PAX5-positive T-cell Anaplastic Large Cell Lymphomas Associated with Extra Copies of the *PAX5* Gene Locus

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Abstract

Cell lineage is the major criterion by which lymphomas are classified. Immunohistochemistry has greatly facilitated lymphoma diagnosis by detecting expression of lineage-associated antigens. However, loss or aberrant expression of these antigens may present diagnostic challenges. Anaplastic large cell lymphoma is a T-cell lymphoma that shows morphologic and phenotypic overlap with classical Hodgkin lymphoma, a tumor of B-cell derivation. Staining for the B-cell transcription factor, PAX5, has been suggested to be helpful in this differential, as it is positive in most classical Hodgkin lymphomas, but absent in anaplastic large cell lymphomas. Herein, we report four systemic T-cell anaplastic large cell lymphomas positive for PAX5 by immunohistochemistry, with weak staining intensity similar to that seen in classical Hodgkin lymphoma. All diagnoses were confirmed by a combination of morphologic, phenotypic, and molecular criteria. Three cases were ALK-negative and one was ALK-positive. PAX5 immunohistochemistry was negative in 198 additional peripheral T-cell lymphomas, including 66 anaplastic large cell lymphomas. Unexpectedly, though *PAX5* translocations were absent, all evaluable PAX5-positive anaplastic large cell lymphomas showed extra copies of the *PAX5* gene locus by fluorescence *in situ* hybridization. In contrast, only 4% of PAX5-negative peripheral T-cell lymphomas had extra copies of *PAX5*. We conclude that aberrant expression of PAX5 occurs rarely in T-cell anaplastic large cell lymphomas, and may be associated with extra copies of the *PAX5* gene. PAX5-positive lymphomas with morphologic features overlapping different lymphoma types should be evaluated with an extensive immunohistochemical panel and/or molecular studies to avoid diagnostic errors that could lead to inappropriate treatment. Since PAX5 overexpression causes T-cell neoplasms in experimental models, PAX5 may have contributed to lymphomagenesis in our cases.

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Keywords

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Cell lineage is the major criterion by which lymphomas are classified.[1] In routine clinical practice, the B- or T-cell origin of lymphomas is determined using immunophenotyping studies to detect lineage-associated antigens expressed by the tumor cells. Occasionally, however, loss or aberrant expression of lineage-associated antigens may present diagnostic challenges. One such challenge is the differential diagnosis between T-cell anaplastic large cell lymphoma and classical Hodgkin lymphoma, a tumor of B-cell derivation.

Anaplastic large cell lymphoma and classical Hodgkin lymphoma can show considerable morphologic overlap.[2,3] Anaplastic large cell lymphomas and other peripheral T-cell lymphomas may have Reed-Sternberg-like cells and a prominent mixed inflammatory background, leading to the introduction of the term, "Hodgkin-like" anaplastic large cell lymphoma.[4,5] Conversely, some cases of classical Hodgkin lymphoma are rich in tumor cells and have a minimal inflammatory background, resembling anaplastic large cell lymphoma.[6] In fact, many of the tumors originally considered "Hodgkin-like" anaplastic large cell lymphomas subsequently were reclassified as classical Hodgkin lymphomas.[7,8]

In addition to their morphologic features, anaplastic large cell lymphoma and classical Hodgkin lymphoma may show striking phenotypic overlap. Classical Hodgkin lymphomas typically express CD30 and CD15, lack expression of multiple B-cell antigens, and may aberrantly coexpress T-cell antigens and cytotoxic proteins.[9,10] Anaplastic large cell lymphomas and some peripheral T-cell lymphomas express CD30, may co-express CD15,[11-14] and often lack expression of multiple T-cell antigens despite having clonal T-cell receptor (TCR) gene rearrangements.[15,16] In addition, occasional peripheral T-cell lymphomas aberrantly express B-lineage markers such as CD20 and CD79a.[17-21] When present, expression of anaplastic lymphoma kinase (ALK) as a result of *ALK* gene translocation is helpful in establishing the diagnosis of anaplastic large cell lymphoma rather than classical Hodgkin lymphoma.[22] However, about 45% of anaplastic large cell lymphomas are ALK-negative.[23,24] Correct diagnosis is critical, since classical Hodgkin lymphoma and ALK-negative anaplastic large cell lymphoma are treated differently, and are associated with an 85% cure rate in the former and <50% 5-year overall survival in the latter.[24,25]

The paired box 5 (PAX5) transcription factor (B-cell-specific activating protein/BSAP) is necessary for B-lineage commitment,[26-28] and has shown excellent specificity for B-cell lineage by immunohistochemistry.[29-33] PAX5 staining may be helpful in the differential diagnosis between classical Hodgkin lymphoma and ALK-negative anaplastic large cell lymphoma, as it shows characteristic weak staining in most classical Hodgkin lymphomas[34] and "should be negative in all cases of anaplastic large cell lymphoma," according to the 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues.[8]

The purpose of this study was to characterize the morphologic, phenotypic, and genetic features of four cases of PAX5-positive anaplastic large cell lymphomas seen in our practice, and to compare these features to 198 additional peripheral T-cell lymphomas. Our findings indicate that PAX5 can be seen in otherwise typical anaplastic large cell lymphomas, and thus cannot be solely relied upon to distinguish anaplastic large cell lymphoma from classical Hodgkin lymphoma. Interestingly, PAX5-positive anaplastic large cell lymphomas showed extra copies of the *PAX5* gene locus, suggesting a possible mechanism for the PAX5 expression, and perhaps contributing to lymphomagenesis in these cases. Our findings support the use of a broad panel of B- and T-cell antigens in assigning lymphoma lineage, with additional molecular studies performed in ambiguous cases.

