

Utility of CD200 immunostaining in the diagnosis of primary mediastinal large B cell lymphoma: comparison with MAL, CD23, and other markers

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CD200, an immunoglobulin superfamily membrane glycoprotein, is expressed in a number of B cell lymphoproliferative disorders, including primary mediastinal large B cell lymphoma, but not diffuse large B cell lymphoma, based on a preliminary study. Here, we compare the expression of CD200 with other markers of primary mediastinal large B cell lymphoma, including MAL and CD23, in formalin-fixed, paraffin-embedded histologic sections from a series of 35 cases of primary mediastinal large B cell lymphoma and 30 cases of diffuse large B cell lymphoma. CD200 exhibits the greatest staining sensitivity of the markers studied: 94%, compared with CD23 (69%), MAL (86%), TRAF (86%), and REL (77%). It exhibits staining specificity of 93%, similar to that of CD23 (93%) and MAL (97%), and greater than that of TRAF (77%) and REL (83%). We conclude that CD200 is a practical and useful marker for the diagnosis of primary mediastinal large B cell lymphoma. *Modern Pathology* (2012) 25, 1637–1643; doi:10.1038/modpathol.2012.129; published online 17 August 2012

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CD200 or OX-2 antigen is an immunoglobulin superfamily membrane glycoprotein¹ widely expressed in multiple cell types, including B cells, a subset of T cells, and a number of non-hematopoietic cells.² It interacts with CD200R, a receptor expressed primarily on myeloid/monocyte lineage cells, and has a suppressive effect on T cell mediated immune response.^{3,4} CD200 is expressed in a number of hematopoietic neoplasms, including acute myeloid leukemia,⁵ multiple myeloma,⁶ and is a marker of poor prognosis when expressed in these neoplasms, probably through its immunosuppressive effect on the host immune system.

We and others have found that CD200 is expressed in a number of B cell-derived neoplasms, including chronic lymphocytic leukemia/small lymphocytic lymphoma, hairy cell leukemia, B lymphoblastic leukemia/lymphoma and classical Hodgkin lymphoma,^{7,8} and by

follicular helper T cells and in a T cell neoplasm, angioimmunoblastic T cell lymphoma.⁹ In our study of CD200 expression in B cell lymphoproliferative disorders, we found that CD200 is expressed by the neoplastic B cells in primary mediastinal large B cell lymphoma (8 of 8 cases), but not in diffuse large B cell lymphoma (0 of 12 cases; $P < 0.0001$; 8).

Primary mediastinal large B cell lymphoma, a large B cell lymphoma, is thought to arise from thymic B cells in the anterior mediastinum. It occurs in young adults with female predominance. Primary mediastinal large B cell lymphoma expresses pan-B cell markers, with variable expression of CD30, Bcl-2, Bcl-6, and CD10. A number of immunophenotypic markers of primary mediastinal large B cell lymphoma (to distinguish it from diffuse large B cell lymphoma) have been described, including CD23, a transmembrane glycoprotein expressed in mature B cells and follicular dendritic cells, MAL, a membrane glycoprotein expressed in thymocytes and thymic medullary B cells, TRAF and REL, nuclear factor κ B signaling pathway molecules recently identified as markers of primary mediastinal large B cell lymphoma by gene expression array analysis. All of these markers have analytical

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or practical limitations, including moderate sensitivity and specificity or lack of commercial reagent availability (10–12; summarized in Table 1).

Based on our preliminary CD200 findings, we studied a large series of cases of primary mediastinal large B cell lymphoma and diffuse large B cell lymphoma to assess the sensitivity and specificity of CD200 as an immunophenotypic marker of primary mediastinal large B cell lymphoma compared with diffuse large B cell lymphoma. We also compared its expression with that of other reported markers of primary mediastinal large B cell lymphoma, to assess the relative diagnostic utility of CD200.

Materials and methods

Case Selection

Cases were obtained from the files of the Department of Pathology at Brigham and Women's Hospital, Boston, MA, with the institutional internal review board's approval. The pathologic diagnoses were originally established according to the criteria of the 2008 WHO classification, based on morphologic and immunohistochemical findings.¹³ Cases of diffuse large B cell lymphoma exhibited a diffuse proliferation of large neoplastic lymphoid cells with nuclear size greater than that of a macrophage nucleus. Within lymphoid tissue, there was complete or partial effacement of nodal architecture, and at extranodal sites, sheets of large lymphoid cells were observed. Neoplastic cells exhibited immunostaining for one or more pan-B cell markers, including CD20, CD79a, and Pax-5/BSAP. Cases of primary mediastinal large B cell lymphoma typically presented with a large anterior mediastinal mass consisting of intermediate to large-sized lymphoid cells with round to oval nuclei with associated fibrosis. Neoplastic cells exhibited immunostaining for one or more pan-B cell markers, including CD20, CD79a, and Pax-5/BSAP. Diagnostic slides from all cases were reviewed by one of the authors of this study (DMD).

