Deep Insight Section

LDI-PCR in Cancer Translocation Mapping

Björn Schneider, Hans G Drexler, Roderick AF MacLeod

DSMZ, German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Inhoffenstr. 7b, 38124 Braunschweig, Germany (BS, HGD, RAFM)

Abstract

Identification of genes in oncogenic chromosome translocations by Fluorescence In Situ Hybridization (FISH) screening using genomic tilepath clones, is often laborious, notably if the region of interest is gene-dense. Other molecular methods for partner identification also suffer limitations; for instance, genomic PCR screening requires prior knowledge of both sets of breakpoints, while Rapid Amplification of cDNA Ends (RACE) is limited to translocations causing mRNA fusion and delivers no breakpoint data. With Long Distance Inverse (LDI)-PCR, however, it is possible to identify unknown translocation partners and to map breakpoints at the base-pair level. Applying LDI-PCR merely requires approximate sequence information on one partner, rendering it ideal for use in combination with FISH to extend and refine cytogenetic breakpoint data.

Introduction

Recurrent chromosomal rearrangements characterize many different types of cancer.

Specific cytogenetic translocations are key events, widely considered to be diagnostically and prognostically significant in leukemia and lymphoma, and increasingly so in solid tumors (Mitelman et al., 2007). Hitherto, most cancer genes have been identified following analysis of recurrent chromosome translocations (Futreal et al., 2004). The pathological significance and usefulness of such rearrangements depend on two key features: a) whether rearrangements display distinct patterns of recurrence within specific tumors, e.g. t(8;14)(q24;q32) which is restricted to B-cell neoplasia; and b) how clustered are the chromosomal breakpoints therein. The significance accorded to breakpoint data depends on their ascertainment precision, from megabase- and kilobase-, down to single base-pair levels, when ascertained by classical cytogenetics, fluorescence in situ hybridization (FISH), and sequence-based methods, respectively.

Chromosome translocations fall into three broad categories. The first causes the physical fusion of the two mRNAs expressed by the participating genes, thus creating novel fusion proteins translated from exons emanating from both genes, e.g. BCR (at [chromosome]-22-[band]-q11) with ABL1 (at 9q34) fused by t(9;22)(q34;q11) in chronic myeloid leukemia (CML) and in some cases of acute lymphoblastic leukemias (ALL) (Turhan, 2008a,b). The second category also fuses mRNA from genes at separate loci but, in this case, serving to deregulate a developmentally silenced partner by exchanging promoters with more active partners, e.g. BCL6 (at 3q27) which is activated by translocations with any one of many partners, chiefly in diffuse large B-cell lymphoma (DLBCL) (Knezevich, 2007).

The third class of chromosome translocation again results in the activation of the normally silent partner, this time by juxtaposition with another constitutively active partner without mRNA fusion, e.g. the neighboring homeobox genes, TLX3 (at 5q35.1) and NKX2-5 (at 5q35.2). According to the proximity of the breakpoint involved, either (but not both) genes may be activated in T-cell ALL by the recurrent t(5;14)(q35;q32.2) by which these are juxtaposed with regulatory regions from BCL11B (at 14q32.2) to stimulate transcription (Bernard et al., 2001; MacLeod et al., 2003; Nagel et al., 2007).

Some "promiscuous" genes engage with multiple partners: notably MLL with 64 known partners (Meyer

et al., 2009b), BCL6 with 28 (Knezevich, 2007), RUNX1 with 39 (Huret and Senon, 2003), NUP98 with 29 (Kearney, 2002), and the IgH-locus with 40 (Lefranc, 2003).

Although, promiscuity may reflect the dependence of tumors on the inappropriate oncogene expression without overly caring how deregulation is accomplished, the role of the partner genes has come under renewed scrutiny. Choice of partner gene may not only reveal in which types of precancerous cells primary oncogenic rearrangements occur, but also by looking for conserved DNA or protein motifs, yield clues to the mechanisms underlying their formation or their functional contribution to neoplasia. In addition, the roles of biologically important genes, e.g. BCL11B, a key regulator of both differentiation and survival during thymocyte development, are often first rendered visible by their participation in cancer rearrangements (MacLeod et al., 2003).

Both the identities of each partner gene and their precise breakpoints at the DNA base-pair level, may be useful not only to characterize potential fusion genes/products but also to ascertain whether additional non protein-coding genomic entities, such as chromosomal fragile sites (Schneider et al., 2008), microRNA loci, putative genes, unspliced "expressed sequence tags", or regulatory non-coding regions may be involved.

Clues to the biological mechanisms generating chromosome rearrangements are given by breakpoint sequences, including T- and B-cell receptor gene (VDJ) rearrangement, Alu-mediated recombination, non-homologous end joining, etc. VDJ genes are flanked by recombination signal sequences composed of heptamers followed, in turn, by a spacer containing either 12 or 23 unconserved nucleotides and a conserved nonamer. Spacers of 12 nucleotides undergo physiological recombination with those containing 23 in order to obey the so-called "12/23 rule". The presence of these or related sequences has been reported in connection with cancer translocations to reveal how physiologic processes may be abused to cause genomic rearrangements (Gu et al., 1992).