Materials and methods

During the period 2007 to 2009, four PAX5-positive anaplastic large cell lymphomas were identified from the hematopathology practice at Mayo Clinic, Rochester, Minnesota; 198 additional peripheral T-cell lymphomas from the years 1987 to 2009 identified from the Mayo Clinic archives were studied. All cases were classified based on 2008 WHO criteria.[1]

PAX5 immunohistochemistry was performed on paraffin embedded tissue sections by pretreating in 1mM EDTA buffer at pH 8.0 for 30 min at 97°C (PT Module; Lab Vision, Fremont, CA) and staining for PAX5 (1:200, clone 24, BD Bioscience) on a Dako (Carpinteria, CA) autostainer using the Advance detection system (Dako) with diaminobenzidine as the chromogen. Immunohistochemistry for other markers was performed as previously described[35] using antibodies shown in Table 1. Aside from CD30 and PAX5 (discussed below), immunostaining was scored as strong or weak, and designated as negative (-, no staining), focal (-/+, <10% of tumor cells), partial (+/-, 10-30% of tumor cells), or positive (+, >30% of tumor cells).

Polymerase chain reaction (PCR) for T-cell receptor (TCR) γ-chain and immunoglobulin gene rearrangements was performed as described previously.[36],[37] FISH for *PAX5* was performed and scored as described previously using a homebrew breakapart probe.[38] Briefly, DNA was isolated from bacterial artificial chromosome probes (ResGen - Invitrogen; Carlsbad, CA) spanning the *PAX5* locus as shown in Fig. 3c. Probes were labeled with SpectrumOrange-dUTP or SpectrumGreen-dUTP by nick translation (Abbott Molecular, Des Plaines, IL) and hybridized to tissue sections. Cases with 4 fusion signals were considered to have extra copies of the *PAX5* gene locus.

Additional peripheral T-cell lymphomas were evaluated by immunohistochemistry and/or FISH as indicated above on tissue microarrays constructed from paraffin blocks as previously described.[39] The study was approved by the Mayo Clinic Institutional Review Board and Biospecimens Committee.

Results

Clinicopathologic Findings of PAX5-positive Anaplastic Large Cell Lymphomas

The clinicopathologic features of the four PAX5-positive anaplastic large cell lymphomas are summarized in Table 2. There were two males and two females with an age range of 31 to 87 years. Three patients (cases 1-3) presented with lymphadenopathy and one (case 4) presented with a pathologic fracture of L4; imaging revealed masses in the neck, chest, and abdomen. Treatment data are available for three patients. One (case 1) had severe cardiac disease precluding systemic chemotherapy. He was treated with palliative radiotherapy for edema caused by bulky inguinal and pelvic adenopathy, and died two months later. Two patients (cases 2 and 4) were treated with cyclophosphamide, doxorubicin hydrochloride, oncovin, and prednisone (CHOP), and achieved a partial response at 6 months (4 cycles) and a complete response at 3 months (3 cycles), respectively.

Morphologic features in all four cases were characteristic of anaplastic large cell lymphoma (Figs. 1 and 2). All showed sheets of medium-sized to large lymphocytes with variably folded or horseshoe-shaped nuclei typical of so-called "hallmark" cells.[40] Reed-Sternberg cells were absent. A sinusoidal pattern of distribution was seen in cases with lymph node material available, most prominently noted in case 2 (Fig. 1e). Occasional inflammatory cells were present in the background, particularly in case 3 (Fig. 2a).

All cases showed uniform, strong staining for CD30 by immunohistochemistry (Figs. 1b, 1f, 2b, and 2f). One case (case 4) was positive for ALK (predominantly cytoplasmic; Fig. 2g). All cases were negative for CD3 and showed variable positivity for other T-cell antigens; of these, CD2 and CD4 were most commonly seen, with at least focal staining seen in 3 and 4 cases, respectively (Figs. 1c and 1g). Cytotoxic marker expression (TIA-1 or granzyme B) was seen at least focally in 3 cases. CD15 expression was seen in one case (case 3). Expression of EMA and clusterin was seen in 2 cases. PAX5 positivity was seen in >80% of tumor cells in all cases, was solely nuclear, and was weaker than that seen in reactive B cells (Figs. 1d and 2d), similar to the typical staining intensity of Reed-Sternberg cells in classical Hodgkin lymphoma. Other surface B-lineage markers (CD19, CD20, CD22, and CD79a) were negative. OCT2 (POU2F2) and BOB1 (POU2AF1, OBF1) were at least focally positive in 2 cases and 1 case, respectively.

All cases were evaluated by PCR for clonal TCR and immunoglobulin gene rearrangements. PCR failed in case 4 (decalcified specimen). Two of the remaining three cases showed clonal TCR gene rearrangements (Figs. 3a and 3b). None showed a clonal immunoglobulin gene rearrangement. Karyotyping was not done. FISH for the *PAX5* gene locus was performed in all cases. Hybridization failed in case 4. Extra copies of *PAX5* were seen in all remaining cases, with copy numbers ranging from 4 in case 1 to >10 in case 3 (Figs. 3e, 3f, and 3g). No *PAX5* translocation was found.

Immunohistochemical and FISH Studies of Additional T-cell Lymphomas

PAX5 was evaluated by immunohistochemistry in 198 additional patients (117 males and 81 females; mean age, 59 years) with the following peripheral T-cell lymphoma subtypes: 25 angioimmunoblastic T-cell lymphomas; 66 anaplastic large cell lymphomas (22 ALK-

positive, 33 ALK-negative, and 11 cutaneous); 82 peripheral T-cell lymphomas, NOS; 10 extranodal NK/T-cell lymphomas, nasal type; 6 cases of mycosis fungoides; 2 subcutaneous panniculitis-like T-cell lymphomas; 2 hepatosplenic T-cell lymphomas; 2 enteropathy-associated T-cell lymphomas; 2 T-cell large granular lymphocytic leukemias; and 1 T-cell prolymphocytic leukemia. All were negative for PAX5. Of these, 109 cases were evaluated by FISH for *PAX5*, and 92 demonstrated hybridization adequate for interpretation. No *PAX5* translocation was found. Four (4%) of the 92 PAX5 protein-negative peripheral T-cell lymphomas had extra copies of the *PAX5* gene locus. All were peripheral T-cell lymphomas, NOS. None resembled anaplastic large cell lymphoma morphologically. CD30 was negative in 3 and partially positive in 1 (10-30% of tumor cells). Other B-cell markers were negative.