Immunohistochemistry

Immunohistochemistry was performed on 5 μ m thick formalin-fixed, paraffin-embedded tissues sec-

tions using a standard indirect avidin-biotin horse-radish peroxidase method and diaminobenzidine color development, as previously described.¹⁴ Briefly, slides were soaked in xylene, passed through graded alcohols and put in distilled water. The sections were blocked for peroxidase activity with 3% hydrogen peroxide in ethanol for 15 min and washed under the running water for 5 min. Slides were then pretreated with 10 mM citrate, pH 6.0 in a pressure cooker for 30 min in preheated buffer, followed by washing in distilled water. All further steps were performed at room temperature in a hydrated chamber.

Immunostaining with goat anti-human CD200 polyclonal antibody (R&D Systems, Minneapolis, MN) was compared with that of goat IgG control antibody diluted to identical protein concentration, to confirm staining specificity, as previously described.^{8,9} MAL antibody was previously described,¹⁰ and staining was compared with that of mouse IgG control antibody diluted to identical protein concentration, to confirm staining specificity. For cases lacking prior immunostaining results for CD23, TRAF, and REL, immunostaining was performed using mouse monoclonal antibodies obtained from The Binding Site, Birmingham, AL; Santa Cruz Biotechnology, Santa Cruz, CA; and Calbiochem, San Diego, CA, respectively, and compared with mouse IgG control antibody diluted to identical protein concentration, to confirm staining specificity.

Case Evaluation

Reactivity for CD200, CD23, TRAF, REL, and MAL was considered as positive if there was more than focal staining of neoplastic cells (>20%). Regarding CD200 staining, staining was considered as homogenous if >80% of the neoplastic cells exhibited staining, and it was considered as heterogenous if 20–80% of the neoplastic cells exhibited staining. Photomicrographs were generated on an Olympus BH2 microscope (Olympus, Center Valley, PA) using an Olympus DP25 digital camera and analyzed with the software Adobe Photoshop 11.0 (Adobe, San Jose, CA).

Table 1 Summary of immunophenotypic markers of primary mediastinal large B cell lymphoma

Marker	Expression in primary mediastinal large B cell lymphoma	Expression in diffuse large B cell lymphoma	Commercially available?	Reference
CD200 (preliminary study)	8/8 (100%)	0/12 (0%)	Yes	9
CD200 (current study)	33/35 (94%)	2/30 (7%)	Yes	Current study
MAL	21/33 (70%)	1/33 (3%)	No	10
CD23	17/24 (71%)	12/100 (12%)	Yes	11
TRAF	48/78 (62%)	20/173 (12%)	Yes	12
REL	31/48 (65%)	28/160 (18%)	Yes	12

Results

A total of 35 cases of primary mediastinal large B cell lymphoma and 30 cases of diffuse large B cell lymphoma were evaluated for expression of CD200 and other markers of primary mediastinal large B cell lymphoma. Patients with primary mediastinal large B cell lymphoma (18 women, 17 men) ranged in age from 21 to 73 years (mean 38 ± 12 years). Thirty-four patients presented with anterior mediastinal masses and underwent biopsy of the mass, including two needle biopsies. In two cases, patients underwent needle biopsies of the lung. One patient underwent biopsy of a chest wall mass. The neoplastic cells were typically medium to large in size with round to oval nuclei, pale cytoplasm and associated fibrosis (Figure 1a). Routine immunohistochemical staining performed as part of the diagnostic evaluation revealed that the neoplastic cells were immunoreactive for pan-B cell markers in all cases, with co-expression of CD30 in 21/29 cases, of which 13 exhibited subset staining and 5 exhibited focal staining. Bcl-2 expression was seen in 19/21 cases, of which 7 exhibited subset staining and one exhibited focal staining. Bcl-6 expression was seen in 26/28 cases, of which 9 exhibited subset staining and 2 exhibited variable staining. CD10 was expressed by 4/24 cases, with 1 exhibiting weak staining and 1 exhibiting rare staining. The Ki-67 proliferation index ranged from 20 to 95% (mean 61.0 ± 23.5).

