Published in final edited form as:

Cytometry B Clin Cytom. 2009 March ; 76(2): 142–149. doi:10.1002/cyto.b.20442.

Novel CD19 Expression in a Peripheral T cell lymphoma: a flow cytometry case report with morphologic correlation

Kathryn Rizzo, D.O., Ph.D.¹, Maryalice Stetler-Stevenson, M.D., Ph.D.¹, Wyndham Wilson, M.D., Ph.D.², and Constance M. Yuan, M.D., Ph.D.^{1,*}

¹Flow Cytometry Unit, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

²Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Abstract

Background—Peripheral T cell lymphomas are uncommon lymphomas that show T cell antigenic loss and clonal T cell receptor gene rearrangement. Rare cases of T cell lymphomas with aberrant expression of CD20 have been described. However, CD19 co-expression in a mature T cell neoplasm has not been reported.

Methods—Histology, immunohistochemistry (IHC), and PCR for T cell receptor gene rearrangement were performed on an excised lymph node specimen and a subsequent fine needle aspiration (FNA) of an additional lymph node. Flow cytometry (FC) was performed on the fine needle aspiration and a peripheral blood specimen.

Results—The lymph node's architecture was effaced by a diffuse atypical lymphoid proliferation, that by IHC was positive for CD3, CD2 and CD43 and negative for CD4, CD5, CD8, TdT, CD1a and B cell associated antigens PAX-5, CD20 and CD79a. A clonal T-cell receptor gene rearrangement was detected. FC performed on a subsequent fine needle aspiration and peripheral blood specimen demonstrated an aberrant T cell population with expression of CD2, CD3, CD27, TCR alpha-beta, CD52, CD38, CD45, CD26 (partial expression) and negative for CD4, CD5, CD7, CD8, CD10, CD30 and CD56. The aberrant T cell population also expressed bright CD19.

Conclusions—Using FC we describe the first case of peripheral T cell lymphoma with aberrant co-expression of CD19.

Keywords

CD19; peripheral T cell lymphoma; immunophenotyping; PTCL-NOS; aberrant expression; flow cytometry

INTRODUCTION

Peripheral T cell lymphomas are an uncommon lymphoma composed of mature T cells. Peripheral T-cell lymphoma unspecified (PTCL-NOS), the most common type, comprises 3.7% of lymphoma cases. (1) T-cell lymphomas can be leukemic or disseminated, extranodal, cutaneous, and/or nodal and are evaluated on the basis of loss of one or more mature T cell antigens and evidence of a clonal T-cell receptor (TCR) gene rearrangement.

*Corresponding author: Constance M. Yuan 10 Center Dr., Building 10, Room 2A33, 9000 Rockville Pike, Bethesda, MD 20892, Telephone: 301-496-4709/Fax : 301-402-0043, yuanc@mail.nih.gov.

Although characteristic cytological features can be seen in a few T cell neoplasms, generally the cytological appearance is variable and can mimic non-neoplastic processes. Thus the diagnosis of a T-cell neoplasm incorporates the overall architecture and location of the lesion, the immunophenotype and molecular studies for TCR gene rearrangement.

T-cell lymphomas are differentiated from B cell lymphomas based on their immunophenotypic profile. However, aberrant immunophenotypic expression is seen in a number of B and T cell lymphomas. B cell lymphomas can aberrantly express T cell associated antigens and vice versa. Expression of CD2 and CD4 is rarely seen in Hodgkin cells (2) and has been reported in pyothorax-associated B cell lymphoma. (3) CD3 is also expressed in a subset of primary effusion lymphomas. (1)

T-cell neoplasms can also express B-cell antigens. There are small case studies which show CD20 expression on mature T cell neoplasms (4–7), demonstrated both by immunohistochemistry (IHC) and flow cytometry (FC). These cases lack additional B-cell antigens. Likewise, a large case series analyzing CD20 and CD79a immunohistochemical expression on various T cell and NK/T cell lymphomas found a small number of cases either expressing CD79a or CD20. (8). A single case report also demonstrated co-expression of CD20 and CD79a in a peripheral T-cell lymphoma. (10) The specificity of certain B and T cell antigens is significant when utilizing these antigens for gating lymphoid populations in FC. The widely used B cell marker, CD19, has not been seen in a mature T cell neoplasm.

