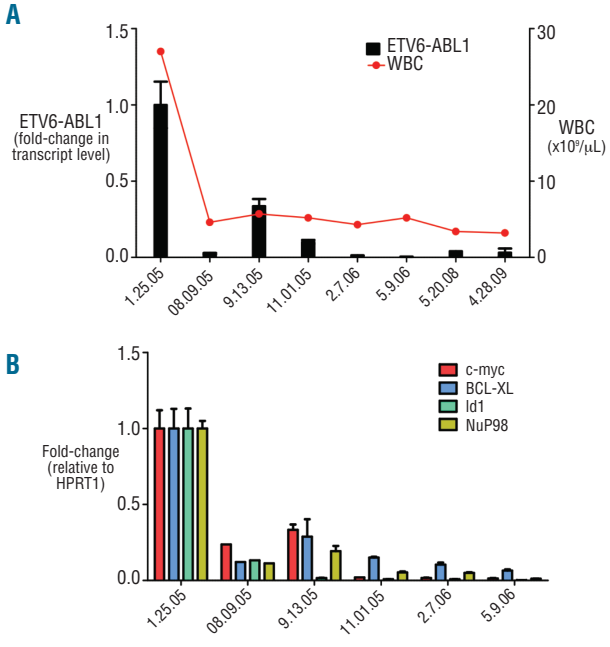

Figure 2. Response to Imatinib in an ETV6-ABL1 positive chronic myeloid leukemia. (A) White blood cell count and peripheral blood qRT-PCR analysis of ETV6-ABL1 transcript levels pre-imatinib and throughout imatinib treatment. (B) Concomitant with decreases in ETV6-ABL1 transcript levels, decreases in C-MYC, ID1, BCL-XL, and NUP-98 transcripts were also seen. The patient remains in hematological remission with no identifiable ETV6-ABL1 transcripts in the peripheral blood after approximately five years of imatinib.

do well on imatinib 400mg/day with no evidence of ETV6-ABL1 transcript by qRT-PCR for the past five years.

Somatic mutations in *UTX*, *ASXL1*, and *TET2* have been reported in chronic myeloid leukemia and mutations in *EZH2* and *IDH1/2* in myeloid malignancies other than CML. We found no somatic alterations in these genes in the DNA extracted from whole blood prior to imatinib treatment, nor when the patient was in a molecular remission.

Our studies indicate that ETV6-ABL1$^+$ "chronic myeloid leukemia" can be sensitive to imatinib and there is significant overlap of molecular targets of ETV6-ABL1 with those of BCR-ABL1, suggesting that the ETV6-ABL1 fusion protein may trigger similar oncogenic cascades as BCR-ABL1. Finally, we were able to exclude mutations in any of the recently identified "myeloid" genes including *UTX*, *ASXL1*, *EZH2*, *TET2* and *IDH1/2* suggesting that the pathogenesis of ETV6-ABL1$^+$ "chronic myeloid leukemia" may be as tyrosine kinase focused as BCR-ABL1 driven disease.