Genomic fusion sequences may also be used to aid cell line authentication - an omnipresent problem confronting cell culturists, given that an unexpectedly (and unacceptably) high percentage of new cell lines has been misidentified, or cross-contaminated by older cell lines (MacLeod et al., 1999). While mRNA fusion sequences are constrained by splicing, their genomic equivalents allow sufficient variation to provide "fingerprints" unique to individual cell lines to serve as potential identifiers. For analyzing patient tumors, knowledge of the exact fusion sequence allows design of patient-specific quantitative (q)PCR used for monitoring minimal residual disease with high sensitivity (Burmeister et al., 2006), to follow up therapeutic responses thus enabling early detection of relapse.
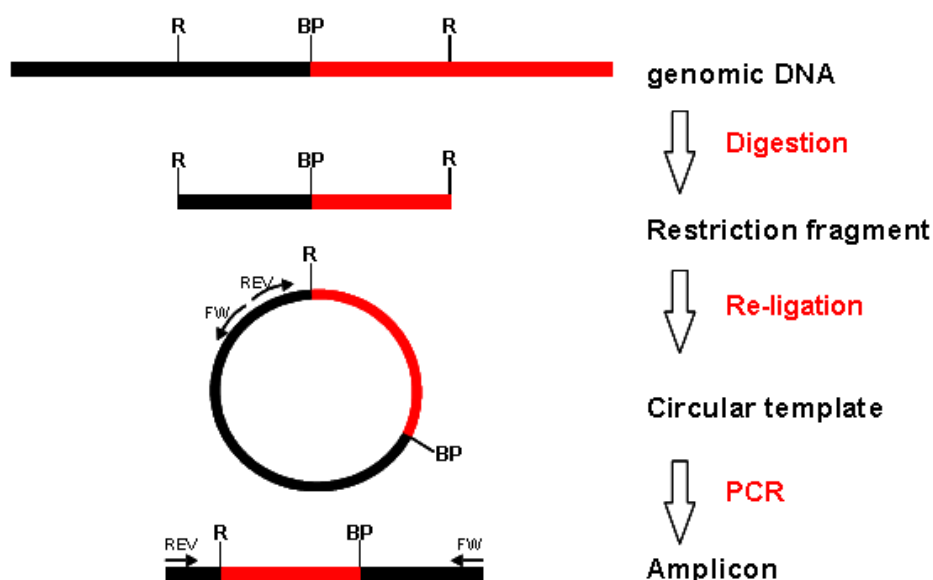

Figure 1: Amplifying Genomic Fusions of Unknown Sequence. The schema summarizes how the genomic DNA is first restricted, then re-ligated to the circular template, and how the resulting amplicon should appear. Note unknown region (red) flanked by known sequences (black). R: restriction site, BP: breakpoint; arrows: forward (FW) and reverse (REV) primers.

Cancer gene promiscuity enables oncogenic chromosome rearrangements, "smoking guns" of cancer genes, to be distinguished from random changes. FISH is initially used to confirm rearrangement of a contextually appropriate oncogene residing at the locus in question. Hence, a breakpoint at 9q34 might throw suspicion onto NOTCH1 in a T-cell-, ABL1 in myeloid- neoplasia, and NUP214 in either entity. While such an approach is less helpful among solid tumors where oncogene rearrangements are less informative, at this locus TSC1 might be deemed a candidate in tuberous sclerosis cells. Even when the index breakpoint is precisely known, determination of its partner by FISH requires time-consuming and laborious procedures for those not afforded immediate blanket tilepath-clone coverage with which to quarter the region of interest.

PCR screening with hit-lists of known and potential partner genes is quite as laborious as FISH, and is liable to miss unknown translocation partners, or those with breakpoints lying outside their respective cluster regions. When there are grounds to suspect transcriptional fusion (as among partners of genes prone to this type of gene rearrangement, e.g. ABL1, ETV6, NUP98, etc.) a mRNA-based method, rapid amplification of cDNA ends (RACE), may be used to detect novel fusion partners (Frohman et al., 1988). A drawback of RACE is its inability to supply genomic breakpoint data, and the risk of overlooking some splice variants. Hence, the technique of choice for identifying unknown partner genes and their breakpoints should not require prior knowledge of the partner gene, yet provide breakpoint data at the DNA base pair level.

Long Distance Inverse (LDI)-PCR satisfies these needs. LDI-PCR was developed from the earlier inverse-PCR (Ochman et al., 1988) to allow the amplification of large DNA fragments comprised of known and unknown sequences (Willis et al., 1997) using re-ligated circular restriction fragments as templates. Primers are set in opposition within the known sequence. The unknown sequence is flanked on both sides by known sequences following re-ligation in the resultant amplicon (Fig. 1). When a restriction fragment length polymorphism (RFLP) distinguishing the wild type and derivative alleles is generated by the genomic alteration, the two resulting amplicons should be separable by gel electrophoresis, enabling their respective sequences to be compared (Fig. 2). Sequencing with one of the PCR-primers directed towards the restriction site allows immediate identification of the partner gene. Sequencing in the other direction allows precise mapping of the breakpoint.
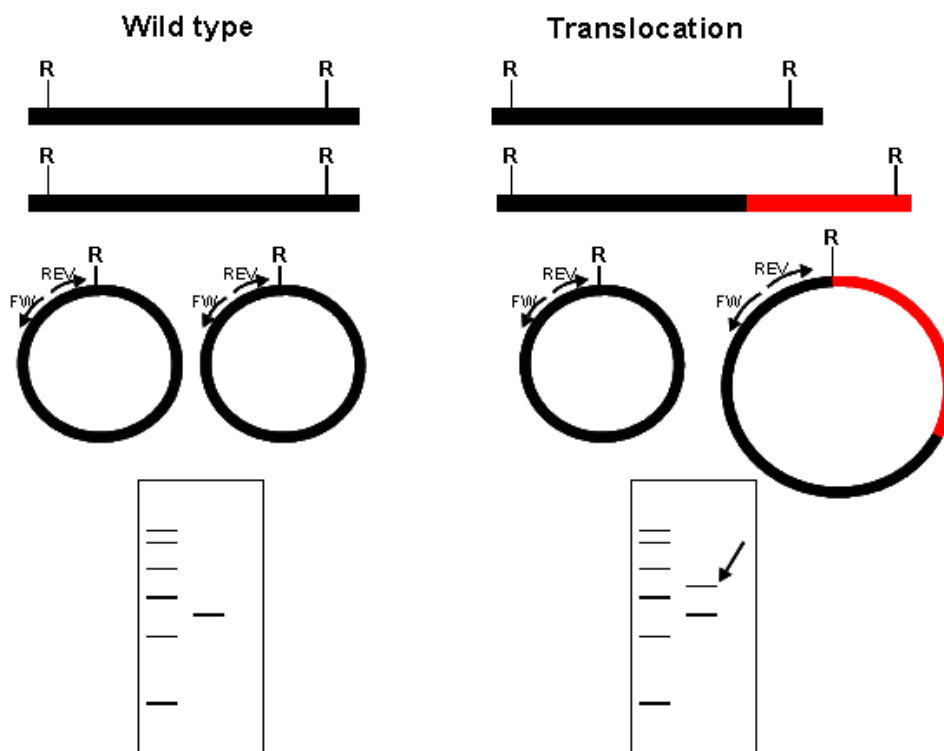

Figure 2: How to Interpret LDI-PCR Gels. Left figure shows the wild type configuration where twin circular templates identical in size would yield a single band by agarose gel electrophoresis. Translocation bearing cells (right figure) yield both wild type and derivative templates, differing in size and detectible as two bands on the gel. The derivative band is indicated by an arrow. Known regions are outlined in black, unknown in red. R: Restriction site, REV: reverse primer, FW: forward primer.