Discussion

We report four cases of PAX5-positive T-cell anaplastic large cell lymphoma. Extra copies of the *PAX5* gene locus were demonstrated in all three cases evaluable by FISH. PAX5 is a transcription factor in the paired-box-containing family, which is involved in control of organ development and tissue differentiation.[41] PAX5 plays an essential role in B-lymphoid lineage commitment,[26-28] and is widely used as a B-cell marker in immunohistochemical evaluation of lymphoid tissues.[30] Anaplastic large cell lymphomas may share morphologic and phenotypic features with B-lineage neoplasms, particularly classical Hodgkin lymphoma. Therefore, our findings have important implications for interpreting PAX5 immunohistochemistry in lymphoma classification.

Our PAX5-positive anaplastic large cell lymphomas had clinical presentations, histologic features, and phenotypes (other than PAX5 expression) characteristic of anaplastic large cell lymphoma, allowing definitive classification despite the unusual positivity for PAX5. Consistent with previously published data,[42] the three ALK-negative cases lacked clonal immunoglobulin gene rearrangements, and two of three had clonal TCR gene rearrangements. Case 3 demonstrated coexpression of CD15, a finding typical of classical Hodgkin lymphoma but which also may be seen in anaplastic large cell lymphoma.[12-14] The other features did not support a diagnosis of classical Hodgkin lymphoma. There were characteristic hallmark cells with only occasional inflammatory cells seen in the background. In addition to the expression of T-cell antigens and cytotoxic markers, the tumor cells expressed BOB1 and (focally) OCT2, transcription factors typically absent in classical Hodgkin lymphoma.[43] Finally, the presence of a clonal TCR gene rearrangement and absence of clonal immunoglobulin gene rearrangement support the diagnosis of anaplastic large cell lymphoma in this case. Case 4 was a decalcified specimen and molecular studies were unsuccessful, but positivity for ALK assisted in confirming the diagnosis of anaplastic large cell lymphoma.

In a study of cases with overlapping features of anaplastic large cell lymphoma and classical Hodgkin lymphoma, Tamaru et al found weak PAX5 expression in 3 of 17 ALK-negative anaplastic large cell lymphomas and 0 of 11 ALK-positive anaplastic large cell lymphomas. [44] Though gene rearrangement studies were not performed to confirm T-cell origin, the three PAX5-positive tumors expressed both CD45 and BOB1, and two expressed EMA. These immunophenotypic features support the diagnosis of ALK-negative anaplastic large cell

lymphoma rather than classical Hodgkin lymphoma. The tumors lacked T-cell antigen expression, except for CD45RO in one case and TIA-1 in another, and were negative for OCT2. The phenotypes of our cases were similar in the intensity of PAX5 staining and variable staining for EMA. We found more consistent positivity for T-cell antigens and observed OCT2 expression in two cases; conversely, BOB1 was seen focally in only one of our cases and CD45 expression was more variable. In addition, one of our cases was ALK-positive.

A single previous case of peripheral T-cell lymphoma, NOS expressing PAX5 was reported by Tzankov et al.[45] No PAX5-positive cases were identified in additional peripheral T-cell lymphomas studied by Tzankov et al (n=43),[45] Krenacs et al (n=20),[31] Foss, et al (n=40),[34] or Torlakovic et al (n=26).[32] We did not identify any additional PAX5-positive cases in 198 peripheral T-cell lymphomas, including 66 additional anaplastic large cell lymphomas. Thus, the overall incidence of PAX5 positivity in peripheral T-cell lymphomas appears low. Nevertheless, PAX5 expression is not entirely specific for B-cell lineage in lymphomas. Furthermore, occasional non-lymphoid neoplasms express PAX5, including t(8;21)-positive acute myelogenous leukemias, small cell carcinomas, and other neuroendocrine tumors.[30]

Translocations between *PAX5* and the immunoglobulin heavy chain gene (*IGH@*) drive PAX5 expression in mature B-cell lymphomas,[46,47] In addition, PAX5 is oncogenic in T cells, since a reconstructed *PAX5/IGH@* translocation induces T-cell lymphoblastic lymphomas in mice.[48] Therefore, to investigate the mechanism for PAX5 expression in anaplastic large cell lymphoma, we performed FISH using a *PAX5* breakapart probe. We did not identify *PAX5* translocations. Unexpectedly, however, all (100%) PAX5-positive anaplastic large cell lymphomas with informative FISH studies had extra copies of the *PAX5* gene locus. In contrast, only 4% of PAX5-negative T-cell lymphomas had extra copies of *PAX5*. No PAX5-negative anaplastic large cell lymphoma had extra copies of *PAX5*, and previous genomic studies of anaplastic large cell lymphoma have not identified recurrent gains of 9p, on which *PAX5* resides.[49-51] These findings suggest a possible association between extra copies of *PAX5* and PAX5 protein expression in anaplastic large cell lymphomas. The finding of rare PAX5-negative T-cell lymphomas with extra copies of *PAX5* (all peripheral T-cell lymphomas, NOS) indicates that factors besides gene dosage influence PAX5 protein expression in T-cell lymphomas. *PAX5* methylation is associated with PAX5 negativity in human tumors[52,53] and might represent a mechanism by which T-cell lymphomas with extra copies of *PAX5* do not express PAX5 protein. However, we did not have adequate material to assess gene methylation in our cases.

In conclusion, recognizing the existence of PAX5-positive anaplastic large cell lymphomas is important to avoid incorrectly assigning B-cell lineage to these rare tumors. Specifically, PAX5 can not always differentiate anaplastic large cell lymphoma from classical Hodgkin lymphoma, particularly since the intensity of staining in PAX5-positive anaplastic large cell lymphomas is similar to that typically seen in classical Hodgkin lymphoma. Diagnostic errors can be avoided by interpreting PAX5 immunohistochemistry in the context of clinical features, morphology (including both cytologic features of the tumor cells and cellular background), and a panel of B- and T-lineage-associated antibodies. Molecular studies are recommended in cases with ambiguous lineage. Extra copies of the *PAX5* gene may

contribute to PAX5 expression in anaplastic large cell lymphomas. Finally, since PAX5 is oncogenic in T cells,[48] PAX5 expression may have contributed to lymphomagenesis in our cases.