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References

1. Andreasson P, Johansson B, Carlsson M, Jarlsfelt I, Fioretos T, Mitelman F, et al. BCR/ABL-negative chronic myeloid leukemia with ETV6/ABL fusion. Genes Chromosomes Cancer. 1997;20(3):299-304.
2. Barbouti A, Ahlgren T, Johansson B, Hoglund M, Lassen C, Turesson I, et al. Clinical and genetic studies of ETV6/ABL1-positive chronic myeloid leukaemia in blast crisis treated with imatinib mesylate. Br J Haematol. 2003;122(1):85-93.
3. Brunel V, Lafage-Pochitaloff M, Alcalay M, Pelicci PG, Birg F. Variant and masked translocations in acute promyelocytic leukemia. Leuk Lymphoma. 1996;22(3-4):221-8.
4. Kawamata N, Dashti A, Lu D, Miller B, Koeffler HP, Schreck R, et al. Chronic phase of ETV6-ABL1 positive CML responds to imatinib. Genes Chromosomes Cancer. 2008;47(10):919-21.
5. Kelly JC, Shahbazi N, Scheerle J, Jahn J, Suchen S, Christacos NC, et al. Insertion (12;9)(p13;q34q34): a cryptic rearrangement involving ABL1/ETV6 fusion in a patient with Philadelphia-negative chronic myeloid leukemia. Cancer Genet Cytogenet. 2009;192(1):36-9.
6. Keung YK, Beaty M, Steward W, Jackle B, Pettnati M. Chronic myelocytic leukemia with eosinophilia, t(9;12)(q34;p13), and ETV6-ABL gene rearrangement: case report and review of the literature. Cancer Genet Cytogenet. 2002;138(2):139-42.
7. Lin H, Guo JQ, Andreeff M, Arlinghaus RB. Detection of dual TEL-ABL transcripts and a Tel-Abl protein containing phosphotyrosine in a chronic myeloid leukemia patient. Leukemia. 2002;16(2):294-7.
8. Tirado CA, Sebastian S, Moore JO, Gong JZ, Goodman BK. Molecular and cytogenetic characterization of a novel rearrangement involving chromosomes 9, 12, and 17 resulting in ETV6 (TEL) and ABL fusion. Cancer Genet Cytogenet. 2005;157(1):74-7.
9. Van Limbergen H, Beverloo HB, van Drunen E, Janssens A, Hahlen K, Poppe B, et al. Molecular cytogenetic and clinical findings in ETV6/ABL1-positive leukemia. Genes Chromosomes Cancer. 2001;30(3):274-82.
10. Janssen JW, Ridge SA, Papadopoulos P, Cotter F, Ludwig WD, Fonatsch C, et al. The fusion of TEL and ABL in human acute lymphoblastic leukaemia is a rare event. Br J Haematol. 1995;90(1):222-4.
11. Zuna J, Zaliova M, Muzikova K, Meyer C, Lizcova L, Zemanova Z et al. Acute leukemias with ETV6/ABL1 (TEL/ABL) fusion: poor prognosis and prenatal origin. Genes Chromosomes Cancer. 2010;49(10):873-84.
12. Nand R, Bryke C, Kroft SH, Divgi A, Bredeson C, Atallah E. Myeloproliferative disorder with eosinophilia and ETV6-ABL gene rearrangement: efficacy of second-generation tyrosine kinase inhibitors. Leuk Res. 2009;33(8):1144-6.

Cytogenetically complex *SEC31A-ALK* fusions are recurrent in ALK-positive large B-cell lymphomas

Fusion tyrosine kinases involving anaplastic lymphoma kinase (ALK) are central to the pathogenesis of numerous malignancies, in which they represent impor-