While easier to perform for genes with well defined, short breakpoint cluster regions, LDI-PCR may be applied to any gene or region involved in a translocation and has, therefore, been applied to a wide variety of translocations involving both frequently rearranged promiscuous oncogenes, but also as single events. Table 1 gives an overview of genes analyzed by LDI-PCR according to literature databases and the respective references.

Limitations are set by the performance of the DNA polymerase since lengthier fragments may resist amplification, and by the placement of the RFLP, as fragments similar in size cannot be readily distinguished by gel electrophoresis. If primary patient tumor material is analyzed, it should be noted that the samples used for analysis not only contain tumor material, but also normal bystander cells devoid of tumor rearrangement. Detection attempted at lower tumor infiltration rates risk false negative results.

In contrast to other PCR methods suitable for detection of unknown fusion sequences, such as panhandle PCR (Megonigal et al., 2000) or analogous techniques requiring adaptor ligations (reviewed in Tonooka and Fujishima, 2009), LDI-PCR is independent of any additional adaptors or anchors which have to be ligated to the restricted fragments, reducing the number of steps required, while remaining sufficiently flexible to allow a wide choice of restriction enzymes.

In the future, translocation analysis by next generation sequencing should overcome these limitations and suitable algorithms have been developed to recognize novel derivative breakpoint-flanking sequences and thereby identify novel cancer translocations and other synonymous rearrangements, including a subset of fusogenic microdeletions (Campbell et al., 2008).

Methology

In principle, LDI-PCR utilizes digested and re-ligated circular templates, which are of different sizes, due to RFLP caused by genomic rearrangements. This size difference renders the amplicons separable by gel electrophoresis (Fig. 2).

For a successful analysis, the LDI-PCR has to be designed carefully. The sequence covering the genomic region of interest should be selected from a genome browser (ENSEMBL, UCSC, NCBI) and then pasted into the query box of a restriction map generator (BioEdit, multiple online tools: SMS, RestrictionMapper). Restriction enzymes should be chosen to yield fragments in a size range of 2-5 kb. If using a double-digest strategy with enzymes producing sticky ends, these ends must be compatible. Ensure that both enzymes perform well in the same buffer and at the same temperature. Primer pairs have to be designed in such a way that one primer is directed towards the restriction site, the other one in the opposite direction (see Figures). The sequence lying between the primer tails is not subject to amplification, so the gap should not be excessive, ideally 30-50 bp. A breakpoint lying therein cannot be detected unless another primer pair, e.g. at the other end of the restriction fragment is used. For longer fragments (greater than 5 kb, say) use of a primer set consisting of one forward and multiple reverse primers (or vice versa) can be helpful. The oligonucleotides should be ~30 bp with a Tm ~65°C and a GC-content of 40-60%.

For LDI-PCR template preparation high quality genomic DNA should be used, meaning high purity (260/280 1.8-2.0 and 260/230 > 2) and high integrity without degradation. One microgram of DNA is then digested with 30-50 U of each restriction enzyme in the presence of the appropriate digestion buffer in a total volume of 100 μl for 3-4 h at the temperature suitable for the chosen enzymes (mostly 37°C), followed by heat inactivation (where applicable) and purification, preferably with a column based purification kit. Phenol / chloroform purification followed by precipitation may also be performed, but residual phenol can disturb downstream processes. To form the circular templates, the restriction fragments are then religated with 5 U T4 ligase overnight at 4-8°C in a total volume of 80 μL, terminated by heat inactivation. These conditions favor the desired self-ligation.

The PCR is performed best using a PCR kit suitable for amplification of long templates and using 5 μL (62.5 ng) of the digested and re-ligated DNA. The PCR products are analyzed by gel electrophoresis. Discrepant bands not corresponding to the calculated amplicon size may represent amplicons of translocated fragments. These are excised from the gel, purified and subjected to sequence analysis and, unless artefacts, may reveal the translocation partner and the exact breakpoint of the rearrangement subject to analysis.

Gene	No. of Partners	References describing LDI-PCR analysis
ALK	14 (Allouche, 2010)	Ma et al., 2000
ANTXR1	1 (Oberthuer et al., 2005)	Oberthuer et al., 2005
API2	1 (Mathijs and Marynen, 2001)	Dierlamm et al., 1999
BCL6	28 (Knezevich, 2007)	Akasaka H et al., 2000; Akasaka T et al., 2000; Kurata et al., 2002; Akasaka et al., 2003; Chen et al., 2003; Montesinos-Rongen et al., 2003; Chen et al., 2006; Schneider et al., 2008
BRD4	1 (Collin, 2007)	Haruki et al., 2005
CALL	1 (Frints et al., 2003)	Frints et al., 2003
E2A	5 (Huret, 1997)	Wiemels et al., 2002a
ETV6	28 (Knezevich, 2005)	Wiemels et al., 1999a; Wiemels et al., 1999b; Wiemels and Greaves, 1999; Wiemels et al., 2008
IGH	40 (Lefranc, 2003)	Willis et al., 1997; Willis et al., 1998; Nardini et al., 2000; Satterwhite et al., 2001; Sonoki et al., 2001; Bichi et al., 2002; Sanchez-Izquierdo et al., 2003; Sonoki et al., 2004; Akasaka et al., 2007; Lenz et al., 2007; Souabni et al., 2007; d'Amore et al., 2008; Ishizaki et al., 2008; Russell et al., 2008; Vieira et al., 2008; Vinatzer et al., 2008; Nagel et al., 2009; Russell et al., 2009; Yin et al., 2009; Hu et al., 2010
let-7a-2, miR-100	1 (Bousquet, 2008)	Bousquet et al., 2008
MLH1	1 (Meyer et al., 2009b)	Meyer et al., 2009a
MLL	64 (Meyer et al., 2009b)	Blanco et al., 2001; Meyer et al., 2005; Teuffel et al., 2005; Attarbaschi et al., 2006; Burmeister et al., 2006; Matsuda et al., 2006; Meyer et al., 2006a; Meyer et al., 2006b; Strehl et al., 2006; Burmeister et al., 2008; Balgobind et al., 2009; Bueno et al., 2009; Burmeister et al., 2009; Matsuda et al., 2009; Meyer and Marschalek, 2009; Cóser et al., 2010; De Braekeleer et al., 2010; Lee et al., 2010
NOTCH1	2 (Suzuki et al., 2009)	Suzuki et al., 2009
PAX5	6 (Strehl, 2005)	An et al., 2008; An et al., 2009
PDGFRA	6 (Dessen, 2009)	Cools et al., 2003
PDGFRB	20 (Vizmanos, 2005)	Walz et al., 2007; Walz et al., 2009
RUNX1 / AML1	39 (Huret and Senon, 2003)	Xiao et al., 2001; Wiemels et al., 2002b

