for 2 h, pelleted them and lysed them in cold lysis buffer containing 1 mM $NaVO_4$ and protease inhibitors. We separated the proteins on NuPAGE Tris-Acetate gels (Invitrogen) and transferred them to PVDF membranes. We used antibodies directed to phosphorylated ABL1, ABL1, phosphorylated CRKL and CRKL (Cell Signaling). Peroxidase-labeled antibodies to mouse or rabbit Ig were from AP Biotech. Antibody to NUP214, directed against the C-terminal part of NUP214 (ref. 25), was provided by G. Grosveld (St Jude Children's Research Hospital, Memphis, Tennessee, USA).

PCR. We carried out RACE as described previously[26]. We synthesized cDNA with ABL1-R1 (primer sequences available on request) and then carried out PCR with the adaptor primers[26] and the nested *ABL1* primers ABL1-R2 and ABL1-R3. We then cloned and sequenced the PCR products. We carried out direct RT-PCR to screen for the presence of *NUP214-ABL1* transcripts using the primers NUP20 (exon 20), NUP28 (exon 28), NUP29 (exon 29) and NUP31 (exon 31), combined with ABL1-R2 and ABL1-R3. All PCR products were sequenced. As control for RNA quality, we amplified *ZNF384* or *ABL1* using the primer pairs ZNF384-F and ZNF384-R, and ABL1-FA2 (exon 2) and ABL1-RA3 (exon 3), respectively.

Northern blotting. We separated 10 μg of total RNA on a 1% formaldehyde agarose gel and transferred it to a nylon filter (Gene Screen, DuPont). We hybridized the filter with a random-primed P^{32}-dCTP labeled probe spanning *ABL1* exons 2 and 3.

URLs. Locations of genes and probes, and gene structures were determined based on Ensembl data (http://www.ensembl.org). Sequence similarities were analyzed using the BLAST algorithm at http://www.ncbi.nlm.nih.gov/BLAST. The BAC clones were obtained from http://bacpac.chori.org.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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