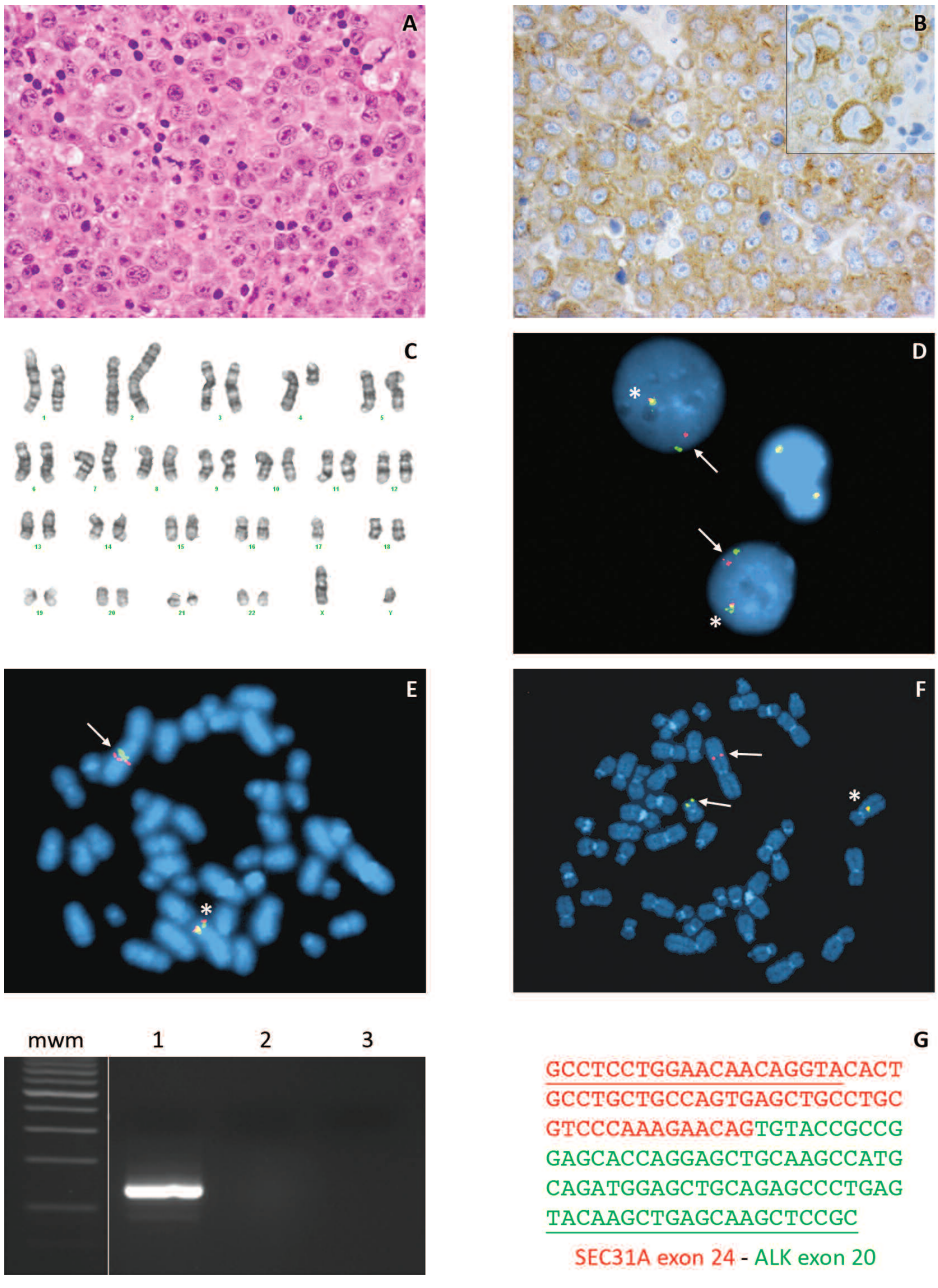

Figure 1. Immunohistological, cytogenetic and molecular analysis. (A) Lymph node biopsy (Haematoxylin & Eosin stain) showing an immunoblastic/plasmablastic lymphoma typical of ALK-positive LBCL. (B) Granular cytoplasmic expression of ALK by lymphoma cells. Immunostaining was performed with anti-ALK mouse monoclonal antibody clone 5A4 (Leica Biosystems Newcastle Ltd, Newcastle upon Tyne, UK) using heat-mediated epitope retrieval on the Ventana Benchmark automated staining platform. Histology images were captured on an Olympus BX51 microscope with Cell A imaging software, magnification x600. (C) Karyotype derived from lymph node biopsy. (D) FISH using *ALK* LSI dual-color breakapart probe (Abbott Molecular, Maidenhead, UK) on interphase cells shows split of one *ALK* allele with green (5', centromeric) and red (3', telomeric) signals remaining in proximity. Arrows: split alleles; stars: intact alleles. (E) FISH using *ALK* LSI dual-color breakapart probe on metaphase cells shows subtle separation of one ALK allele on der(2). Arrow: split allele; star: intact allele. (F) FISH using an in-house *SEC31A* dual-color breakapart probe (BAC RP11-57B24, Spectrum Green, centromeric to *SEC31A*; BAC RP11-429022, Spectrum Red, telomeric to *SEC31A*; labeled probes obtained from Genome Resources Facility, The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Canada) on metaphase cells shows split of one *SEC31A* allele, with the red (5', telomeric) signal translocated to der(2) and the green (3', centromeric) signal remaining on der(4). Arrows: split allele; star: intact allele. Cytogenetic images were acquired on a Nikon Eclipse 80i microscope with Cytovision 4.5.2 image analysis software (Genetix Europe Ltd, Gateshead, UK). (G) RT-PCR using forward *SEC31A* exon 24 primers and reverse *ALK* exon 20 primers, and sequencing of the resulting product, identified a *SEC31A-ALK* fusion transcript identical to that previously identified.[2-3] Left panel: mwm, 100 base pair ladder; 1, patient cDNA; 2, negative control (no cDNA); 3, negative control (control cDNA). mwm and column 1 were run non-adjacent on the same gel, and have been juxtaposed during preparation of the figure. RNA was extracted from formalin-fixed paraffin-embedded lymphoma tissue using the Ambion RecoverAll kit (Applied Biosystems, Warrington, UK) and cDNA synthesised using the First-Strand cDNA synthesis kit (GE Healthcare, Little Chalfont, UK) with random hexamers. Forward primers: external SEC31A-F1; 5' *CAGGAGCTCCACCATCATCT* 3', internal SEC31A-F2; 5' *GCCTCCTGGAACAACAGGTA* 3'; reverse primers: external ALK-R1; 5' *TTGGGGTTGTAGTCGGTCAT* 3', internal ALK-R2; 5' *CGGAGCTTGCTCAGCTTGTA* 3'. Right panel: *SEC31A-ALK* fusion cDNA sequence. Red, *SEC31A* exon 24; green, *ALK* exon 20. Primer sequences are underlined. Sequencing of the internal 140 bp PCR product was by Beckman Dye Terminator Cycle Sequencing with primers SEC31A-F2 & ALK-R2 on a Beckman CEQ8000 sequencer.