Table 1: Genes involved in translocations analyzed by LDI-PCR, numbers of yet known translocation partner genes and references wherein the analysis of the particular gene is described.

References

Frohman MA, Dush MK, Martin GR. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc Natl Acad Sci U S A. 1988 Dec;85(23):8998-9002

Ochman H, Gerber AS, Hartl DL. Genetic applications of an inverse polymerase chain reaction. Genetics. 1988 Nov;120(3):621-3

Gu Y, Cimino G, Alder H, Nakamura T, Prasad R, Canaani O, Moir DT, Jones C, Nowell PC, Croce CM. The (4;11)(q21;q23) chromosome translocations in acute leukemias involve the VDJ recombinase. Proc Natl Acad Sci U S A. 1992 Nov 1;89(21):10464-8

Huret JL.. TCF3 (transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)). Atlas Genet Cytogenet Oncol Haematol. December 1997.URL: http://AtlasGeneticsOncology.org/Genes/E2A.html

Willis TG, Jadayel DM, Coignet LJ, Abdul-Rauf M, Treleaven JG, Catovsky D, Dyer MJ. Rapid molecular cloning of rearrangements of the IGHJ locus using long-distance inverse polymerase chain reaction. Blood. 1997 Sep 15;90(6):2456-64

Willis TG, Zalcberg IR, Coignet LJ, Wlodarska I, Stul M, Jadayel DM, Bastard C, Treleaven JG, Catovsky D, Silva ML, Dyer MJ. Molecular cloning of translocation t(1;14)(q21;q32) defines a novel gene (BCL9) at chromosome 1q21. Blood. 1998 Mar 15;91(6):1873-81

Dierlamm J, Baens M, Wlodarska I, Stefanova-Ouzounova M, Hernandez JM, Hossfeld DK, De Wolf-Peeters C, Hagemeijer A, Van den Berghe H, Marynen P. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood. 1999 Jun 1;93(11):3601-9

MacLeod RA, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. Int J Cancer. 1999 Nov 12;83(4):555-63

Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, Saha V, Biondi A, Greaves MF. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet. 1999 Oct 30;354(9189):1499-503

Wiemels JL, Ford AM, Van Wering ER, Postma A, Greaves M. Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion in utero. Blood. 1999 Aug 1;94(3):1057-62

Wiemels JL, Greaves M. Structure and possible mechanisms of TEL-AML1 gene fusions in childhood acute lymphoblastic leukemia. Cancer Res. 1999 Aug 15;59(16):4075-82

Akasaka H, Akasaka T, Kurata M, Ueda C, Shimizu A, Uchiyama T, Ohno H. Molecular anatomy of BCL6 translocations revealed by long-distance polymerase chain reaction-based assays. Cancer Res. 2000 May 1;60(9):2335-41

Akasaka T, Ueda C, Kurata M, Akasaka H, Yamabe H, Uchiyama T, Ohno H. Nonimmunoglobulin (non-Ig)/BCL6 gene fusion in diffuse large B-cell lymphoma results in worse prognosis than Ig/BCL6. Blood. 2000 Oct 15;96(8):2907-9

Ma Z, Cools J, Marynen P, Cui X, Siebert R, Gesk S, Schlegelberger B, Peeters B, De Wolf-Peeters C, Wlodarska I, Morris SW. Inv(2)(p23q35) in anaplastic large-cell lymphoma induces constitutive anaplastic lymphoma kinase (ALK) tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis. Blood. 2000 Mar 15;95(6):2144-9

Megonigal MD, Rappaport EF, Wilson RB, Jones DH, Whitlock JA, Ortega JA, Slater DJ, Nowell PC, Felix CA. Panhandle PCR for cDNA: a rapid method for isolation of MLL fusion transcripts involving unknown partner genes. Proc Natl Acad Sci U S A. 2000 Aug 15;97(17):9597-602

Nardini E, Aiello A, Giardini R, Colnaghi MI, Ménard S, Balsari A. Detection of aberrant isotype switch recombination in low-grade and high-grade gastric MALT lymphomas. Blood. 2000 Feb 1;95(3):1032-8

Bernard OA, Busson-LeConiat M, Ballerini P, Mauchauffé M, Della Valle V, Monni R, Nguyen Khac F, Mercher T, Penard-Lacronique V, Pasturaud P, Gressin L, Heilig R, Daniel MT, Lessard M, Berger R. A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. Leukemia. 2001 Oct;15(10):1495-504

Blanco JG, Dervieux T, Edick MJ, Mehta PK, Rubnitz JE, Shurtleff S, Raimondi SC, Behm FG, Pui CH, Relling MV. Molecular emergence of acute myeloid leukemia during treatment for acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2001 Aug 28;98(18):10338-43

Mathijs B, Marynen P.. BIRC3 baculoviral IAP repeat-containing 3. Atlas Genet Cytogenet Oncol Haematol. September 2001. URL: http://AtlasGeneticsOncology.org/Genes/BIRC3ID239.html

Satterwhite E, Sonoki T, Willis TG, Harder L, Nowak R, Arriola EL, Liu H, Price HP, Gesk S, Steinemann D, Schlegelberger B, Oscier DG, Siebert R, Tucker PW, Dyer MJ. The BCL11 gene family: involvement of BCL11A in lymphoid malignancies. Blood. 2001 Dec 1;98(12):3413-20

