To the Editor:

Anaplastic large-cell lymphoma (ALCL) is frequently associated with the recurrent translocation t(1;2)(p13;q35) that results in activation of the anaplastic lymphoma kinase (ALK) gene at 2p23 by fusion to the ubiquitously expressed gene encoding the nucleolar phosphoprotein nucleophosmin (NPM) at 5q35. This translocation leads to aberrant nuclear (and cytoplasmic) expression of ALK, which is normally silent in hematopoietic tissues.1,2

Approximately 20% of ALK-positive ALCLs do not express ALK in
the nucleus but show aberrant expression of ALK restricted to the cytoplasm. These “cytoplasmic ALK only” ALCL do not contain the t(2;5), suggesting that other genetic abnormalities can result in aberrant ALK expression. So far, only very limited data on cytogenetic alterations of this subtype of ALCL are available. Nevertheless, in very recent issues of Blood, “cytoplasmic ALK only” ALCL cases have been described to contain variant rearrangements of the chromosomal region 2p23. Wlodarska et al reported 3 cases with cryptic inv(2)(p23q35). Rosenwald et al described 1 case each with t(1;2)(q21; p23) and t(2;3)(p23;q21). Finally, Lamant et al reported a t(1;2)(q25; p23) in 1 patient. Cloning of the translocation breakpoint showed a fusion of ALK to the TPM3 gene in 1q25 encoding a nonmuscle tropomyosin. A TPM3-ALK fusion transcript was detectable by reverse transcriptase-polymerase chain reaction (RT-PCR) in 2 of 3 additional ALCL, with ALK staining restricted to the cytoplasm.

We recently investigated 3 cases of “cytoplasmic ALK only” ALCL.
by means of fluorescence in situ hybridization (FISH). Whereas 2 cases turned out to contain the previously described inv(2)(p23q35), we report here a cryptic insertion into the ALK gene observed in the third patient. The lymph node biopsy specimen was obtained from a 5-year-old male patient with stage IVB disease. Histological evaluation showed a CD30(+)Ki-1-positive ALCL of null cell phenotype. Using the ALK1 antibody (Dako, Glostrup, Denmark), only 20% of the tumor cells stained positive, with strong cytoplasmic ALK protein expression but lack of any nuclear ALK expression.

Chromosome analysis of R-banded metaphases derived from unstimulated short-term lymph node cultures showed clonal aberrations in 6 of 16 metaphases. The karyotype was 50 − 51, XY, +X, del(1)(q21), +2, del(2)(p25p21)(p25p11)dup(2)(p11p25) × 2, +, 6, +7, +17 [cpg]/14, XY [10]. To investigate the case for a cryptic ALK rearrangement, we performed FISH with the LSI ALK assay (Vysis, Downers Grove, IL) according to the manufacturer’s instructions. The LSI ALK assay consists of 2 differentially labeled probes for the centromeric (5’) and telomeric (3’) regions of the ALK gene that showed to be separated in cases with t(2;5) and inv(2). Using this assay, 38 of 100 interphase cells each displayed 2 isolated signals for the centromeric (green) and telomeric (red) ALK probes, indicating 2 rearranged copies of the ALK gene in addition to 1 colocalization of each 1 signal derived from an intact ALK locus. FISH on metaphases followed by subsequent R-banding analysis showed the short arms of the intact chromosome 2 and both der(2) chromosomes each to contain 1 signal for the centromeric and the telomeric ALK probe. Nevertheless, the spatial separation of the 2 ALK probes on the der(2) chromosome suggests an insertion of genomic material into the ALK gene on the der(2) (Fig 1A through D).

As for R-banding analysis, there was no evidence for material from chromosomes other than chromosome 2 to be contained in the der(2) chromosomes, suggesting that a complex inv(2) might lead to insertion of 2q35 material into the ALK locus. To test this hypothesis, FISH with YAC probes 884F10, 770F5, and 914E7 flanking and spanning the 2q35 breakpoint of the inv(2), respectively, was applied.5 These hybridizations did not provide evidence for the genomic material inserted into the ALK locus to be derived from a complex inv(2).

Considering the loss of chromosome 1 material due to the del(1)(q21) in the tumor cells, it was also tempting to speculate that the material inserted into the ALK locus might be derived from the long arm of chromosome 1. Consequently, FISH on archived R-banded slides was performed with a whole chromosome 1 painting probe (AGS, Heidelberg, Germany). Surprisingly, this analysis showed a major part of the short arms of the der(2) chromosomes to be derived from chromosome 1 (Fig 1B, small picture). Thus, the present case contains a complex t(1;2) translocation that is hardly detectable by banding analyses due to the underlying multiple break events.