We describe a case of a 29 year old male with a diagnosis of peripheral T cell lymphoma, unspecified with co-expression of bright CD19 by FC. This aberrant phenotype was identified on a lymph node fine needle aspirate and peripheral blood specimen. To date, this is the first case describing an occurrence of CD19 co-expression in a mature T cell lymphoma.

CASE HISTORY

A 29 year old male initially presented with an enlarged left cervical lymph node and a 2.5cm inguinal lymph node. The lymphadenopathy waxed and waned. He subsequently developed pruritis and drenching night sweats. Physical examination showed bilateral cervical, axillary and bilateral inguinal lymphadenopathy. An abdominal sonogram showed hepatosplenomegaly. Chest x-ray, Complete blood count (CBC) and liver function tests were all within normal limits. Testing for human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV 1/2) and Epstein-Barr virus (EBV) were negative. Positron emission tomography(PET)/ computerized tomography (CT) scan showed increased uptake in multiple deep and superficial lymph nodes and the right inguinal lymph node was excised.

The patient was then seen at the National Institutes of Health (NIH) where a fine needle aspiration (FNA) performed on a right epitrochlear lymph node showed atypical lymphoid cells, morphologically consistent with the patient's history of malignant T-cell lymphoma. FC performed on the lymph node aspirate and a peripheral blood sample showed an aberrant T cell population. The right inguinal lymph node was reviewed at the NIH and diagnosed as Peripheral T cell lymphoma, unspecified.

MATERIALS AND METHODS

Histologic and Immunohistochemical Studies

The hematoxylin and eosin (H&E) stained slide and unstained slides were reviewed by the Hematopathology Section, Laboratory of Pathology, National Cancer Institute, NIH. The morphologic features of the case studied were assessed on H&E stained sections of

formalin-fixed, paraffin-embedded tissue. Fine needle aspiration specimen was assessed on Diff Quick stained slides.

Immunohistochemical (IHC) analyses were performed by the avidin-biotin peroxidase complex method using standard manual methods or an automated immunostaining machine (Ventana-Biotech, Tucson, AZ). Tissue sections were stained with antibodies directed against CD3, CD20, T-cell receptor beta framework-1 (beta-F1), cytotoxic granule-associated RNA binding protein (TIA-1), CD4, CD5, CD8, granzyme B, and CD52. CD19 IHC was performed on a cytopsin preparation of the lymph node fine needle aspiration specimen. Appropriate positive and negative controls were used for all immunohistochemical stains. The sources of antibodies used are as follows: Polyclonal anti-CD3 (Dako), anti-CD20 (L26, Dako), beta-F1 (8A3, Endogen), TIA-1 (Immunotech), CD4 (1F6, Novocastra), CD5 (4C7, Novocastra), CD8 (Dako), granzyme B (GrB-7, Monosan), CD19 (HD37, DAKO) and CD52 (YTH34.5, Serotec). Staining was performed on an automated immunostaining machine (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's instructions. Prior antigen retrieval was performed using microwave method or Ventana's proprietary protease-1 treatment.

Immunophenotyping by Flow Cytometry

FC (BD FACS Calibur) was performed on the FNA and a peripheral blood specimen. Erythrocytes were lysed by incubating with lysing solution (150 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA) for 10 minutes at room temperature at a ratio of 1:9 (volume of sample: volume of lysing solution). After incubation, cells were pelleted by centrifugation (500xg for 5 minutes at room temperature), the media was aspirated, and the cells washed twice in a phosphate-buffered saline (PBS) solution containing 0.1% NaN₃. The following fluorochromes are abbreviated as follows: Fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC) and Peridinin-chlorophyll-protein Complex (PerCP). 4-color analysis was performed on the following antibodies: CD3 APC (Immunotech), CD19 PerCP Cy5.5 (SJ25C1, BDIS), CD14 FITC (BDIS), CD56+16 PE (BDIS), CD4 FITC (BDIS), CD8 PE (BDIS), CD45 PerCP (BDIS), CD3 PerCP (BDIS), CD5 APC (BDIS), CD7 FITC (Coulter), CD2 PE (Coulter), CD5 FITC (BDIS), CD38 PE (BDIS), kappa FITC (BDIS), lambda PE (BDIS), CD45 APC (BDIS).