Acknowledgments

References

1. Swerdlow, S.; Campo, E.; Harris, N., et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, World Health Organization Classification of Tumours. 4. Bosman, F.; Jaffe, E.; Lakhani, S.; Ohgaki, H., editors. Lyon: International Agency for Research on Cancer; 2008.

2. Stein H, Foss HD, Durkop H, et al. CD30(+) anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood. 2000; 96:3681–3695. [PubMed: 11090048]

3. Vassallo J, Lamant L, Brugieres L, et al. ALK-positive anaplastic large cell lymphoma mimicking nodular sclerosis Hodgkin's lymphoma: report of 10 cases. Am J Surg Pathol. 2006; 30:223–229. [PubMed: 16434897]

4. Leoncini L, Del Vecchio M, Kraft R, et al. Hodgkin's disease and CD30-positive anaplastic large cell lymphomas - a continuous spectrum of malignant disorders. Am J Pathol. 1990; 137:1047–1057. [PubMed: 2173409]

5. Pileri S, Bocchia M, Baroni C, et al. Anaplastic large cell lymphoma (CD30+/Ki-1+): results of a prospective clinicopathologic study of 69 cases. Br J Haematol. 1994; 86:513–523. [PubMed: 7519036]

6. Stein, H.; Delsol, G.; Pileri, SA., et al. Classical Hodgkin lymphoma, introduction, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4. Swerdlow, S.; Campo, E.; Harris, N.; Jaffe, E.; Pileri, S.; Stein, H.; Thiele, J.; Vardiman, J., editors. Lyon: International Agency for Research on Cancer; 2008. p. 326-329.

7. Jaffe ES. Anaplastic large cell lymphoma: the shifting sands of diagnostic hematopathology. Mod Pathol. 2001; 14:219–228. [PubMed: 11266530]

8. Mason, DY.; Harris, NL.; Delsol, G., et al. Anaplastic large cell lymphoma, ALK-negative, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Swerdlow, S.; Campo, E.; Harris, N.; Jaffe, E.; Pileri, S.; Stein, H.; Thiele, J.; Vardiman, J., editors. Lyon: International Agency for Research on Cancer; 2008. p. 317-319.

9. Tzankov A, Bourgau C, Kaiser A, et al. Rare expression of T-cell markers in classical Hodgkin's lymphoma. Mod Pathol. 2005; 18:1542–1549. [PubMed: 16056244]

10. Asano N, Oshiro A, Matsuo K, et al. Prognostic significance of T-cell or cytotoxic molecules phenotype in classical Hodgkin's lymphoma: a clinicopathologic study. J Clin Oncol. 2006; 24:4626–4633. [PubMed: 16954517]

11. Barry TS, Jaffe ES, Sorbara L, et al. Peripheral T-cell lymphomas expressing CD30 and CD15. Am J Surg Pathol. 2003; 27:1513–1522. [PubMed: 14657710]

12. Felgar RE, Salhany KE, Macon WR, et al. The expression of TIA-1+ cytolytic-type granules and other cytolytic lymphocyte-associated markers in CD30+ anaplastic large cell lymphomas (ALCL): correlation with morphology, immunophenotype, ultrastructure, and clinical features. Hum Pathol. 1999; 30:228–236. [PubMed: 10029454]

13. Gorczyca W, Tsang P, Liu Z, et al. CD30-positive T-cell lymphomas co-expressing CD15: an immunohistochemical analysis. Int J Oncol. 2003; 22:319–324. [PubMed: 12527929]

14. Perkins PL, Ross CW, Schnitzer B. CD30-positive, anaplastic large-cell lymphomas that express CD15 but lack CD45. A possible diagnostic pitfall. Arch Pathol Lab Med. 1992; 116:1192–1196. [PubMed: 1359849]

15. Foss HD, Anagnostopoulos I, Araujo I, et al. Anaplastic large-cell lymphomas of T-cell and null-cell phenotype express cytotoxic molecules. Blood. 1996; 88:4005–4011. [PubMed: 8916967]

16. Bonzheim I, Geissinger E, Roth S, et al. Anaplastic large cell lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling. Blood. 2004; 104:3358–3360. [PubMed: 15297316]

17. Quintanilla-Martinez L, Preffer F, Rubin D, et al. CD20+ T-cell lymphoma. Neoplastic transformation of a normal T-cell subset. Am J Clin Pathol. 1994; 102:483–489. [PubMed: 7524302]

18. Blakolmer K, Vesely M, Kummer JA, et al. Immunoreactivity of B-cell markers (CD79a, L26) in rare cases of extranodal cytotoxic peripheral T- (NK/T-) cell lymphomas. Mod Pathol. 2000; 13:766–772. [PubMed: 10912936]

19. Yao X, Teruya-Feldstein J, Raffeld M, et al. Peripheral T-cell lymphoma with aberrant expression of CD79a and CD20: a diagnostic pitfall. Mod Pathol. 2001; 14:105–110. [PubMed: 11235901]

20. Went P, Agostinelli C, Gallamini A, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. J Clin Oncol. 2006; 24:2472–2479. [PubMed: 16636342]

21. Sen F, Kang S, Cangiarella J, et al. CD20 positive mycosis fungoides: a case report. J Cutan Pathol. 2008; 35:398–403. [PubMed: 18261116]

22. Wellmann A, Otsuki T, Vogelbruch M, et al. Analysis of the t(2;5) (p23;q35) translocation by reverse trasnscription-polymerase chain reaction in CD 30+ anaplastic large-cell lymphomas, in other non-Hodgkin's of T-cell phenotype, and in Hodgkin's disease. Blood. 1995; 86:2321–2328. [PubMed: 7662979]

23. Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol. 2008; 26:4124–4130. [PubMed: 18626005]

