ALK-Positive Plasmablastic B-Cell Lymphoma with Expression of the NPM-ALK Fusion Transcript: Report of Two Cases

Mihaela Onciu, Frederick G. Behm, James R. Downing, Sheila A. Shurtleff, Susana C. Raimondi, Zhigui Ma, Stephan W. Morris, Wren Kennedy, Sandra C. Jones, and John T. Sandlund

From the Departments of Pathology (M.O., F.G.B., J.R.D., S.A.S., S.C.R., Z.M., S.W.M.) and Hematology/Oncology (W.K., S.C.J., J.T.S.) at St. Jude Children’s Research Hospital, Memphis, TN and The University of Tennessee Health Sciences Center, Memphis, TN. (F.G.B., J.R.D., S.C.R., J.T.S.)

Supported in part by the National Institutes of Health (grants CA21765 and CA69129) (S.W.M.) and by the American Lebanese Syrian Associated Charities (ALSAC) of St. Jude Children’s Research Hospital.

Corresponding author/Reprints: Mihaela Onciu, MD, St. Jude Children’s Research Hospital, Department of Pathology, 332 North Lauderdale Street, Memphis, TN 38105; Telephone: (901) 495 5347, Fax: (901) 495-3100, E-mail: mihaela.onciu@stjude.org

Keywords: Non-Hodgkin lymphoma, B-cell, ALK, NPM-ALK.

Running heads: Left: ONCIU et al Right: NPM-ALK-Positive B-cell lymphoma

Journal Section: Special section - Brief report Scientific section - NEOPLASIA

Word counts

Abstract: 139
Text: 1300
ABSTRACT

While most ALK-positive Non-Hodgkin lymphomas (NHL) are of T-cell lineage, a small number of B-lineage tumors with plasmablastic morphology and expression of the full-length ALK protein have been described in the literature. All of these reported tumors lacked the NPM-ALK fusion transcript. There is controversy regarding the existence of ALK fusion-positive B-cell NHL, many investigators contending that ALK fusions are expressed uniquely in T- or null-cell lymphomas. Here we describe two well-characterized cases of ALK-positive B-cell lymphoma expressing the NPM-ALK fusion. Both tumors occurred in pediatric patients and showed poor response to chemotherapy. Each had plasmablastic morphology, showed IgA restriction, and was ALK-positive and CD30-negative by immunohistochemistry. One tumor showed the t(2;5)(p23;q35) chromosomal translocation by conventional cytogenetics. Both were positive for NPM-ALK by RT-PCR. Thus, ALK-positive plasmablastic B-cell lymphomas are more heterogeneous at the molecular level than previously recognized.
BACKGROUND

ALK-positive anaplastic large cell lymphoma (ALCL) is classified by the World Health Organization (WHO) as a subtype of T-lineage Non-Hodgkin lymphoma characterized by expression of the anaplastic lymphoma kinase (ALK) and of CD30. ALK overexpression is the result of the t(2;5)(p23;q35) chromosomal translocation that leads to fusion of the nucleophosmin, NPM, gene at 5q35 to the ALK gene at 2p23. In 1997, Delsol and colleagues described an unusual subtype of large B-cell lymphoma with plasmablastic morphology and expression of full-length ALK. Clinically, these tumors occurred most often in older adult patients and had a poor response to chemotherapy. By immunohistochemistry (IHC), the tumor cells were CD20-negative, IgA-restricted and lacked CD30 expression. A significant subset of those tumors also expressed weak CD4 and CD57. All of these tumors were negative for the NPM-ALK transcript. The ALK expression, as detected by IHC, had a membrane and speckled cytoplasmic distribution, different from the homogeneous cytoplasmic and/or nuclear pattern characteristic for the classic T/null-lineage ALCL containing NPM-ALK. To date, a total of ten cases of ALK-positive B-cell lymphoma of this type have been described in the literature. The WHO classification has placed these tumors under the large B-cell lymphoma category, where they are designated as “diffuse large B-cell lymphomas with expression of full-length ALK.”

We describe the clinicopathologic features of two cases of large B-cell lymphoma with plasmablastic morphology and expression of the NPM-ALK transcript. One of these cases was included in a series of patients previously reported by our institution.
MATERIALS AND METHODS

Patients. The patients reported here were identified through a search of the St. Jude Department of Pathology files for NPM-ALK-positive tumors that were CD30-negative and/or of B-cell lineage. Ethical approval of the Institutional Review Board was obtained prior to the medical records review for these two patients. Histologic examination was performed on formalin- and B5-fixed, paraffin-embedded tissue sections that were stained with hematoxylin and eosin (H&E). Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissue sections following heat-induced epitope retrieval using the avidin-biotin peroxidase technique and the Ventana Nexes IHC Staining System (Ventana, Tucson, AZ) or DAKO Autostainer (DAKO, Glostrup, Denmark). Antibody specificities, along with their sources were as follows: ALK-1, CD3, CD4, CD8, CD30, CD79a, EMA, EBV-LMP1, Immunoglobulin alpha, mu, and gamma heavy chains (DAKO, Glostrup, Denmark), CD20, CD45, CD45RO/UCHL-1, CD56 (Ventana, Tucson, AZ), and CD57 (Novocastra, Newcastle upon Tyne, UK). In situ hybridization studies for EBV (EBER) and immunoglobulin kappa and lambda light chains were performed on formalin-fixed paraffin-embedded tissue sections following pretreatment with proteinase K, using specific fluorescein-conjugated RNA probes, the DAKO PNA ISH detection kit (all from DAKO, Glostrup, Denmark) and the Nexes Discovery staining system (Ventana, Tucson, AZ). Cytogenetic analysis was performed using direct preparations and overnight unstimulated cultures, followed by banding with Trypsin-Wright’s as described elsewhere and the karyotypes were written according to the ISCN 1995 Nomenclature. Reverse Transcriptase-Polymerase Chain Reaction (RT PCR) for ALK and NPM/ALK Total RNA extracted from frozen (Patient 1) or paraffin-embedded tissue (Patient 2) was reverse-transcribed using random primer (Promega, Madison, WI). The cDNA quality was checked by amplifying a 321 bp portion of NPM gene using the primers NPM/R5’ - TCGATGGACATGGACATGAGC-3’ and NPM/R (5’- ATGCACTTGCCCTGAACCACAC-3’). Detection of NPM-ALK mRNA. RT-PCR was performed in a standard reaction using the primers 5’ NPM/R and 3’ ALK-3 (5’- CGAGGTGCGGAGCTTGCTCAG-3’). Detection of ALK mRNA. Transcripts encoding the cytoplasmic portion of ALK (3’ ALK) were detected using primers based on the ALK cDNA sequence: ALK-4201/F: 5’ -GCTTTGCTGGCAAGACCTCCTC- 3’ (position 4201-4123 of the full-length normal ALK cDNA; clone RMS17-2); and ALK-4342/R: 5’- GGCTTGGCTGCTGCTGGCACTC-3’ (position 4342-4321). Transcripts encoding the extracellular portion of ALK (5’ALK) were detected using two primers: ALK-EC1: 5’ –CCATCTCCTTCCCTGATTATTTT -3’ (position 1611-1634); ALK-EC2: 5’ - CACTGCAGA CAAGCAGGGTT -3’ (position 2162-2142).
RESULTS AND DISCUSSION

Patient 1
A 16-year-old black male presented with a 5-cm mass of the scalp and parietal bone, fatigue, and a five-pound weight loss. Additional evaluation showed cervical, axillary and inguinal lymphadenopathy, and multiple lytic skeletal lesions. A biopsy of the scalp mass showed large B-cell lymphoma. The patient was treated according to LMB89 chemotherapy protocol\textsuperscript{10} with poor initial response. Four months after completion of chemotherapy the patient developed recurrent disease. Weekly vinblastine, intrathecal chemotherapy, and palliative radiation therapy led to only a partial response. The patient died of disease two years from initial diagnosis.

Patient 2
A 10 year old white male presented with a laryngeal supraglottic mass and cervical and submandibular lymphadenopathy. Additional evaluation showed no other sites of disease. The laryngeal mass was resected and showed large B-cell lymphoma. The patient was enrolled in the POG 8719 protocol,\textsuperscript{11} with persistent disease (in the larynx and cervical lymph nodes) at the end of induction and consolidation therapy. He then received radiation therapy (total 39 Gy) to the sites of persistent disease and three courses of DAHP (dexamethasone, cytarabine, cisplatinum), with complete response. He is currently in complete continuous remission, 13 years from initial diagnosis.
Figure 1

Morphologic and immunophenotypic features of ALK-positive large B-cell lymphoma (A-H). Characteristic plasmablastic and immunoblastic morphology (Hematoxylin and eosin staining, original magnification 400x) (A). On immunohistochemical staining (B-F), the tumor strongly expresses ALK, with a diffuse cytoplasmic and nuclear pattern (B), CD79, with a weak and focal membrane pattern (C), and cytoplasmic immunoglobulin (Ig) alfa heavy chains (D) and lacks expression of Ig gamma (E) and Ig mu (F) heavy chains. (Immunoperoxidase staining with hematoxylin counterstain). By in situ hybridization for immunoglobulin lambda (G) and kappa (H) light chain RNA the tumor shows lambda light chain restriction.
Figure 2.
RT-PCR analysis demonstrating the presence of the *NPM-ALK* fusion transcript and the absence of mRNA for the extracellular portion of ALK in two cases of ALK-positive B-cell lymphoma. RT-PCR was performed on total RNA extracted from the patient tissue samples, a known positive control, and a known negative control. The presence of amplifiable RNA was confirmed by RT-PCR amplification of a 321 bp portion of the ubiquitously expressed *NPM* gene. The analysis demonstrates the presence of 429 bp *NPM/ALK* and 121 bp 3’*ALK* (encoding for the intracellular portion of ALK, also present in the NPM-ALK) transcripts and is negative for the 552 bp 5’*ALK* transcript (encoding for the extracellular portion of ALK).
The tumors from both patients showed similar histologic features, closely resembling the morphology and immunophenotype described by Delsol et al.\textsuperscript{4} and Reichard et al.\textsuperscript{6} Both tumors had a diffuse growth pattern largely replacing the underlying tissue in the scalp lesion and the laryngeal tumor, and partially effacing the architecture of involved lymph node, with an interfollicular distribution (in a lymph node obtained from patient 2 following chemotherapy). Tumor cells were predominantly plasmablastic in appearance. (See Figure 1A) Occasional large, bizarre, multinucleated cells and variable numbers of mature plasma cells were present throughout the tumors.

Immunophenotypically, these tumors expressed the leukocyte common antigen and were positive for ALK, CD79a (weak, focal), IgA, and EMA. The ALK staining was homogeneous, nuclear and cytoplasmic in patient 2 (See Figure 1) and cytoplasmic-only in patient 1 (data not shown). By \textit{in situ} hybridization, there was immunoglobulin light chain restriction (Ig kappa in patient 1 and Ig lambda in patient 2). The tumors were negative for CD30, CD3, CD8, CD20, CD56, CD57, IgM, IgD, and EBV (EBER and LMP-1). On staining for CD4, the tumor from patient 2 was positive, while the one from patient 1 was negative.

Cytogenetic analysis was available only in patient 2 (performed from lymph node biopsy material obtained following chemotherapy), with the following karyotype: 45,XY,der(1;11)(q10;q10),t(2;5)(p23;q35)[3]/46,XY[10].

In both tumors RT-PCR demonstrated the presence of the \textit{NPM-ALK} and the 3' \textit{ALK} transcripts (the latter encoding for the intracellular portion of ALK and present in the \textit{NPM-ALK} fusion transcript) and was negative for the 5' \textit{ALK} mRNA (encoding for the extracellular portion of the ALK protein) (See Figure 2).

To our knowledge the cases described here represent the first report of \textit{NPM-ALK}-positive plasmablastic large B-cell lymphoma. A single case of ALK- positive, CD30-negative, IgA kappa-restricted B-cell lymphoma that was reported to be t(2;5) positive but \textit{NPM-ALK} negative by RT-PCR was mentioned in a larger series of cases by Gascoyne et al.\textsuperscript{12} It is possible that the \textit{NPM-ALK} was not detected in that case due to breakpoint heterogeneity that prevented amplification with the set of primers used in that study.

The two cases of \textit{NPM-ALK}-positive plasmablastic lymphoma described here are morphologically, immunophenotypically and clinically very similar to the cases previously described by Delsol et al. However, the present cases differ markedly in their ALK staining pattern from the latter tumors, showing diffuse staining versus a membrane and speckled pattern. The existence of \textit{NPM-ALK}-positive B-cell tumors is not surprising in light of the observation that enforced expression of \textit{NPM-ALK} in mice has been reported to induce T-lineage lymphoblastic lymphomas or B-lineage plasmacytic/plasmablastic tumors with high frequency.\textsuperscript{13-15}
The cases presented here, very similar to the previously described ALK-positive plasmablastic lymphomas (currently classified as diffuse large B-cell lymphomas with expression of full-length ALK), suggest that this family of tumors is molecularly more heterogeneous than previously thought, and should be more appropriately designated as “diffuse large B-cell lymphoma expressing ALK.”
REFERENCES

ALK-positive plasmablastic B-cell lymphoma with expression of the 
NPM-ALK fusion transcript: Report of two cases

Mihaela Onciu, Frederick G Behm, James R Downing, Sheila A Shurtleff, Susana C Raimondi, Zhigui Ma, 
Stephan W Morris, Wren Kennedy, Sandra C Jones and John T Sandlund