The flow cytometry antibody combinations performed for each tube are listed below, with combinations containing CD19 appearing in bold print. The flow cytometry antibody combinations in the lymph node FNA specimen are as follows:

Tube	FITC	PE	Per CP	APC
1	CD14	CD16+56	CD19	CD3
2	CD4	CD8	CD45	CD3
3	CD57	CD56	CD45	CD3
4	TCR a/b	TCR g/d	CD3	CD5
5	CD7	CD2	CD3	CD5
6	CD5	CD38	CD19	CD34
7	CD30	CD25	CD4	CD2
8	CD26	CD27	CD4	CD3
9	CD5	CD13	CD3	CD10

Tube	FITC	PE	Per CP	APC
10	kappa	lambda	CD19	CD45
11	CD7	CD25	CD4	CD3
12	CD7	CD52	CD4	CD3
13	CD7	CD25	CD3	CD5
14	CD7	CD52	CD3	CD5

The flow cytometry antibody combinations in the peripheral blood specimen are as follows:

Tube	FITC	PE	Per CP	APC
1	CD14	CD16+56	CD19	CD3
2	CD4	CD8	CD45	CD3
3	CD57	CD56	CD45	CD3
4	TCR a/b	TCR g/d	CD3	CD5
5	CD7	CD2	CD3	CD5
6	CD5	CD38	CD19	CD34
7	CD30	CD25	CD4	CD2
8	CD26	CD27	CD4	CD3
9	CD5	CD13	CD3	CD10
10	kappa	lambda	CD19	CD45
11	CD7	CD25	CD4	CD3
12	CD7	CD52	CD4	CD3
13	CD7	CD25	CD3	CD5
14	CD7	CD52	CD3	CD5

Per tube, acquisition was based on 5,000 lymphoid cells. The total events varied from 10,724 to 14,328 for the lymph node FNA. The total events varied from 71,186 to 122,865 for the peripheral blood analysis. Data acquisition and analysis were performed using CellQuest Pro software and FCS Express software was used to generate data figures.

Specific gating strategy occurred in the following stepwise fashion. The first step involved utilizing a lymphoid cell gate. Lymphocytes were identified by forward and side scatter characteristics. Secondly, the CD3 population was identified. The CD3 gate was drawn based on CD3 positivity and side scatter characteristics. The third step involved identification of the CD19 population. The CD19 gate was drawn based on CD19 positivity and side scatter characteristics. CD3 and CD19 positive events were subsequently colored blue and pink, respectively. The CD3 and CD19 cell gates were then re-analyzed based on forward and side scatter characteristics via the blue CD3 population and the pink CD19 population.

Molecular Testing

Molecular testing to detect a TCR gene rearrangement was performed by TCR Southern blot analysis (Brigham and Women's Hospital, Boston, MA). Genomic DNA was isolated from a frozen portion of the right inguinal lymph node tissue (Gentra PureGene DNA Isolation Kit,

Qiagen, Minneapolis, MN). The DNA was digested with restriction endonucleases (Invitrogen, Carlsbad, CA), size-fractionated in a 0.8% agarose gel and transferred to a nylon membrane (Sure Blot, Millipore, Billerica, MA). The Southern blots were hybridized with ³²P labeled probes to the joining region of the immunoglobulin heavy chain, the joining region of the immunoglobulin kappa light chain gene, the constant region of the beta T cell receptor (all from DAKO, Carpinteria, CA) and the joining region of the gamma T cell receptor (Dr. Jeffery Sklar, Yale Medical School) and autoradiographed.