Sonoki T, Harder L, Horsman DE, Karran L, Taniguchi I, Willis TG, Gesk S, Steinemann D, Zucca E, Schlegelberger B, Solé F, Mungall AJ, Gascoyne RD, Siebert R, Dyer MJ. Cyclin D3 is a target gene of t(6;14)(p21.1;q32.3) of mature B-cell malignancies. Blood. 2001 Nov 1;98(9):2837-44

Xiao Z, Greaves MF, Buffler P, Smith MT, Segal MR, Dicks BM, Wiencke JK, Wiemels JL. Molecular characterization of genomic AML1-ETO fusions in childhood leukemia. Leukemia. 2001 Dec;15(12):1906-13

Bichi R, Shinton SA, Martin ES, Koval A, Calin GA, Cesari R, Russo G, Hardy RR, Croce CM. Human chronic lymphocytic leukemia modeled in mouse by targeted TCL1 expression. Proc Natl Acad Sci U S A. 2002 May 14;99(10):6955-60

Kearney L.. NUP98 (nucleoporin 98 kDa). Atlas Genet Cytogenet Oncol Haematol. March 2002. URL: http://AtlasGeneticsOncology.org/Genes/NUP98.html

Kurata M, Maesako Y, Ueda C, Nishikori M, Akasaka T, Uchiyama T, Ohno H. Characterization of t(3;6)(q27;p21) breakpoints in B-cell non-Hodgkin's lymphoma and construction of the histone H4/BCL6 fusion gene, leading to altered expression of Bcl-6. Cancer Res. 2002 Nov 1;62(21):6224-30

Wiemels JL, Leonard BC, Wang Y, Segal MR, Hunger SP, Smith MT, Crouse V, Ma X, Buffler PA, Pine SR. Site-specific translocation and evidence of postnatal origin of the t(1;19) E2A-PBX1 fusion in childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2002 Nov 12;99(23):15101-6

Wiemels JL, Xiao Z, Buffler PA, Maia AT, Ma X, Dicks BM, Smith MT, Zhang L, Feusner J, Wiencke J, Pritchard-Jones K, Kempski H, Greaves M. In utero origin of t(8;21) AML1-ETO translocations in childhood acute myeloid leukemia. Blood. 2002 May 15;99(10):3801-5

Akasaka T, Lossos IS, Levy R. BCL6 gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood. 2003 Aug 15;102(4):1443-8

Chen YW, Liang AC, Au WY, Chu KM, Wong KY, Hu X, Lu L, Tang JC, Chan KW, Beh SL, Kwong YL, Liang RH, Srivastava G. Multiple BCL6 translocation partners in individual cases of gastric lymphoma. Blood. 2003 Sep 1;102(5):1931-2; author reply 1932

Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, Griffin JD, Cross NC, Tefferi A, Malone J, Alam R, Schrier SL, Schmid J, Rose M, Vandenberghe P, Verhoef G, Boogaerts M, Wlodarska I, Kantarjian H, Marynen P, Coutre SE, Stone R, Gilliland DG. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003 Mar 27;348(13):1201-14

Frints SG, Marynen P, Hartmann D, Fryns JP, Steyaert J, Schachner M, Rolf B, Craessaerts K, Snellinx A, Hollanders K, D'Hooge R, De Deyn PP, Froyen G. CALL interrupted in a patient with non-specific mental retardation: gene dosage-dependent alteration of murine brain development and behavior. Hum Mol Genet. 2003 Jul 1;12(13):1463-74

Huret JL, Senon S.. RUNX1 (runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)). Atlas Genet Cytogenet Oncol Haematol. January 2003. URL: http://AtlasGeneticsOncology.org/Genes/AML1.html

Lefranc MP.. IGH@ (Immunoglobulin Heavy). Atlas Genet Cytogenet Oncol Haematol. September 2003. URL: http://AtlasGeneticsOncology.org/Genes/IgHID40.html

MacLeod RA, Nagel S, Kaufmann M, Janssen JW, Drexler HG. Activation of HOX11L2 by juxtaposition with 3'-BCL11B in an acute lymphoblastic leukemia cell line (HPB-ALL) with t(5;14)(q35;q32.2). Genes Chromosomes Cancer. 2003 May;37(1):84-91

Montesinos-Rongen M, Akasaka T, Zühlke-Jenisch R, Schaller C, Van Roost D, Wiestler OD, Siebert R, Deckert M. Molecular characterization of BCL6 breakpoints in primary diffuse large B-cell lymphomas of the central nervous system identifies GAPD as novel translocation partner. Brain Pathol. 2003 Oct;13(4):534-8

Sanchez-Izquierdo D, Buchonnet G, Siebert R, Gascoyne RD, Climent J, Karran L, Marin M, Blesa D, Horsman D, Rosenwald A, Staudt LM, Albertson DG, Du MQ, Ye H, Marynen P, Garcia-Conde J, Pinkel D, Dyer MJ, Martinez-Climent JA. MALT1 is deregulated by both chromosomal translocation and amplification in B-cell non-Hodgkin lymphoma. Blood. 2003 Jun 1;101(11):4539-46

Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR. A census of human cancer genes. Nat Rev Cancer. 2004 Mar;4(3):177-83

Sonoki T, Willis TG, Oscier DG, Karran EL, Siebert R, Dyer MJ. Rapid amplification of immunoglobulin heavy chain switch (IGHS) translocation breakpoints using long-distance inverse PCR. Leukemia. 2004 Dec;18(12):2026-31

Haruki N, Kawaguchi KS, Eichenberger S, Massion PP, Gonzalez A, Gazdar AF, Minna JD, Carbone DP, Dang TP. Cloned fusion product from a rare t(15;19)(q13.2;p13.1) inhibit S phase in vitro. J Med Genet. 2005 Jul;42(7):558-64

Knezevich S.. ETV6 (ETS variant gene 6 (TEL oncogene)). Atlas Genet Cytogenet Oncol Haematol. June 2005. URL: http://AtlasGeneticsOncology.org/Genes/ETV6ID38.html