tant diagnostic and therapeutic targets.[1] Van Roosbroeck *et al.* recently reported in this Journal the elegant characterization of a cytogenetically cryptic *SEC31A-ALK* fusion arising from complex chromosomal rearrangements in a case of ALK-positive large B-cell lymphoma (LBCL).[2] As this fusion had previously been identified only in a single case of inflammatory myofibroblastic tumor,[3] the authors proceeded to show that it produced a constitutively active fusion tyrosine kinase able to transform hematopoietic cells *in vitro* and susceptible to an ALK-selective small molecule kinase inhibitor. However, whether the *SEC31A-ALK* fusion is a recurrent oncogenic event in lymphoma remained unknown. We now report a second case of ALK-positive LBCL harboring a complex *SEC31A-ALK* fusion, confirming it as an important lymphomagenic oncogene and further highlighting difficulties in its cytogenetic identification.

ALK-positive LBCL is a rare tumor of post-germinal center B cells which occurs most frequently in adult males, many of whom present with advanced disease and pursue an aggressive clinical course.[4-5] Most cases carry a t(2;17)(p23;q23)/*CLTC-ALK* while a minority harbor a t(2;5)(p23;q35)/*NPM-ALK*.[4-8] The patient reported here was clinically typical. He was a 66-year old man with a short history of weight loss, night sweats and dysphagia. Serial imaging showed rapid development of widespread lymphadenopathy, and numerous lymphomatous deposits in the liver and bones. An inguinal lymph node biopsy showed a typical diffuse and sinusoidal infiltrate of large, EBV-negative, immunoblastic/plasmablastic lymphoid cells (Figure 1A) which expressed CD138, IRF4, EMA, CD4, CD45 and perforin, but not several other B- or T-cell antigens, and showed lambda immunoglobulin light chain restriction. ALK was expressed with a granular cytoplasmic staining pattern (Figure 1B). Bone marrow and duodenal biopsies were similarly involved (Ann Arbor stage 4B). The patient was treated with multi-agent chemotherapy but died three weeks after diagnosis.

Cytogenetic analysis revealed the karyotype 45,XY,der(1;17)(q10;q10),t(2;4)(p2?4;q21) (Figure 1C). Although expression of ALK by the neoplastic cells suggested a translocation involving *ALK* at 2p23, the breakpoint on chromosome 2 appeared to be at 2p24-25, telomeric to *ALK*. Fluorescence *in situ* hybridization (FISH) using an *ALK* breakapart probe nevertheless showed a split signal pattern in which the 5' (centromeric) and 3' (telomeric) elements were clearly separated in both interphase and metaphase cells. However, both signals remained nearby in interphase cells and in metaphases they were seen to be in proximity, in the normal orientation, on the p arm of der(2) (Figure 1D-E). These results suggested a complex rearrangement on der(2) involving *ALK* and a gene at 4q21-qter. Prompted by the report of Van Roosbroeck *et al.*,[2] we investigated the involvement of *SEC31A* on 4q21 in the formation of a *SEC31A-ALK* fusion. FISH using an in-house *SEC31A* breakapart probe showed a split signal in which the 5' (telomeric) element hybridized to der(2) and the 3' (centromeric) element remained on der(4) (Figure 1F). RT-PCR was subsequently performed on RNA isolated from fixed lymphoma tissue using *SEC31A* exon 24 and *ALK* exon 20 primers, designed to identify the previously reported *SEC31A-ALK* fusion.[2-3] This yielded a correctly-sized PCR product which, when sequenced, confirmed the expected in frame *SEC31A-ALK* fusion transcript (Figure 1G). In all three *SEC31A-ALK* translocations now reported, complex rearrangements involving the two partner genes have been observed.[2-3] These were probably required to generate a functional *SEC31A-ALK* fusion, as the relative transcriptional orientation of the two genes precludes its formation by a simple reciprocal translocation. The requirement for a complex rearrangement probably underlies the comparative rarity of *SEC31A-ALK* amongst *ALK* fusions. A simple scenario that may be postulated in the present case is t(2;4)(p2?4;q21) followed by inversion of a segment of der(2) including the 3' ends of *SEC31A* and *ALK*, bringing together the 5' end of *SEC31A* and the 3' end of *ALK*. Unfortunately, whole chromosome painting to further characterize the der(2) gave equivocal results and we were unable to detect the reciprocal *ALK-SEC31A* transcript by RT-PCR.

This report complements that of Van Roosbroeck *et al.*,[2] confirming *SEC31A-ALK* as a recurrent event in ALK-positive LBCL. Recognition of this translocation in clinical practice is important for diagnosis of these lymphomas, which are probably under-recognised by histopathology alone, as they often have an aggressive clinical course which may warrant a modified treatment approach and as they may be susceptible to newly developed ALK kinase inhibitors. Cytogeneticists should be aware of the spectrum of complex rearrangements which may underlie *SEC31A-ALK* fusions. In particular, since ALK breakapart probes may be only minimally separated, vigilance is necessary in the FISH analysis.

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References

1. Webb TR, Slavish J, George RE, Look AT, Xue L, Jiang Q, et al. Anaplastic lymphoma kinase: role in cancer pathogenesis and small-molecule inhibitor development for therapy. Expert Rev Anticancer Ther. 2009;9(3):331-56.
2. Van Roosbroeck K, Cools J, Dierickx D, Thomas J, Vandenberghe P, Stul M, et al. ALKpositive large B-cell lymphomas with cryptic SEC31A-ALK and NPM1-ALK fusions. Haematologica. 2010;95(3): 509-13.
3. Panagopoulos I, Nilsson T, Domanski HA, Isaksson M, Lindblom P, Mertens F, et al. Fusion of the SEC31L1 and ALK genes in an

inflammatory myofibroblastic tumor. Int J Cancer. 2006;118(5): 1181-6.
4. Delsol G, Campo E, Gascoyne RD. ALK-positive large B-cell lymphoma. In: Serdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al, editors. WHO Classification of Haematopoietic and Lymphoid Tissues, 4th edition. Lyon: IARC Press; 2008. p. 254-5.
5. Laurent C, Do C, Gascoyne RD, Lamant L, Ysebaert L, Laurent G, et al. Anaplastic Lymphoma Kinase–Positive Diffuse Large B-Cell Lymphoma: A Rare Clinicopathologic Entity With Poor Prognosis. J Clin Oncol. 2009;27(25):4211-6.
6. De Paepe P, Baens M, van Krieken H, Verhasselt B, Stul M, Simons A, et al. ALK activation by the CLTC-ALK fusion is a recurrent event in large B-cell lymphoma. Blood. 2003;102(7): 2638-41.
7. Gascoyne RD, Lamant L, Martin-Subero JI, Lestou VS, Harris NL, Muller-Hermelink H-K, et al. ALK-positive diffuse large B-cell lymphoma is associated with Clathrin-ALK rearrangements: report of 6 cases. Blood. 2003;102(7):2568-73.
8. Onciu M, Behm FG, Downing JR, Shurtleff SA, Raimondi SC, Ma Z, et al. ALK-positive plasmablastic B-cell lymphoma with expression of the NPM-ALK fusion transcript: report of 2 cases. Blood. 2003;102(7):2642-4.