RESULTS

Histological Findings

The H&E stained sections of the excision of the right inguinal lymph node showed complete effacement of the nodal architecture by an atypical lymphoid proliferation. (Figure 1A-D) The atypical lymphoid cells ranged in size from small to medium with occasional large cells. Some of the cells contain irregular nuclei, vesicular chromatin and distinct nucleoli. (Figure 1B)

IHC stains performed on the lymph node showed the neoplastic cells to be positive for CD3 and negative for CD20. (Figure 1 C, D). The neoplastic cells were also positive for CD2, CD43, beta-F1 and TIA-1 and negative for CD4, CD5, CD7, CD8, TdT, CD30, ALK-1, CD1a, CD79a, PAX-5, granzyme B and EBV in situ hybridization. Molecular testing by southern blot for T-cell receptor gene rearrangement at the outside institution showed a clonal rearrangement of TCR-beta and TCR-gamma. IgH did not show a clonal rearrangement.

The results of the immunohistochemical and molecular studies showed that the neoplastic cells were positive for CD3 and for T cell receptor gene rearrangement and negative for B cell antigens (CD20, CD79a, PAX-5) and IgH clonal rearrangement, thus confirming that the cells were of T cell origin. The lack of T cell antigens such as CD5 and CD7 and lack of both CD4 and CD8, further demonstrated the neoplastic nature of the T cell population. The lack of staining for TdT and CD1a showed that it was a mature T cell neoplasm. The additional negative staining for CD30 and ALK-1 further defined the diagnosis as peripheral T cell lymphoma, unspecified.

FNA of the right epitrochlear lymph node was subsequently performed at NIH (Figure 1 E, F). The cellular specimen was composed of numerous atypical lymphoid cells with irregular nuclei, prominent nucleoli and basophilic cytoplasm, consistent with the patient's history of malignant lymphoma.

Immunophenotyping by Flow Cytometry

FC was performed on the fine needle aspiration specimen. The specimen was analyzed on cells which fulfilled the lymphoid cell gate, based on forward and side scatter characteristics and the CD3 positive cell gate (Figure 2A-E).

Although debris is noted (low side and forward scatter characteristics), most of the viable cells were within the lymphoid cell gate (95%) (Figure 2A). Of the lymphocytes in the specimen, 97% were T cells, 1.5% were B cells and 1.5% were natural killer cells.

FC demonstrated an aberrant T cell population comprising approximately 41% of the lymphoid cells. This aberrant population was within the lymphoid cell gate, thus having similar forward scatter properties as the residual normal lymphoid cells. The aberrant T cell population expressed CD3, dim CD2, TCR alpha-beta, CD52, CD27 and partial expression of CD26 and was negative for CD4, CD5, CD7, CD8, TCR gamma-delta, CD56, CD16, and

CD57 (Figure 2). CD45 expression was also bright within this aberrant population (data not shown).

The aberrant T cell population also co-expressed CD19 (Figure 2 Q, R, S). The co-expression of CD19 in the aberrant T-cell population was present in two separate tubes, (see Immunophenotyping by flow cytometry section, Materials and Methods). In a 3rd tube, the aberrant CD19 expressing T-cell population was identified as the population lacking expression of surface kappa and lambda light chains (Figure 2 S). The level of CD19 expression on the aberrant population was of the same intensity as residual polyclonal B cells, (Figure 2 S, T). CD14 expression was also negative in the aberrant T cell population (Figure 2 U).

Subsequent FC analysis of a peripheral blood sample showed similar findings. Of the lymphocytes in the peripheral blood specimen, 80 % were T-cells, 3 % were B-cells and 17 % were natural killer cells (data not shown). In the lymphoid gate (Figure 3 A, C), there was an aberrant T-cell population comprising 1–2 % of the lymphocytes. This aberrant T-cell population showed the same immunophenotype as the lymph node specimen; most notably, co-expression of CD19 was also demonstrated (Figure 3 F, G). Table 1 summarizes the immunophenotypic results of the aberrant T-cell population as seen by flow cytometry and immunohistochemistry.

DISCUSSION

Peripheral T cell lymphoma, unspecified, is a lymphoma of mature T cells characterized by atypical morphology, antigenic loss and clonal T cell receptor gene rearrangement. T cell neoplasms can be diagnostically challenging on H&E stained sections. Cytologic morphology varies, especially within the subgroup of peripheral T cell lymphoma, unspecified. Reactive conditions, as well as some non-T cell lymphomas, may exhibit cytologic atypia within the T cells. Thus, immunophenotyping in the evaluation of a potential neoplastic process is essential.

Aberrant B cell antigen expression has also been described in mature T cell neoplasms. Although rare, aberrant expression of the CD20 B-cell marker has been observed on peripheral T cell lymphomas, and in some cases identified both by IHC and FC. Immunohistochemical analysis on one case report showed the neoplastic T cells to be positive for CD3, CD4, CD5, CD8, CD45RO and CD20 and negative for other B-cell markers such as CD79a and PAX-5 (5). Flow cytometry showed expression of CD20 and FMC-7 with lack of expression for CD79a and TdT. Another report described a peripheral T cell lymphoma expressing CD3, CD4, CD5, CD45RO and CD20 (9). By flow cytometry the cells were negative for CD19, CD22 and surface immunoglobulin. A clonal T cell receptor gamma chain gene rearrangement was detected. A review of the literature revealed 6 cases of CD20 positive T cell lymphomas that were negative for CD19 by flow cytometry (9). All of these cases demonstrate that CD20 can be rarely expressed in T cell lymphomas. Additionally, CD20 expression has been identified by FC on a subset of normal T-cells, but no normal T-cell subsets have been described that express CD19 (10). CD19 is an important and informative marker of B-cell lineage and maturation, and FC analysis relies heavily on the specificity of CD19 for defining B cell populations. CD19 is a transmembrane receptor protein which binds to CD21 for B cell activation. CD19 can be co-expressed in other neoplastic processes, such as acute myeloid leukemia harboring a t(8;21) translocation (1). However, to date, there have been no case reports in the literature describing co-expression of CD19 on a mature T-cell lymphoma.

The mechanism of aberrant CD19 expression may be related to expression of the transcription factor B-cell specific activator protein (BSAP), which is encoded by the PAX5 gene. In normal B-cell development, PAX5 plays a dual role in the commitment of bone marrow multipotent progenitor cells to the B lymphocyte lineage. PAX5 has a repressor role, inhibiting transcription of non- B cell hematopoietic differentiation. PAX5 also induces V_H-DJ_H recombination and transcriptional activation of CD19 and CD79a in B-cells(11). Aberrant CD19 expression in t(8;21) AML is well documented, and more recently, expression of BSAP in this AML subtype has been described ((12,13). In a recent series, CD19 expression was detected in 26/28 cases of t(8;21) AML, all of which expressed BSAP. Interestingly, this series also observed a case of CD19(+) T-cell ALL with expression of BSAP (13). Furthermore, a rare case of a mature T-cell lymphoma with aberrant expression of BSAP has been described (14). These findings would suggest a strong correlation between aberrant CD19 expression and PAX5. However, the mechanism of CD19 expression in our particular T-cell lymphoma case may fall outside of this potential mechanism, as immunohistochemistry for BSAP expression was negative in the neoplastic T-cells.

In our current case, the patient presented with diffuse superficial and deep lymphadenopathy, constitutional symptoms, hepatosplenomegaly and bone marrow and peripheral blood involvement by a neoplastic process. H&E stained sections showed effacement of the architecture by this atypical lymphoid proliferation. FC and IHC demonstrated an aberrant T cell lymphoid population in two separate lymph nodes and in the peripheral blood. The atypical lymphoid population consisted of a CD3 positive population expressing CD2, CD43, CD27, CD52, TCR alpha beta and partial expression of CD26 with loss of CD4, CD5, CD7 and CD8. The lack of staining for TdT and CD1a demonstrated that this was a mature T cell neoplasm. The clinical presentation and lack of additional markers such as CD30 and ALK-1 showed that this neoplasm was consistent with PTCL-NOS.

Molecular studies for T-cell receptor gene rearrangement were positive for a clonal process. CD16, CD56 and EBV in situ hybridization were negative, excluding an NK cell lymphoma. Immunohistochemical staining for specific B cell markers, CD20, CD79a and PAX-5 were negative, further excluding a neoplasm of B cell lineage. CD19 was attempted on the lymph node FNA specimen, but could not be successfully performed due to lack of viable cells remaining in the sample. Altogether, the immunophenotypic and molecular findings confirmed the neoplasm's T-cell lineage.

FC demonstrated unusual co-expression of CD19 in the neoplastic T-cells. In FC, CD19 is a key antigen used to define B cell populations; therefore, an FC panel with some redundancy built into its design is useful to confirm antigen expression and exclude an artifact of some kind. In this case, the CD19 co-expression on the aberrant T-cells was confirmed in two separate tubes containing CD19 and either CD3 or CD5, in both the FNA and peripheral blood specimens, and the findings were further supported in a 3rd tube, in which the aberrant CD19+ T-cell population was identified by its lack of surface kappa and lambda light chain immunoglobulin. The percentage of aberrant T cells of the total lymphoid cells was similar between the three separate tubes.

We also ruled out additional artifacts such as non-specific binding by monocyte Fc receptors by gating on the aberrant T cell population and demonstrating lack of CD14 expression. Non-specific binding by debris was excluded by analyzing the forward and side scatter characteristics of the aberrant T cell population, and confirming that the scatter properties were consistent with lymphoid cells, as the aberrant population fell within the lymphoid cell gate.

This is the first described case of a mature T cell neoplasm co-expressing CD19. This is an extremely rare event; however it is important to be aware of this rare phenomenon as it may cause a diagnostic dilemma.

Acknowledgments

We thank Catharine McCoy, Gregory Jasper and Linda Weaver for their technical assistance and the Molecular Diagnostics Laboratory, Department of Pathology, Brigham and Women's Hospital, Boston, MA, for information on molecular testing. This research was supported by the Intramural Research Program of the NIH, National Cancer Institute.

REFERENCES

1. Jaffe, ESHN.; Stein, H.; Vardiman, JW., editors. World Health Organization Classification of Tumours. Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001.
2. Tzankov A, Bourgau C, Kaiser A, Zimpfer A, Maurer R, Pileri SA, Went P, Dirnhofer S. Rare expression of T-cell markers in classical Hodgkin's lymphoma. *Mod Pathol.* 2005; 18(12):1542–1549. [PubMed: 16056244]
3. Lee HW, Kim K, Kim W, Ko YH. ALK-positive diffuse large B-cell lymphoma: report of three cases. *Hematol Oncol.* 2008
4. Quintanilla-Martinez L, Preffer F, Rubin D, Ferry JA, Harris NL. CD20+ T-cell lymphoma. Neoplastic transformation of a normal T-cell subset. *Am J Clin Pathol.* 1994; 102(4):483–489. [PubMed: 7524302]
5. Sun T, Akalin A, Rodacker M, Braun T. CD20 positive T cell lymphoma: is it a real entity? *J Clin Pathol.* 2004; 57(4):442–444. [PubMed: 15047755]
6. Magro CM, Seilstad KH, Porcu P, Morrison CD. Primary CD20+CD10+CD8+ T-cell lymphoma of the skin with IgH and TCR beta gene rearrangement. *Am J Clin Pathol.* 2006; 126(1):14–22. [PubMed: 16753590]
7. Mohrmann RL, Arber DA. CD20-Positive peripheral T-cell lymphoma: report of a case after nodular sclerosis Hodgkin's disease and review of the literature. *Mod Pathol.* 2000; 13(11):1244–1252. [PubMed: 11106083]
8. Blakolmer K, Vesely M, Kummer JA, Jurecka W, Mannhalter C, Chott A. Immunoreactivity of B-cell markers (CD79a, L26) in rare cases of extranodal cytotoxic peripheral T- (NK/T-) cell lymphomas. *Mod Pathol.* 2000; 13(7):766–772. [PubMed: 10912936]
9. Yokose N, Ogata K, Sugisaki Y, Mori S, Yamada T, An E, Dan K. CD20-positive T cell leukemia/lymphoma: case report and review of the literature. *Ann Hematol.* 2001; 80(6):372–375. [PubMed: 11475154]
10. Hultin LE, Hausner MA, Hultin PM, Giorgi JV. CD20 (pan-B cell) antigen is expressed at a low level on a subpopulation of human T lymphocytes. *Cytometry.* 1993; 14(2):196–204. [PubMed: 7679964]
11. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. *Nat Immunol.* 2007; 8(5):463–470. [PubMed: 17440452]
12. Tiaci E, Pileri S, Orleth A, Pacini R, Tabarrini A, Frenguelli F, Liso A, Diverio D, Lo-Coco F, Falini B. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. *Cancer Res.* 2004; 64(20):7399–7404. [PubMed: 15492262]
13. Valbuena JR, Medeiros LJ, Rassidakis GZ, Hao S, Wu CD, Chen L, Lin P. Expression of B cell-specific activator protein/PAX5 in acute myeloid leukemia with t(8;21)(q22;q22). *Am J Clin Pathol.* 2006; 126(2):235–240. [PubMed: 16891199]
14. Tzankov AS, Went PT, Munst S, Papadopoulos T, Jundt G, Dirnhofer SR. Rare expression of BSAP (PAX-5) in mature T-cell lymphomas. *Mod Pathol.* 2007; 20(6):632–637. [PubMed: 17431414]

Figure 1. Inguinal lymph node

A. Lymph node architecture is effaced by a diffuse atypical lymphoid proliferation (H&E, $\times 40$) **B.** The proliferation is composed of atypical medium to large sized lymphoid cells (Hematoxylin and eosin $\times 400$) **C.** CD3 stains numerous atypical T cells ($\times 100$) **D.** CD20 stains few residual B cells ($\times 100$). **E.** FNA of the right epitrochlear lymph node (Diff Quick $\times 200$). **F.** Medium to large sized lymphoid cells are present (Diff Quick $\times 400$)

Figure 2. Flow cytometry analysis of the epitrochlear lymph node FNA specimen

Analysis is performed on cells consistent with lymphocytes by FSC vs SSC (**Panel A**), with subsequent gating of CD3 + cells (highlighted in blue) (**Panel B, C, D, E**) and CD19+ cells (highlighted in pink) (**Panel O,P**), as described. Flow cytometry demonstrates an aberrant T-cell population with the following immunophenotype: CD3+ (**Panel B, D**), CD2 dim positive, CD52+, TCR alpha beta +, CD27 + and partially positive for CD26 (**Panel G, J, K, M**), CD5-, CD7-, CD4-, CD8-, TCR gamma-delta- (**Panel F, H, I, L**). CD56 and CD16 show a minor population of NK cells, but are not expressed on the aberrant T-cells (**Panel**

N, V). The aberrant T cell population is also positive for CD19 (**Panel Q, R, T**) and negative for kappa and lambda light chains and CD14 (**Panel S, U**).

\$watermark-text

\$watermark-text

\$watermark-text

Figure 3. Flow cytometry analysis of the peripheral blood specimen

Analysis is performed on cells consistent with lymphocytes by FSC vs SSC (**Panel A**), with subsequent gating of CD3 + cells (highlighted in blue) (**Panel B, C**) and CD19+ cells (highlighted in pink) (**Panel D, E**), as described. Flow cytometry demonstrates an aberrant T-cell population co-expressing CD19 and CD3 (**Panel F, G**), with an immunophenotype identical to that of the lymph node FNA.

Table 1

Summary of Flow Cytometry and Immunohistochemical phenotypic results of the aberrant T cell population

	Flow cytometry Lymph Node FNA and Peripheral Blood	Immunohistochemical stains Inguinal lymph node
Positive Antigens	CD45, CD19, CD3, dim CD2, TCR alpha-beta, CD52, CD27, partial expression of CD26	CD2, CD3, CD43, beta-F1, TIA-1
Negative Antigens	CD4, CD5, CD7, CD8, TCR gamma-delta, CD56, CD16, CD57	CD20, CD4, CD5, CD7, CD8, TdT, CD30, ALK-1, CD1a, CD79a, PAX-5, granzyme B, EBV in situ hybridization