Meyer C, Schneider B, Reichel M, Angermueller S, Strehl S, Schnittger S, Schoch C, Jansen MW, van Dongen JJ, Pieters R, Haas OA, Dingermann T, Klingebiel T, Marschalek R. Diagnostic tool for the identification of MLL rearrangements including unknown partner genes. Proc Natl Acad Sci U S A. 2005 Jan 11;102(2):449-54

Oberthuer A, Skowron M, Spitz R, Kahlert Y, Westermann F, Mehler K, Berthold F, Fischer M. Characterization of a complex genomic alteration on chromosome 2p that leads to four alternatively spliced fusion transcripts in the neuroblastoma cell lines IMR-5, IMR-5/75 and IMR-32. Gene. 2005 Dec 19;363:41-50

Strehl S.. PAX5 (paired box gene 5). Atlas Genet Cytogenet Oncol Haematol. August 2005. URL: http://AtlasGeneticsOncology.org/Genes/PAX5ID62.html

Teuffel O, Betts DR, Thali M, Eberle D, Meyer C, Schneider B, Marschalek R, Trakhtenbrot L, Amariglio N, Niggli FK, Schäfer BW. Clonal expansion of a new MLL rearrangement in the absence of leukemia. Blood. 2005 May 15;105(10):4151-2

Vizmanos JL.. PDGFRB (platelet-derived growth factor receptor, beta polypeptide). Atlas Genet Cytogenet Oncol Haematol. July 2005. URL: http://AtlasGeneticsOncology.org/Genes/PDGFRBID21ch5q32.html

Attarbaschi A, Mann G, König M, Steiner M, Strehl S, Schreiberhuber A, Schneider B, Meyer C, Marschalek R, Borkhardt A, Pickl WF, Lion T, Gadner H, Haas OA, Dworzak MN. Mixed lineage leukemia-rearranged childhood pro-B and CD10-negative pre-B acute lymphoblastic leukemia constitute a distinct clinical entity. Clin Cancer Res. 2006 May 15;12(10):2988-94

Burmeister T, Marschalek R, Schneider B, Meyer C, Gökbuget N, Schwartz S, Hoelzer D, Thiel E. Monitoring minimal residual disease by quantification of genomic chromosomal breakpoint sequences in acute leukemias with MLL aberrations. Leukemia. 2006 Mar;20(3):451-7

Chen YW, Hu XT, Liang AC, Au WY, So CC, Wong ML, Shen L, Tao Q, Chu KM, Kwong YL, Liang RH, Srivastava G. High BCL6 expression predicts better prognosis, independent of BCL6 translocation status, translocation partner, or BCL6-deregulating mutations, in gastric lymphoma. Blood. 2006 Oct 1;108(7):2373-83

Matsuda K, Hidaka E, Ishida F, Yamauchi K, Makishima H, Ito T, Suzuki T, Imagawa E, Sano K, Katsuyama T, Ota H. A case of acute myelogenous leukemia with MLL-AF10 fusion caused by insertion of 5' MLL into 10p12, with concurrent 3' MLL deletion. Cancer Genet Cytogenet. 2006 Nov;171(1):24-30

Meyer C, Kowarz E, Schneider B, Oehm C, Klingebiel T, Dingermann T, Marschalek R. Genomic DNA of leukemic patients: target for clinical diagnosis of MLL rearrangements. Biotechnol J. 2006 Jun;1(6):656-63

Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S, Schoch C, Jansen MW, van Dongen JJ, den Boer ML, Pieters R, Ennas MG, Angelucci E, Koehl U, Greil J, Griesinger F, Zur Stadt U, Eckert C, Szczepański T, Niggli FK, Schäfer BW, Kempski H, Brady HJ, Zuna J, Trka J, Nigro LL, Biondi A, Delabesse E, Macintyre E, Stanulla M, Schrappe M, Haas OA, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. The MLL recombinome of acute leukemias. Leukemia. 2006 May;20(5):777-84

Strehl S, König M, Meyer C, Schneider B, Harbott J, Jäger U, von Bergh AR, Loncarevic IF, Jarosova M, Schmidt HH, Moore SD, Marschalek R, Haas OA. Molecular dissection of t(11;17) in acute myeloid leukemia reveals a variety of gene fusions with heterogeneous fusion transcripts and multiple splice variants. Genes Chromosomes Cancer. 2006 Nov;45(11):1041-9

Akasaka T, Balasas T, Russell LJ, Sugimoto KJ, Majid A, Walewska R, Karran EL, Brown DG, Cain K, Harder L, Gesk S, Martin-Subero JI, Atherton MG, Brüggemann M, Calasanz MJ, Davies T, Haas OA, Hagemeijer A, Kempski H, Lessard M, Lillington DM, Moore S, Nguyen-Khac F, Radford-Weiss I, Schoch C, Struski S, Talley P, Welham MJ, Worley H, Strefford JC, Harrison CJ, Siebert R, Dyer MJ. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2007 Apr 15;109(8):3451-61

Collin A.. BRD4 (bromodomain containing 4). Atlas Genet Cytogenet Oncol Haematol. February 2007. URL: http://AtlasGeneticsOncology.org/Genes/BRD4ID837ch19p13.html

Knezevich S.. BCL6 (B-Cell Lymphoma 6). Atlas Genet Cytogenet Oncol Hematol. 2007. URL: http://AtlasGeneticsOncology.org/Genes/BCL6ID20.html

Lenz G, Nagel I, Siebert R, Roschke AV, Sanger W, Wright GW, Dave SS, Tan B, Zhao H, Rosenwald A, Muller-Hermelink HK, Gascoyne RD, Campo E, Jaffe ES, Smeland EB, Fisher RI, Kuehl WM, Chan WC, Staudt LM. Aberrant immunoglobulin class switch recombination and switch translocations in activated B cell-like diffuse large B cell lymphoma. J Exp Med. 2007 Mar 19;204(3):633-43

Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer. 2007 Apr;7(4):233-45

Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, MacLeod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and

coregulation by PU.1 and HMGA1. Cancer Res. 2007 Feb 15;67(4):1461-71

Souabni A, Jochum W, Busslinger M. Oncogenic role of Pax5 in the T-lymphoid lineage upon ectopic expression from the immunoglobulin heavy-chain locus. Blood. 2007 Jan 1;109(1):281-9