24. Savage KJ, Harris NL, Vose JM, et al. ALK- anaplastic large-cell lymphoma is clinically and immunophenotypically different from both ALK+ ALCL and peripheral T-cell lymphoma, not otherwise specified: report from the International Peripheral T-Cell Lymphoma Project. Blood. 2008; 111:5496–5504. [PubMed: 18385450]

25. Diehl V, Engert A, Re D. New strategies for the treatment of advanced-stage Hodgkin's lymphoma. Hematol Oncol Clin North Am. 2007; 21:897–914. [PubMed: 17908627]

26. Nutt SL, Heavey B, Rolink AG, et al. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. Nature. 1999; 401:556–562. [PubMed: 10524622]

27. Mikkola I, Heavey B, Horcher M, et al. Reversion of B cell commitment upon loss of Pax5 expression. Science. 2002; 297:110–113. [PubMed: 12098702]

28. Cobaleda C, Jochum W, Busslinger M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. Nature. 2007; 449:473–477. [PubMed: 17851532]

29. Mhawech-Fauceglia P, Saxena R, Zhang S, et al. Pax-5 immunoexpression in various types of benign and malignant tumors: a high-throughput tissue microarray analysis. J Clin Pathol. 2007; 60:709–714. [PubMed: 16837628]

30. Feldman AL, Dogan A. Diagnostic uses of Pax5 immunohistochemistry. Adv Anat Pathol. 2007; 14:323–334. [PubMed: 17717432]

31. Krenacs L, Himmelmann AW, Quintanilla-Martinez L, et al. Transcription factor B-cell-specific activator protein (BSAP) is differentially expressed in B cells and in subsets of B-cell lymphomas. Blood. 1998; 92:1308–1316. [PubMed: 9694719]

32. Torlakovic E, Torlakovic G, Nguyen PL, et al. The value of anti-pax-5 immunostaining in routinely fixed and paraffin-embedded sections: a novel pan pre-B and B-cell marker. Am J Surg Pathol. 2002; 26:1343–1350. [PubMed: 12360049]

33. Tiacci E, Pileri S, Orleth A, et al. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. Cancer Res. 2004; 64:7399–7404. [PubMed: 15492262]

34. Foss HD, Reusch R, Demel G, et al. Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. Blood. 1999; 94:3108–3113. [PubMed: 10556196]

35. Kurtin PJ, Hobday KS, Ziesmer S, et al. Demonstration of distinct antigenic profiles of small B-cell lymphomas by paraffin section immunohistochemistry. Am J Clin Pathol. 1999; 112:319–329. [PubMed: 10478136]

36. Morice WG, Katzmann JA, Pittelkow MR, et al. A comparison of morphologic features, flow cytometry, TCR-Vbeta analysis, and TCR-PCR in qualitative and quantitative assessment of peripheral blood involvement by Sezary syndrome. Am J Clin Pathol. 2006; 125:364–374. [PubMed: 16613339]

37. McClure RF, Kaur P, Pagel E, et al. Validation of immunoglobulin gene rearrangement detection by PCR using commercially available BIOMED-2 primers. Leukemia. 2006; 20:176–179. [PubMed: 16307010]

38. Remstein ED, Law M, Mollejo M, et al. The prevalence of IG translocations and 7q32 deletions in splenic marginal zone lymphoma. Leukemia. 2007

39. Feldman AL, Law M, Grogg KL, et al. Incidence of TCR and TCL1 gene translocations and isochromosome 7q in peripheral T-cell lymphomas using fluorescence in situ hybridization. Am J Clin Pathol. 2008; 130:178–185. [PubMed: 18628085]

40. Benharroch D, Meguerian-Bedoyan Z, Lamant L, et al. ALK-positive lymphoma: a single disease with a broad spectrum of morphology. Blood. 1998; 91:2076–2084. [PubMed: 9490693]

41. Walther C, Guenet JL, Simon D, et al. Pax: a murine multigene family of paired box-containing genes. Genomics. 1991; 11:424–434. [PubMed: 1685142]

42. Tan BT, Seo K, Warnke RA, et al. The frequency of immunoglobulin heavy chain gene and T-cell receptor gamma-chain gene rearrangements and Epstein-Barr virus in ALK+ and ALK- anaplastic large cell lymphoma and other peripheral T-cell lymphomas. J Mol Diagn. 2008; 10:502–512. [PubMed: 18832464]

43. Loddenkemper C, Anagnostopoulos I, Hummel M, et al. Differential Emu enhancer activity and expression of BOB.1/OBF.1, Oct2, PU.1, and immunoglobulin in reactive B-cell populations, B-cell non-Hodgkin lymphomas, and Hodgkin lymphomas. J Pathol. 2004; 202:60–69. [PubMed: 14694522]

44. Tamaru J, Tokuhira M, Nittsu N, et al. Hodgkin-like anaplastic large cell lymphoma (previously designated in the REAL classification) has same immunophenotypic features to classical Hodgkin lymphoma. Leuk Lymphoma. 2007; 48:1127–1138. [PubMed: 17577776]

45. Tzankov AS, Went PT, Munst S, et al. Rare expression of BSAP (PAX-5) in mature T-cell lymphomas. Mod Pathol. 2007; 20:632–637. [PubMed: 17431414]

46. Busslinger M, Klix N, Pfeffer P, et al. Deregulation of PAX-5 by translocation of the Emu enhancer of the IgH locus adjacent to two alternative PAX-5 promoters in a diffuse large-cell lymphoma. Proc Natl Acad Sci U S A. 1996; 93:6129–6134. [PubMed: 8650231]

47. Poppe B, De Paepe P, Michaux L, et al. PAX5/IGH rearrangement is a recurrent finding in a subset of aggressive B-NHL with complex chromosomal rearrangements. Genes Chromosomes Cancer. 2005; 44:218–223. [PubMed: 15942942]

48. 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; 109:281–289. [PubMed: 16968900]

49. Salaverria I, Bea S, Lopez-Guillermo A, et al. Genomic profiling reveals different genetic aberrations in systemic ALK-positive and ALK-negative anaplastic large cell lymphomas. Br J Haematol. 2008; 140:516–526. [PubMed: 18275429]

50. Mao X, Orchard G, Lillington DM, et al. Genetic alterations in primary cutaneous CD30+ anaplastic large cell lymphoma. Genes Chromosomes Cancer. 2003; 37:176–185. [PubMed: 12696066]

51. Zettl A, Rudiger T, Konrad MA, et al. Genomic profiling of peripheral T-cell lymphoma, unspecified, and anaplastic large T-cell lymphoma delineates novel recurrent chromosomal alterations. Am J Pathol. 2004; 164:1837–1848. [PubMed: 15111330]

52. Lazzi S, Bellan C, Onnis A, et al. Rare lymphoid neoplasms coexpressing B- and T-cell antigens. The role of PAX-5 gene methylation in their pathogenesis. Hum Pathol. 2009; 40:1252–1261. [PubMed: 19368954]

53. Palmisano WA, Crume KP, Grimes MJ, et al. Aberrant promoter methylation of the transcription factor genes PAX5 alpha and beta in human cancers. Cancer Res. 2003; 63:4620–4625. [PubMed: 12907641]
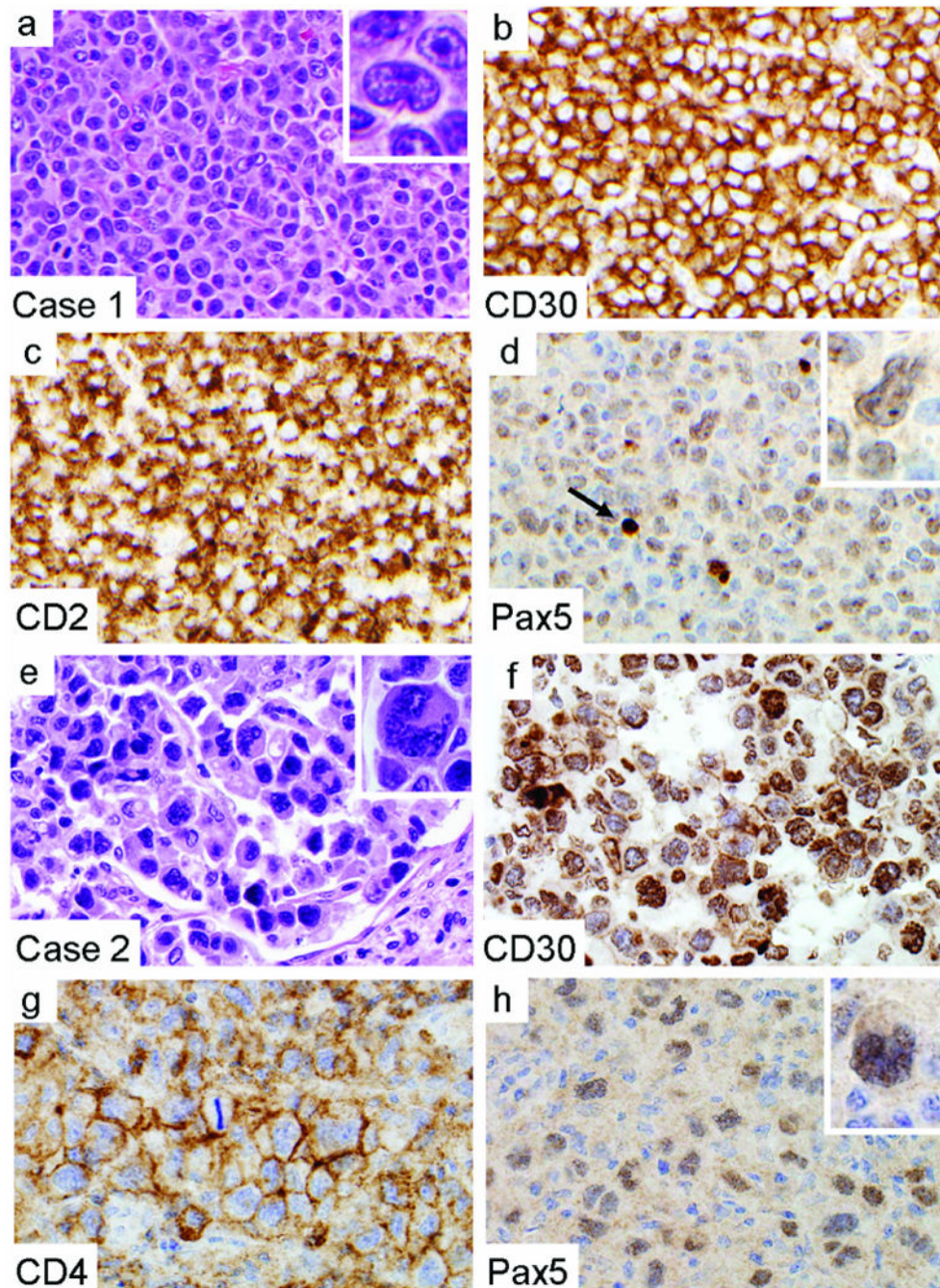
Figure 1.
Histologic and immunophenotypic features of PAX5-positive anaplastic large cell lymphomas (original magnification x400; insets, x1000). (**a-d**) Case 1: ALK-negative anaplastic large cell lymphoma. Hematoxylin and eosin (H&E) -stained slides of a lymph node (**a**) shows sheets of hallmark cells without a significant inflammatory background. The tumor cells are positive for CD30 (**b**) and CD2 (**c**). PAX5 (**d**) shows weak nuclear positivity in the large tumor cells, compared with strong positivity in occasional small B cells (arrow). (**e-h**) Case 2: ALK-negative anaplastic large cell lymphoma. H&E-stained slides of a lymph
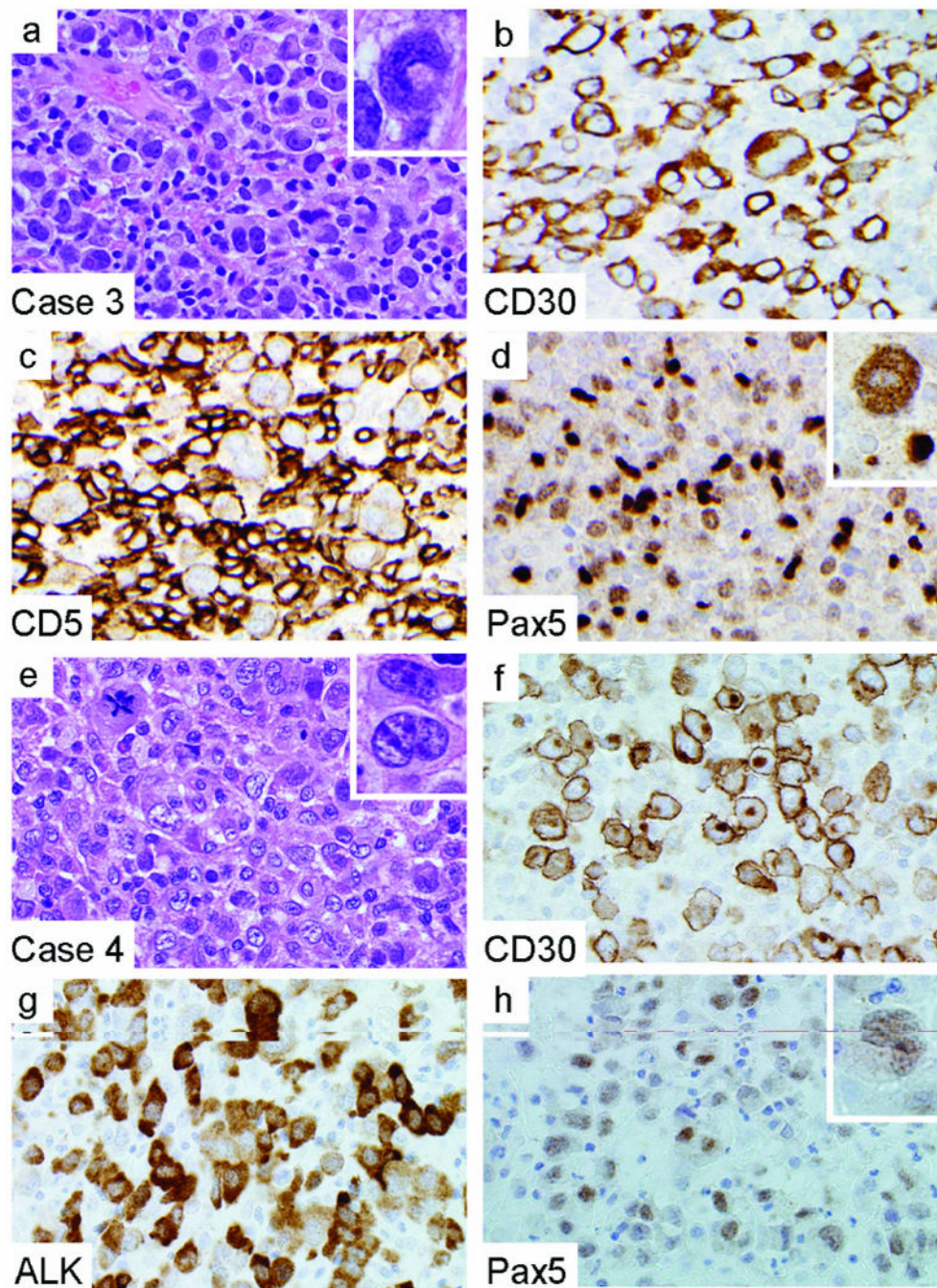
node show hallmark cells within sinuses (**e**). The tumor cells are positive for CD30 (**f**) and CD4 (**g**), and are weakly positive for PAX5 (**h**).

Figure 2.
Histologic and immunophenotypic features of PAX5-positive anaplastic large cell lymphomas, *continued* (original magnification x400; insets, x1000). (**a-d**) Case 3: ALK-negative anaplastic large cell lymphoma. H&E-stained slides of a lymph node show numerous hallmark cells (**a**). The tumor cells are positive for CD30 (**b**) and CD5 (**c**). PAX5 (**d**) is more weakly positive in the tumor cells (inset, upper left) than in admixed small B cells (inset, lower right). (**e-h**) Case 4: ALK-positive anaplastic large cell lymphoma. H&E-
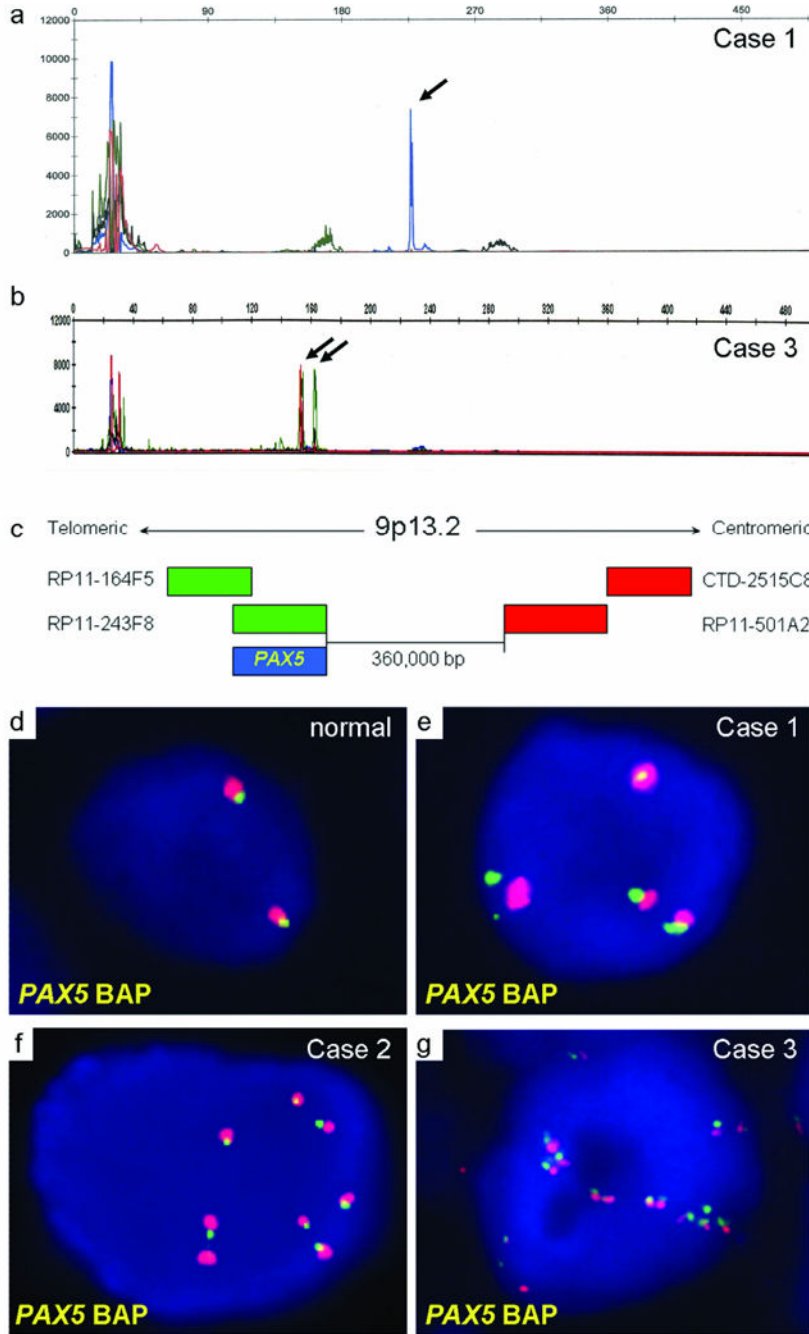
stained slides of an L4 vertebral mass show numerous hallmark cells (**e**). The tumor cells are positive for CD30 (**f**) and ALK (**g**), and are weakly positive for PAX5 (**h**).

Figure 3.
Molecular features of PAX5-positive anaplastic large cell lymphomas. (**a,b**) PCR for T-cell receptor γ-chain gene rearrangement in cases 1 (**a**) and 3 (**b**) show clonal peaks (arrows). (**c**) FISH was performed using a breakapart probe for the *PAX5* gene locus on 9p13.2, with bacterial artificial chromosome (BAC) designations as shown. Centromeric and telomeric BACs were labeled red and green, respectively. Relative location of *PAX5* is shown in blue. (**d**) A normal cell shows 2 fusion signals by FISH. (**e-g**) Cells from PAX5-positive anaplastic large cell lymphomas show extra copies of the *PAX5* gene locus.

Table 1

Antibodies Used in Immunophenotypic Analyses.

Antigen	Clone	Dilution	Source
ALK	ALK1	1:100	Dako (Carpinteria, CA)
BetaF1	8A3	1:100	Endogen (Woburn, MA)
BOB1	TG14	1:200	Novocastra (Newcastle upon Tyne, England)
CD2	AB75	1:100	Novocastra
CD3	PS1	1:50	Novocastra
CD4	4B12	1:600	Novocastra
CD5	4C7	1:300	Novocastra
CD7	LP15	1:200	Novocastra
CD8	C8/144B	1:100	Dako
CD15	MMA	1:50	BD Biosciences (Franklin Lakes, NJ)
CD19	LE-CD19	1:200	Dako
CD20	L26	1:200	Dako
CD22	FPC1	1:200	Novocastra
CD30	Ber-H2	1:20	Dako
CD43	L60	1:10000	BD Biosciences
CD45	2B11+PD7/26	1:1500	Dako
CD56	123C3	1:25	Monosan/Caltag (Burlingame, CA)
CD79a	JCB117	1:50	Dako
Clusterin	41D	1:200	Upstate (Lake Placid, NY)
EMA	E29	1:50	Dako
Granzyme B	GRB-7	1:100	Monosan/Caltag
OCT2	OCT-207	1:100	Novocastra
PAX5	24	1:200	BD Biosciences
TIA-1	TIA-1	1:200	Immunotech (Fullerton, CA)

Table 2

Clinical Features and Results of Immunohistochemistry and Molecular Studies in PAX5-positive Anaplastic Large Cell Lymphomas.

	Case 1	Case 2	Case 3	Case 4
Clinical Features				
Age (yr)/Gender	87/M	31/F	45/F	53/M
Biopsy site	inguinal lymph node	axillary lymph node	axillary lymph node	L4 vertebra
Stage	III	IIIA	unknown	IVB
Outcome	died of disease	alive, PR	unknown	alive, CR
Follow-up (mos)	2	6	-	3
Immunohistochemistry[*]				
ALK	-	-	-	+
BetaF1	-	nd	-	-
BOB1	-	-	-/+(w)	-
CD2	+	-/+	+/-	-
CD3	-	-	-	-
CD4	+	+	+/-	+/-(w)
CD5	-	-	+	-
CD7	-	-/+	nd	-
CD8	-	-	nd	-
CD15	-	nd	+	-
CD19	-	-	-	-
CD20	-	-	-	-
CD22	-	-	-	-
CD30	+	+	+	+
CD43	+	nd	+/-	-
CD45	+ (w)	-	+/-	+ (w)
CD79a	-	-	-	-
Clusterin	-	-/+(w)	-	+
EMA	-	+/-(w)	-	+
Granzyme B	-	nd	-/+	-/+
OCT2	-	-	+ (w)	-/+(w)
PAX5	+ (w)	+ (w)	+ (w)	+ (w)
TIA-1	-	-/+	-/+	-
Gene rearrangement (PCR)				
T-cell receptor	positive	negative	positive	failed
Immunoglobulin	negative	negative	negative	failed
FISH				
4 copies of *PAX5*	yes	yes	yes	failed

[*] Immunohistochemical staining intensity was strong unless otherwise indicated [(w)=weak]. -, negative; -/+, <10% of tumor cells; +/-, 10-30%; +, >30%. PR, partial response; CR, complete response; nd, not done.