Patients with diffuse large B cell lymphoma (19 women, 11 men) ranged in age from 34 to 85 years (mean 62 ± 11 years). Patients presented with lymphadenopathy in 14 cases, including 2 involving the mediastinum, and with masses at various anatomic sites in the remainder. Five of the patients underwent needle biopsies. The neoplastic cells were large in size with round to oval to irregular vesicular nuclei with distinct to prominent nucleoli and scant to moderate cytoplasm. Routine immunohistochemical staining performed as part of the diagnostic evaluation revealed that the neoplastic cells were immunoreactive for pan-B cell markers in all cases with co-expression of CD30 in 1/2 cases, Bcl-2 in 16/17 cases (3 with subset staining), Bcl-6 in 18/21 cases (1 with subset staining), and CD10 in 15/27 cases (1 with subset staining and 1 with weak staining). The Ki-67 proliferation index ranged from 20 to 95% (mean 72.1 ± 21.7).

The majority of primary mediastinal large B cell lymphoma cases exhibited immunostaining for CD200 (33/35; 94% sensitivity; summarized in Table 2), typically with a strong membrane and cytoplasmic staining pattern (Figure 1b). Staining was homogenous in 18 cases, including 3 of the needle biopsies and heterogenous in 15 cases, including 1 of the needle biopsies. Two cases of primary mediastinal large B cell lymphoma were CD200 negative, but positive for at least two other markers of primary mediastinal large B cell lymphoma (Table 2). The

patients were 44- and 73-year-old men and their neoplasms were negative for CD10 and CD30 and positive for BCL-6 (data not shown). In contrast, 28 of 30 cases of diffuse large B cell lymphoma did not exhibit immunostaining for CD200 (93% specificity; $P < 0.0001$; summarized in Table 3). Both CD200-positive cases exhibited necrosis, one focal and one extensive, and were immunoreactive for one other marker of primary mediastinal large B cell lymphoma (Table 3). One biopsy specimen exhibited homogenous staining for CD200, and the other, a needle biopsy, exhibited heterogenous CD200 staining. The patients were 54- and 85-year-old men with CD10-negative diffuse large B cell lymphoma of the tonsil and psoas muscle, respectively.

We studied several additional markers of primary mediastinal large B cell lymphoma to compare CD200 staining results with that of other markers of primary mediastinal large B cell lymphoma. CD23 exhibited strong membrane and cytoplasmic staining in 24/35 cases of primary mediastinal large B cell lymphoma (69% sensitivity; Figure 1c). Ten of eleven CD23-negative cases were positive for CD200, and all were positive for at least two additional markers of primary mediastinal large B cell lymphoma (Table 2). The CD23-negative cases did not exhibit any specific staining pattern for CD30, CD10, Bcl-2, and Bcl-6, although all were non-immunoreactive for CD10 (7/7 and data not shown). A total of 2 of 30 cases (7%) of diffuse large B cell lymphoma were CD23 positive. Both cases were CD200 negative, exhibited staining for at least one additional marker of primary mediastinal large B cell lymphoma (Table 3), and were immunoreactive for Bcl-6.

MAL exhibited strong membrane and cytoplasmic staining in 30/35 cases (86%) of primary mediastinal large B cell lymphoma (Figure 1d). Four of five MAL-negative cases were immunoreactive for CD200 and all were immunoreactive for at least two additional markers of primary mediastinal large B cell lymphoma (Table 2). The MAL-negative cases did not exhibit any specific staining pattern for CD30, CD10, Bcl-2, and Bcl-6 (data not shown). Only 1 of the 30 diffuse large B cell lymphoma cases (3%) was MAL positive. This case was CD200 negative, exhibited staining for two other markers of primary mediastinal large B cell lymphoma (Table 3), and was of follicular origin (immunoreactive for CD10, Bcl-6, and Bcl-2).

We found that TRAF exhibited cytoplasmic staining in 30 of 35 cases of primary mediastinal large B cell lymphoma (86%; Figure 1e). All five TRAF-negative neoplasms were immunoreactive for CD200 and at least one additional marker of primary mediastinal large B cell lymphoma (Table 2). The TRAF-negative cases did not exhibit any specific staining pattern for CD30, CD10, Bcl-2, and Bcl-6, although all exhibited subset staining for CD30 (data not shown). In contrast, 7 of 30 cases of diffuse large B cell lymphoma (23%) were immunoreactive for TRAF, and, in 4/7 cases, at least 1 additional

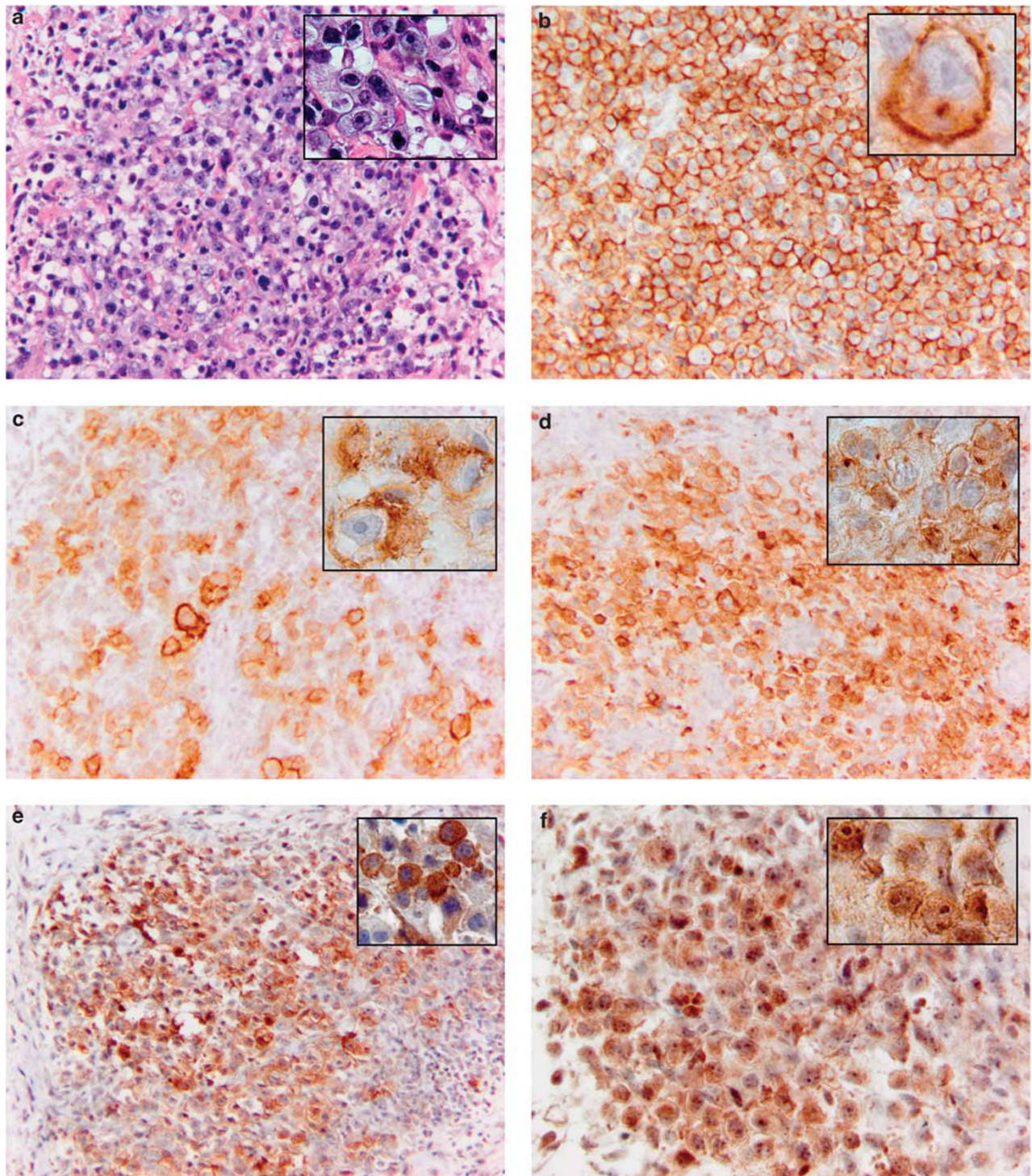


Figure 1 Primary mediastinal large B cell lymphoma (a) contains sheets of medium to large-sized neoplastic cells with round to oval nuclei, pale cytoplasm and associated fibrosis. The neoplastic cells exhibit membrane and cytoplasmic staining for CD200 (b), membrane and cytoplasmic staining for CD23 (c), membrane and cytoplasmic staining for MAL (d), primarily cytoplasmic staining for TRAF (e), and nuclear and cytoplasmic staining for REL (f). (a–e) $\times 200$, (f) $\times 500$; insets $\times 1000$.

primary mediastinal large B cell lymphoma marker (Table 3). All cases studied were immunoreactive for BCL-6 (6/6) and Bcl-2 (4/4). REL exhibited nuclear and cytoplasmic staining in 27 of 35 cases of

primary mediastinal large B cell lymphoma (77%; Figure 1f). Seven of eight REL-negative neoplasms were immunoreactive for CD200 and all were immunoreactive for at least 3 additional markers

Table 2 Summary of primary mediastinal large B cell lymphoma immunostaining results.^a

Case no.	CD200	CD23	MAL	TRAF	REL
1					
2					
3					
4					
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32					
33					
34					
35					
sensitivity	33/35 = 94%	24/35 = 69%	30/35 = 86%	30/35 = 86%	27/35 = 77%

^aDark cells: $\geq 20\%$ immunoreactivity; light cells: $< 20\%$ immunoreactivity.

of primary mediastinal large B cell lymphoma (Table 3). The REL-negative cases did not exhibit any specific staining pattern for CD30, CD10, Bcl-2, and Bcl-6, although cases studied exhibited immunostaining for Bcl-6 (6/6) and were non-immunoreactive for CD10 (5/5; data not shown). In contrast, 5 of 30 cases of diffuse large B cell lymphoma (17%) were immunoreactive for TRAF, and, in 2/5 cases, at least 1 additional primary mediastinal large B cell lymphoma marker (Table 3).

Table 3 Summary of diffuse large B cell lymphoma immunostaining results^a

Case no.	CD200	CD23	MAL	TRAF	REL
1					
2					
3					
4					
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30					
specificity	28/30 = 93%	28/30 = 93%	29/30 = 97%	23/30 = 77%	25/30 = 83%

^aDark cells: $\geq 20\%$ immunoreactivity; light cells: $< 20\%$ immunoreactivity.

Discussion

CD200, an immunoglobulin superfamily membrane glycoprotein, is expressed in a range of hematopoietic neoplasms and is a useful immunophenotypic marker for immunohistochemical staining in diagnostic hematopathology.^{8,9} In addition, CD200 immunoreactivity can be assessed by flow-cytometric analysis in addition to immunohistochemical staining (Palumbo *et al*¹⁵ and Alapat *et al*¹⁶; Pillai *et al*, manuscript in preparation). A number of B cell-derived neoplasms are immunoreactive for CD200, including chronic lymphocytic leukemia/small lymphocytic lymphoma, hairy cell leukemia, B lymphoblastic leukemia/lymphoma, multiple

myeloma, and classical Hodgkin lymphoma.^{7,8} CD200 is useful in the differential diagnosis in a number of B cell-derived neoplasms, because it is not expressed in mantle cell lymphoma (*vs* chronic lymphocytic leukemia/small lymphocytic lymphoma), Burkitt lymphoma (*vs* B lymphoblastic leukemia/lymphoma), or nodular lymphocyte predominant Hodgkin lymphoma (*vs* classical Hodgkin lymphoma; Dorfman and Shahsafaei⁸, Palumbo *et al*¹⁵; Pillai *et al*, in preparation). In a recent flow-cytometric study, we found that CD200 staining can differentiate hairy cell leukemia from hairy cell leukemia variant as well (Pillai *et al*, in preparation). In our initial study of CD200 expression in B cell lymphoproliferative disorders, we found that CD200 is expressed in primary mediastinal large B cell lymphoma but not in diffuse large B cell lymphoma.⁸ The current study substantiates the preliminary observation and suggests that CD200 is a useful marker for the differential diagnosis of large cell neoplasms of the mediastinum.

Our findings also are consistent with earlier studies on CD23, MAL, TRAF, and REL as markers useful for the distinction of primary mediastinal large B cell lymphoma from diffuse large B cell lymphoma. Calaminici *et al*¹¹ found that 17/24 cases (70%) of primary mediastinal large B cell lymphoma were immunoreactive for CD23, while only 8/52 cases of nodal diffuse large B cell lymphoma (15%) and 4/48 cases of extranodal diffuse large B cell lymphoma (9%) were CD200 positive (Table 1). This is similar to our finding of CD200 immunoreactivity in 69% of primary mediastinal large B cell lymphoma cases and 7% of diffuse large B cell lymphoma cases. In all, 21 of 33 cases of primary mediastinal large B cell lymphoma (70%) and only 1/33 cases of diffuse large B cell lymphoma (3%) were initially found to immunoreactive for MAL.¹⁰ Similarly, we found that 86% of primary mediastinal large B cell lymphoma and 3% of diffuse large B cell lymphoma cases were MAL positive. Finally, TRAF and REL were found to be immunoreactive in 48/78 cases (62%) and 31/48 cases (65%) of primary mediastinal large B cell lymphoma, respectively, and 20/173 cases (12%) and 28/160 cases (18%) of diffuse large B cell lymphoma.¹² Similarly, we found that these markers were immunoreactive in 86 and 77% of primary mediastinal large B cell lymphoma cases, respectively, and 12 and 17% of diffuse large B cell lymphoma cases, respectively.

We previously found that the neoplastic cells in classical Hodgkin lymphoma and in primary mediastinal large B cell lymphoma, but not in nodular lymphocyte predominant Hodgkin lymphoma, are positive for CD200 in the majority of cases.⁸ Therefore, CD200 immunostaining should be helpful in the differential diagnosis of classical Hodgkin lymphoma *vs* nodular lymphocyte predominant Hodgkin lymphoma and diffuse large B cell lymphoma. However, additional markers are needed to discriminate classical Hodgkin lymphoma from

primary mediastinal large B cell lymphoma on immunophenotypic grounds. Useful immunophenotypic markers for this purpose include CD15, a well-established marker of classical Hodgkin lymphoma that was found to be expressed in 17/20 cases (85%) of classical Hodgkin lymphoma but none of 27 cases of primary mediastinal large B cell lymphoma in a recent study by Salama *et al*.¹⁸ That study also found that CD23, one of the immunophenotypic markers useful for distinguishing primary mediastinal large B cell lymphoma from diffuse large B cell lymphoma, was expressed in 23/27 cases (85%) of primary mediastinal large B cell lymphoma but only 2/18 cases (11%) of classical Hodgkin lymphoma, in rare, scattered Reed-Sternberg cells.¹⁸ MAL, another primary mediastinal large B cell lymphoma marker, was found to be expressed in only 3/31 cases of classical Hodgkin lymphoma,¹⁰ so it too may be useful for this application. In contrast, TRAF and REL fail to distinguish primary mediastinal large B cell lymphoma from classical Hodgkin lymphoma: 21/25 cases (84%) of classical Hodgkin lymphoma were found to be immunoreactive for TRAF and 23/25 cases (92%) were immunoreactive for REL.¹⁹

Anti-CD200 targeted therapy may serve as a treatment for CD200-positive neoplasms, including chronic lymphocytic leukemia/small lymphocytic lymphoma. In an animal model of chronic lymphocytic leukemia, anti-CD200 antibody administration inhibits tumor growth, thought to be due to blocking of CD200 receptor–ligand interaction as well as elimination of activated T cells involved in immune mediated killing of neoplastic cells.^{20–22} ALXN6000, a humanized, murine-derived anti-human CD200 antibody is in clinical trial as a potential novel immunotherapeutic agent to treat chronic lymphocytic leukemia/small lymphocytic lymphoma and multiple myeloma (Kretz-Rommel and Bowdish²²; NCT00648739). If such therapy is found to be efficacious for these CD200-positive neoplasms, then it may be suitable to treat other CD200-positive B cell-derived neoplasms such as primary mediastinal large B cell lymphoma.

In conclusion, CD200 is a sensitive and specific marker for distinguishing primary mediastinal large B cell lymphoma from diffuse large B cell lymphoma in formalin-fixed, paraffin-embedded histologic sections. Its sensitivity as a marker of primary mediastinal large B cell lymphoma exceeds that of other immunophenotypic markers studied and its staining specificity is comparable to that of other immunophenotypic markers of primary mediastinal large B cell lymphoma. CD200 is a useful addition to the armamentarium of immunophenotypic markers for the assessment of mediastinal lymphomas and large cell lymphomas at other sites of possible mediastinal origin. In addition, CD200 expression by the majority of cases of primary mediastinal large B cell lymphoma raises the possibility of a new immunotherapeutic treatment for this disease.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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