Walz C, Metzgeroth G, Haferlach C, Schmitt-Graeff A, Fabarius A, Hagen V, Prümmer O, Rauh S, Hehlmann R, Hochhaus A, Cross NC, Reiter A. Characterization of three new imatinib-responsive fusion genes in chronic myeloproliferative disorders generated by disruption of the platelet-derived growth factor receptor beta gene. Haematologica. 2007 Feb;92(2):163-9

An Q, Wright SL, Konn ZJ, Matheson E, Minto L, Moorman AV, Parker H, Griffiths M, Ross FM, Davies T, Hall AG, Harrison CJ, Irving JA, Strefford JC. Variable breakpoints target PAX5 in patients with dicentric chromosomes: a model for the basis of unbalanced translocations in cancer. Proc Natl Acad Sci U S A. 2008 Nov 4;105(44):17050-4

Bousquet M, Quelen C, Rosati R, Mansat-De Mas V, La Starza R, Bastard C, Lippert E, Talmant P, Lafage-Pochitaloff M, Leroux D, Gervais C, Viguié F, Lai JL, Terre C, Beverlo B, Sambani C, Hagemeijer A, Marynen P, Delsol G, Dastugue N, Mecucci C, Brousset P. Myeloid cell differentiation arrest by miR-125b-1 in myelodysplastic syndrome and acute myeloid leukemia with the t(2;11)(p21;q23) translocation. J Exp Med. 2008 Oct 27;205(11):2499-506

Burmeister T, Meyer C, Thiel G, Reinhardt R, Thiel E, Marschalek R. A MLL-KIAA0284 fusion gene in a patient with secondary acute myeloid leukemia and t(11;14)(q23;q32). Blood Cells Mol Dis. 2008 Sep-Oct;41(2):210-4

Campbell PJ, Stephens PJ, Pleasance ED, O'Meara S, Li H, Santarius T, Stebbings LA, Leroy C, Edkins S, Hardy C, Teague JW, Menzies A, Goodhead I, Turner DJ, Clee CM, Quail MA, Cox A, Brown C, Durbin R, Hurles ME, Edwards PA, Bignell GR, Stratton MR, Futreal PA. Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. Nat Genet. 2008 Jun;40(6):722-9

d'Amore F, Chan E, Iqbal J, Geng H, Young K, Xiao L, Hess MM, Sanger WG, Smith L, Wiuf C, Hagberg O, Fu K, Chan WC, Dave BJ. Clonal evolution in t(14;18)-positive follicular lymphoma, evidence for multiple common pathways, and frequent parallel clonal evolution. Clin Cancer Res. 2008 Nov 15;14(22):7180-7

Ishizaki A, Sugahara K, Tsuruda K, Hasegawa H, Yanagihara K, Tsukasaki K, Yamada Y, Kamihira S. Usefulness of long-distance inverse polymerase chain reaction for molecular detection of 14q32 translocation in a clinical setting. Scand J Clin Lab Invest. 2008;68(7):519-25

Russell LJ, Akasaka T, Majid A, Sugimoto KJ, Loraine Karran E, Nagel I, Harder L, Claviez A, Gesk S, Moorman AV, Ross F, Mazzullo H, Strefford JC, Siebert R, Dyer MJ, Harrison CJ. t(6;14)(p22;q32): a new recurrent IGH@ translocation involving ID4 in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2008 Jan 1;111(1):387-91

Schneider B, Nagel S, Kaufmann M, Winkelmann S, Bode J, Drexler HG, MacLeod RA. T(3;7)(q27;q32) fuses BCL6 to a non-coding region at FRA7H near miR-29. Leukemia. 2008 Jun;22(6):1262-6

Turhan AG.. BCR (Breakpoint cluster region). Atlas Genet Cytogenet Oncol Haematol. 2008a. URL: http://AtlasGeneticsOncology.org/Genes/BCR.html

Turhan AG.. ABL1 (v-abl Abelson murine leukemia viral oncogene homolog 1). Atlas Genet Cytogenet Oncol Haematol. 2008b. URL:

http://AtlasGeneticsOncology.org/Genes/ABL.html

Vieira L, Martinho A, Antunes O, Silva E, Ambrósio AP, Geraldes MC, Nascimento R, Silva C, Pereira JM, Júnior EC, Jordan P. Combined molecular diagnosis of B-cell lymphomas with t(11;14)(q13;q32) or t(14;18)(q32;q21) using multiplex- and long distance inverse-polymerase chain reaction. Diagn Mol Pathol. 2008 Jun;17(2):73-81

Vinatzer U, Gollinger M, Müllauer L, Raderer M, Chott A, Streubel B. Mucosa-associated lymphoid tissue lymphoma: novel translocations including rearrangements of ODZ2, JMJD2C, and CNN3. Clin Cancer Res. 2008 Oct 15;14(20):6426-31

Wiemels JL, Hofmann J, Kang M, Selzer R, Green R, Zhou M, Zhong S, Zhang L, Smith MT, Marsit C, Loh M, Buffler P, Yeh RF. Chromosome 12p deletions in TEL-AML1 childhood acute lymphoblastic leukemia are associated with retrotransposon elements and occur postnatally. Cancer Res. 2008 Dec 1;68(23):9935-44

An Q, Wright SL, Moorman AV, Parker H, Griffiths M, Ross FM, Davies T, Harrison CJ, Strefford JC. Heterogeneous breakpoints in patients with acute lymphoblastic leukemia and the dic(9;20)(p11-13;q11) show recurrent involvement of genes at 20q11.21. Haematologica. 2009 Aug;94(8):1164-9

Balgobind BV, Zwaan CM, Meyer C, Marschalek R, Pieters R, Beverloo HB, Van den Heuvel-Eibrink MM. NRIP3: a novel translocation partner of MLL detected in a pediatric acute myeloid leukemia with complex chromosome 11 rearrangements. Haematologica. 2009 Jul;94(7):1033

Bueno C, Catalina P, Melen GJ, Montes R, Sánchez L, Ligero G, García-Pérez JL, Menendez P. Etoposide induces MLL rearrangements and other chromosomal abnormalities in human embryonic stem cells. Carcinogenesis. 2009 Sep;30(9):1628-37