Because this complex aberration might be a variant of the recently cloned translocation t(1;2)(q25;p23) leading to a TPM3-ALK fusion, we aimed to investigate the case for this hybrid transcript.3 No fresh material for molecular analyses was available from initial diagnosis and the patient is in continuous complete remission for 2.5 years now after initial treatment according to the German NHL-BFM95 trial (group III-K3).3 Thus, RNA was extracted from cells in Carnoy’s fixative by means of RNeasy (WAK Chemie, Bad Homburg, Germany), and first-strand synthesis was performed with pooled ALK-specific primers (5’-AGC ACA CCT TGC GCA GGG TCT TCA CAG CCA-3’ and 5’-CAT TCC GGA CAT GTC TCC ATC ACA CTC TC-3’) using the Reverse Transcription System (Promega, Madison, WI). Nested PCR according to Lamant et al3 indeed amplified the characteristic PCR product of approximately 300 bp (Fig 1E). Sequencing of this PCR product confirmed the presence of the TPM3-ALK fusion transcript.

In summary, we identified a cryptic ALK gene rearrangement due to an insertion that, by molecular cytogenetics and RT-PCR analysis, turned out to be a complex variant translocation t(1;2) leading to TPM3-ALK fusion. On the one hand, the present case confirms TPM3-ALK fusion to be a recurrent mode of ALK activation in ALCL with ALK expression restricted to the cytoplasm.2 On the other hand, this case again indicates that ALK gene rearrangements might be cytogenetically hidden, eg, due to complex breakage events. Indeed, in the present case as well as in the cases with inv(2), the ALK rearrangements would not have been cytogenetically detected without applying molecular cytogenetics. Thus, in our opinion, FISH with an ALK-specific probe should be generally integrated into the cytogenetic analysis of “cytoplasmic ALK only” ALCL so that cryptic ALK rearrangements are not missed.

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REFERENCES

Detection of TT Virus DNA in Plasma-Derived Clotting Factor Concentrates

To The Editor:

Clotting factors such as factor VIII and factor IX have been used as replacement therapy in patients with hemophilia to control hemorrhagic episodes. Since around 1980, when the delayed announcement of possible human immunodeficiency virus (HIV) contamination in such products became a serious social problem in Japan, patients with hemophilia have been concerned about various viral contamination of blood products.

Recently, an unenveloped, single-stranded DNA virus, named the TT virus (TTV), was identified as a transfusion-transmissible agent reported to be associated with non–A–G hepatitis. The reported prevalence rates of TTV have varied widely, depending on the primers used in the polymerase chain reaction (PCR) detection system. The primer set used by Takahashi et al is 10 to 100 times more sensitive than the one described by Okamoto et al, which is commonly used. Takahashi et al detected TTV DNA in 92 (92%) of 100 individuals at a routine health screening, suggesting that TTV is not pathogenic.

In our hospital, 3 brands of plasma-derived clotting factor concentrates are available (Table 1). We measured TTV DNA in the products using the most sensitive of Takahashi’s primer set. For viral inactivation, ion-exchange chromatography, nano-filtration (35 nm), and dry heat at 65°C for 96 hours are unlikely to be sufficient for complete elimination of TTV DNA, although infectivity of attenuated virions might be diminished or lost. Immunoaffinity chromatography, which was designed for selection of targeted materials using monoclonal antibodies, is likely to achieve complete elimination of TTV DNA.

Unenveloped viruses tend to be more difficult to inactivate than enveloped viruses. Enveloped viruses, such as vesicular stomatitis virus (VSV) and Sindbis virus, are easily inactivated, whereas unenveloped viruses, such as ECHO virus and poliovirus, are not inactivated by solvent/detergent treatment. Some viruses, such as parvovirus, are thought to be resistant to heat. TTV, an unenveloped and putative parvovirus-like virus, may be resistant to methods commonly used for viral inactivation. Immunoaffinity chromatography may be recommended for the elimination of viruses resistant to inactivation.

Table 1. Characteristics of 3 Clotting Factor Concentrates

<table>
<thead>
<tr>
<th>Brand*</th>
<th>Clotting Factor</th>
<th>Production Steps After Source (viral inactivation)</th>
<th>TT Viral DNA Positive/Total Investigated Lots (%)</th>
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<tbody>
<tr>
<td>A</td>
<td>Factor VIII</td>
<td>(1) Ion-exchange chromatography</td>
<td>28/28 (100%)</td>
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<td></td>
<td></td>
<td>(2) Nano-filtration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) Dry heat 65°C, 96 h</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Factor VIII</td>
<td>(1) Solvent/detergent† treatment</td>
<td>0/24 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) Immunoaffinity chromatography</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3) Ion-exchange chromatography</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Factor IX</td>
<td>(1) Immunoaffinity chromatography</td>
<td>0/25 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) Ion-exchange chromatography</td>
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<td>(3) Nano-filtration</td>
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<td></td>
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<td>(4) Dry heat 65°C, 96 h</td>
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*All products are made in Japan from plasma source taken from domestic, screened volunteers. Individual donor plasma is screened using serologic tests for syphilis, HIV1, HIV2, HTLV-1, HBsAg, HBCAb, HCVAb, and ALT. Abnormal plasma is excluded. Brand A and C are made by the same company.

†Tri-(n-butyl)-phosphate (TNBP)/Triton X-100.

REFERENCES

Complex Variant Translocation t(1;2) With TPM3-ALK Fusion Due to Cryptic ALK Gene Rearrangement in Anaplastic Large-Cell Lymphoma

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