Burmeister T, Meyer C, Schwartz S, Hofmann J, Molkentin M, Kowarz E, Schneider B, Raff T, Reinhardt R, Gökbuget N, Hoelzer D, Thiel E, Marschalek R. The MLL recombinome of adult CD10-negative B-cell precursor acute lymphoblastic leukemia: results from the GMALL study group. Blood. 2009 Apr 23;113(17):4011-5

Dessen P.. PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) Atlas Genet Cytogenet Oncol Haematol. 2009. URL: http://atlasgeneticsoncology.org/Genes/GC_PDGFRA.html

Matsuda K, Tanaka M, Araki S, Yanagisawa R, Yamauchi K, Koike K. Cryptic insertion into 11q23 of MLLT10 not involved in t(1;15;11;10)(p36;q11;q23;q24) in infant acute biphenotypic leukemia. Cancer Genet Cytogenet. 2009 Apr 15;190(2):113-20

Meyer C, Brieger A, Plotz G, Weber N, Passmann S, Dingermann T, Zeuzem S, Trojan J, Marschalek R. An interstitial deletion at 3p21.3 results in the genetic fusion of MLH1 and ITGA9 in a Lynch syndrome family. Clin Cancer Res. 2009 Feb 1;15(3):762-9

Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, Ben Abdelali R, Macintyre E, De Braekeleer E, De Braekeleer M, Delabesse E, de Oliveira MP, Cavé H, Clappier E, van Dongen JJ, Balgobind BV, van den Heuvel-Eibrink MM, Beverloo HB, Panzer-Grümayer R, Teigler-Schlegel A, Harbott J, Kjeldsen E, Schnittger S, Koehl U, Gruhn B, Heidenreich O, Chan LC, Yip SF, Krzywinski M, Eckert C, Möricke A, Schrappe M, Alonso CN, Schäfer BW, Krauter J, Lee DA, Zur Stadt U, Te Kronnie G, Sutton R, Izraeli S, Trakhtenbrot L, Lo Nigro L, Tsaur G, Fechina L, Szczepanski T, Strehl S, Ilencikova D, Molkentin M, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. New insights to the MLL

recombinome of acute leukemias. Leukemia. 2009 Aug;23(8):1490-9

Meyer C, Marschalek R. LDI-PCR: identification of known and unknown gene fusions of the human MLL gene. Methods Mol Biol. 2009;538:71-83

Nagel I, Akasaka T, Klapper W, Gesk S, Böttcher S, Ritgen M, Harder L, Kneba M, Dyer MJ, Siebert R. Identification of the gene encoding cyclin E1 (CCNE1) as a novel IGH translocation partner in t(14;19)(q32;q12) in diffuse large B-cell lymphoma. Haematologica. 2009 Jul;94(7):1020-3

Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, Chandrasekaran T, Chapiro E, Gesk S, Griffiths M, Guttery DS, Haferlach C, Harder L, Heidenreich O, Irving J, Kearney L, Nguyen-Khac F, Machado L, Minto L, Majid A, Moorman AV, Morrison H, Rand V, Strefford JC, Schwab C, Tönnies H, Dyer MJ, Siebert R, Harrison CJ. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009 Sep 24;114(13):2688-98

Suzuki S, Nagel S, Schneider B, Chen S, Kaufmann M, Uozumi K, Arima N, Drexler HG, MacLeod RA. A second NOTCH1 chromosome rearrangement: t(9;14)(q34.3;q11.2) in T-cell neoplasia. Leukemia. 2009 May;23(5):1003-6

Tonooka Y, Fujishima M. Comparison and critical evaluation of PCR-mediated methods to walk along the sequence of genomic DNA. Appl Microbiol Biotechnol. 2009 Nov;85(1):37-43

Walz C, Haferlach C, Hänel A, Metzgeroth G, Erben P, Gosenca D, Hochhaus A, Cross NC, Reiter A. Identification of a MYO18A-PDGFRB fusion gene in an eosinophilia-associated atypical myeloproliferative neoplasm with a t(5;17)(q33-34;q11.2). Genes Chromosomes Cancer. 2009 Feb;48(2):179-83

Yin CC, Lin KI, Ketterling RP, Knudson RA, Medeiros LJ, Barron LL, Huh YO, Luthra R, Keating MJ, Abruzzo LV. Chronic lymphocytic leukemia With t(2;14)(p16;q32) involves the BCL11A and IgH genes and is associated with atypical morphologic features and unmutated IgVH genes. Am J Clin Pathol. 2009 May;131(5):663-70

Allouche M.. ALK (anaplastic lymphoma receptor tyrosine kinase). Atlas Genet Cytogenet Oncol Haematol. February 2010. URL: http://AtlasGeneticsOncology.org/Genes/ALK.html

Cóser VM, Meyer C, Basegio R, Menezes J, Marschalek R, Pombo-de-Oliveira MS. Nebulette is the second member of the nebulin family fused to the MLL gene in infant leukemia. Cancer Genet Cytogenet. 2010 Apr 15;198(2):151-4

De Braekeleer E, Meyer C, Douet-Guilbert N, Morel F, Le Bris MJ, Berthou C, Arnaud B, Marschalek R, Férec C, De Braekeleer M. Complex and cryptic chromosomal rearrangements involving the MLL gene in acute leukemia: a study of 7 patients and review of the literature. Blood Cells Mol Dis. 2010 Apr 15;44(4):268-74

Hu XT, Chen YW, Liang AC, Au WY, Wong KY, Wan TS, Wong ML, Shen L, Chan KK, Guo T, Chu KM, Tao Q, Chim CS, Loong F, Choi WW, Lu L, So CC, Chan LC, Kwong YL, Liang RH, Srivastava G. CD44 activation in mature B-cell malignancies by a novel recurrent IGH translocation. Blood. 2010 Mar 25;115(12):2458-61

Lee SG, Park TS, Won SC, Song J, Lee KA, Choi JR, Marschalek R, Meyer C. Three-way translocation involving MLL, MLLT1, and a novel third partner, NRXN1, in a patient with acute lymphoblastic leukemia and t(2;19;11)(p12;p13.3;q23). Cancer Genet Cytogenet. 2010 Feb;197(1):32-8

This article should be referenced as such: