RESEARCH ARTICLE

Expression of the Class II and III Beta-Tubulin in Neoplastic and Non-Neoplastic Lymphoid Tissues

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Abstract

Aim: Abnormal expression patterns of beta-tubulin isotypes may provide a molecular rationale for the behaviour of lymphoma subtypes. In the present study class II and III beta-tubulin expression was assessed in non-neoplastic and neoplastic lymphoid tissues with reference to potential utility as new tumour biomarkers. **Methods and results:** In this cross-sectional study class II and III beta-tubulin expression was assessed in 304 neoplastic and 20 normal lymphoid tissues using qualitative and semi-quantitative immunohistochemistry. Class II beta-tubulin was found to be positive in the germinal centres, mantle zone and interfollicular regions of normal lymphoid tissues. It was also expressed in 15/15 (100%) lymphoblastic lymphomas, 229/231 (99%) mature B cell lymphomas, 22/22 (100%) T/NK-cell lymphomas and 36/36(100%) classical Hodgkin lymphomas. Class III beta-tubulin in contrast was germinal centre restricted and more selective, being found mainly in classical Hodgkin lymphomas (34/36 (94%)). It was also expressed in 58/171(34%) DLBCL, 11/12 (92%) mantle cell lymphomas and 6/6 (100%) Burkitt lymphomas. Other mature B cell, T/NK cell lymphomas and precursor lymphoblastic lymphomas were usually negative. **Conclusions:** Class II beta-tubulin shows ubiquitous expression in neoplastic and non-neoplastic lymphoisd tissues. In contrast, Class III beta-tubulin is germinal centre-restricted. Its consistent expression in classical Hodgkin lymphomas may point to use in the identification of Reed-Sternberg and Hodgkin cells. Its expression in a proportion of DLBCL, Burkitt and mantle cell lymphomas is of interest as this may be related to their aggressiveness.

Keywords: lymphoid lesion- lymphoma- immunohistochemistry

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Introduction

Lymphomas comprise nearly 50% of hematological neoplasms with higher prevalence in developed countries(Roman and Smith, 2011; Siegel et al., 2014)and with one third of estimated cases occurring in Asia(Jemal et al., 2011).Due to the diversity in their prognosis, accurate classification of lymphoma subtype is essential(Harris et al., 2000; van Dongen and Orfao, 2012). This is based on clinical, morphological, immunophenotype and genetic features. However in some cases the immunophenotyping may lack precision (Campo et al., 2011). This warrants the identification of novel markers and recently the differential expression of Beta-tubulin subclasses has been studied for this purpose (Parker et al., 2014).

Microtubules are dynamic filamentous structures composed of Alpha and Beta- tubulin heterodimers that are important in cell proliferation, intracellular trafficking, signalling and migration in eukaryotic cells (Dumontet C, 2010). Diverse changes in the microtubule network have been identified and characterized in a wide variety of cancers. These changes are often associated with chemotherapy resistance and poor outcome(Parker et al., 2014). Class III Beta-tubulin over expression is associated with poor prognosis in epithelial cancers and non-Hodgkin lymphoma (Choi et al., 2012; Roque et al., 2013; Parker et al., 2014; Roque et al., 2014) while Class II, IVa and IVb Beta-tubulin are associated with non-small cell lung cancer, neuroblastoma, breast cancer and acute lymphoblastic leukemia (Verrills et al., 2003; Don et al., 2004; Cucchiarelli et al., 2008; Gan and Kavallaris, 2008; Gan et al., 2011; Lobert et al., 2011).

Although the association between Class II/IIII Beta-tubulins and chemotherapy resistance has been reported in certain lymphomas, there is a scarcity of data on their expression in non-neoplastic lymphoid tissues and other lymphoma subtypes (Verrills et al., 2003; Yoon et al., 2010; Choi et al., 2012).The aim of this study was to assess Class II and IIII Beta-tubulin expression in a wide variety of neoplastic and non-neoplastic lymphoid tissues.

Materials and Methods

This study was approved by the Universiti Kebangsaan

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Tissue samples

Formalin-fixed paraffin-embedded tissue samples were obtained from 15 cases of precursor lymphoid neoplasms (nine B lymphoblastic lymphomas and six T lymphoblastic lymphomas), 171 diffuse large B cell lymphoma (DLBCL) 60 mature small B-cell lymphomas, 22 T/Natural killer (NK) cell lymphomas(including eight peripheral T-cell lymphomas, five extranodal NK/T-cell lymphomas-nasal type, four angioimmunoblastic T-cell lymphomas, three subcutaneous panniculitis-like T-cell lymphomas and two ALK- negative anaplastic large cell lymphomas) and 36 classical Hodgkin lymphomas (Table 1). Similarly, formalin-fixed, paraffin-embedded tissues of 20 non-neoplastic lymphoid tissues (lymph node = 4, tonsil = 4, spleen = 4, adenoid = 3, appendix = 3, ileum = 1, thymus = 1) were retrieved, along with their corresponding H&E stained slides.

Tissue preparation

Tissue microarrays were constructed using a tissue microarrayer (Alphelys MTA Booster, Plaisir France) for lymphoma cases where duplicated 1 mm tissue cores were taken from each tumour. Three µm thick whole paraffin sections were prepared from the 20 non-neoplastic lymphoid tissues.

Antibodies

Rabbit polyclonal antibody against Class II Beta-tubulin (Cat. No. ab103667, Abcam England) was used at a dilution of 1:50with normal human brain tissue as the positive control.Rabbit monoclonal [EP1569Y] antibody against Class III Beta-tubulin (Cat. No. ab52623, Abcam) was used at a dilution of 1:100 with human breast carcinoma as the positive control.

Immunohistochemistry

Immunohistochemical staining was performed on the tissue microarray (TMA) sections and paraffin sections using the protocol from EnVisionTM FLEX Mini Kit, High pH (Dako Denmark).

Immunostaining analysis

The staining for Class II Beta-tubulin and Class III Beta-tubulin was scored quantitatively and qualitatively at x400 magnification.

Staining was considered positive when cytoplasmic labelling was observed in more than 10% of tumour cells with equal or greater intensity to the internal positive controls. The absolute number of positive cells was also counted. Results were categorised into four groups as shown in Table 2. For Hodgkin and Reed-Sternberg (HRS) cells, expression was interpreted as positive when cytoplasmic membrane staining or at least paranuclear dot-like accentuation was observed in any number.

Statistical analysis

Data analysis was performed using the statistical package for social sciences (SPSS) version 19.0 (IBM Inc. Chicago, Il, USA). Pearson chi-square test was used to determine the association between different variables. P values of <0.05 were considered statistically significant.

Results

Normal lymphoid tissues

Class II Beta-tubulin

Class II Beta tubulin expression was observed in the follicles and interfollicular region. Germinal centre cells were strongly stained while cells in the mantle zone show a weaker expression (Figure 1A). It was also observed that scattered large cells in the interfollicular region were strongly positive while the smaller lymphocytes were weakly labelled (Figure 1B).

Class III Beta-tubulin

The expression of Class III Beta-tubulin was restricted to the germinal centre, staining predominantly the larger cells (Figure 1C) but not the smaller centrocytes. Cells in the mantle zone and the interfollicular region were negative.

The pattern of immunoreactivity for Class II and Class III Beta-tubulin is cytoplasmic and membrane associated . In addition to the lymphoid cells, their expression was also observed in the endothelial cells of blood vessels (Figure 1D).

Neoplastic lymphoid tissues

A total of 304 cases (consisting 164 males and 140

Table 1. Lymphoma Subtypes and the Number of Cases

Type of lymphoma	No of cases
Precursor lymphoid neoplasms	
B lymphoblastic lymphoma NOS	9
T lymphoblastic lymphoma	6
Mature B-cell neoplasms	
Diffuse large B cell lymphoma	171
Follicular lymphoma	20
Extranodal marginal zone lymphoma	12
Nodal marginal zone lymphoma	3
Mantle cell lymphoma	12
Chronic lymphocytic leukaemia/Small lymphocytic lymphoma	7
Burkitt lymphoma	6
Mature T- and NK cell neoplasms	
Peripheral T cell lymphoma	8
Extranodal NK/T-cell lymphoma, nasal type	5
Angioimmunoblastic T-cell lymphoma	4
Subcutaneous panniculitis-like T-cell lymphoma	3
Anaplastic large cell lymphoma, ALK negative	2
Classical Hodgkin Lymphoma	
Mixed cellularity	15
Nodular sclerosis	15
Lymphocyte depleted	1
Unclassifiable	5

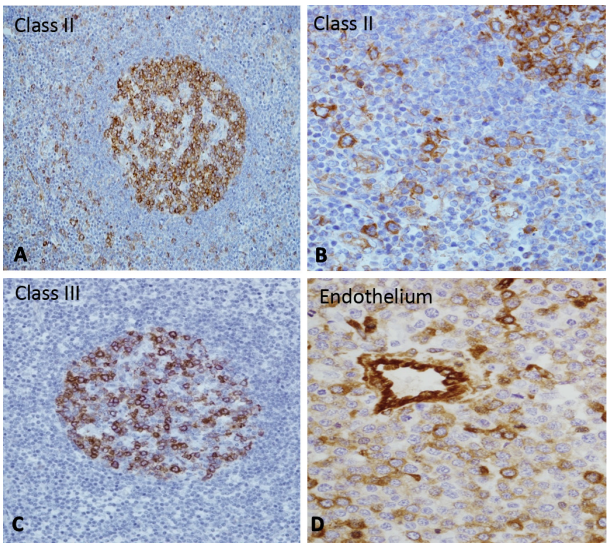

Figure 1. Immunohistochemical expression of Class II and III Beta-Tubulin in Normal Lymphoid Tissue: A) Class II Beta-tubulin exhibit strong expression in the germinal center and weak labelling in the mantle zone (X200). B). Isolated large cells in the interfollicular region are strongly positive for Class II Beta-tubulin (X200). C) Class III Beta-tubulin is positive in the germinal center but not in the mantle zone and interfollicular region (X200). D) The blood vessel endothelial cells show Class III Beta-tubulin expression(X400).

females) were included in this study.The race distribution is composed of 146 Malays, 90 Chinese, 58 Indians and 9 from other races.

Class II Beta-tubulin was expressed in all lymphoma categories, being positive in302/304cases (99%).There was no significant difference in the expression of this protein between mature B-cell lymphomas and acute lymphoblastic lymphoma (p=0.97), or with T-and NK-cell lymphomas (p=0.97) or Hodgkin lymphoma (p=0.96).

Class III Beta-tubulin expression however was more restricted ,being expressed in less than half of cases (128/304, 42%) with statistically significant difference in the expression between mature B-cell neoplasms and classical Hodgkin lymphoma ($p<0.05$). The results are summarised in Table 3 and 4.

Diffuse Large B Cell Lymphoma
Class II Beta-tubulin

Class II Beta-tubulin was expressed in 169/171 cases (99%) where almost all positive cases were in groups 3 and 4 (Table 3) i.e., exhibiting high percentage of positivity. The two negative cases showed weak cytoplasmic staining in less than 10% of tumour cells.

Class III Beta tubulin

In contrast to Class II Beta-tubulin,the expression of Class III Beta-tubulin in DLBCL is more heterogeneous. It is positive in only 58/171 cases (36%) with a spectrum of positivity ranging from group 1 to group 4 (Table 4). The majority of positive cases (47/58, 81%) were in group 1 or group2, in which less than 50% of cells were stained. Subgrouping according to the cell of origin(Sabattini et al., 2010)was possible in 37/58 cases. Of these, 7/58 (12%) positive cases were germinal centre B cell-like (GCB) subtype while 30/58 (52%) cases were non-GCB subtype.

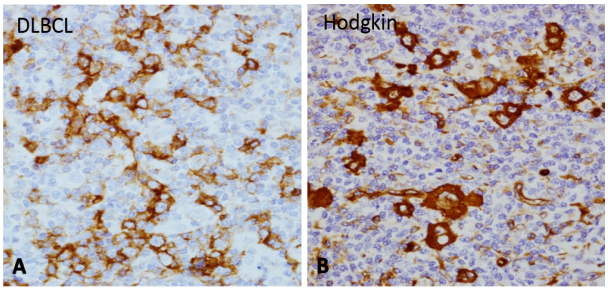

Figure 2. A) Heterogenous expression of Class III Beta-tubulin in tumour cells of DLBCL(X400). B) The Reed-Sternberg and Hodgkin cells show strong cytoplasmic staining for Class III Beta-tubulin in Hodgkin lymphoma. The background lymphocytes are negative (X400).

Table 2. Percentage of Positive Tumour Cells in Each Group

Group	Percentage of positive cells
1	11- 25%
2	26 - 50%
3	51 - 75%
4	more than 75%

It can be summarised that almost all DLBCL were positive for Class II Beta-tubulin but only a proportion of cases were positive for Class III Beta-tubulin and when positive for the latter, they are usually in group 1 or 2 where the staining is heterogenous and low (Figure 2A).

Burkitt Lymphoma

All six cases of Burkitt lymphomas were positive for class II (strong expression) and class III Beta-tubulin with the latter showing a spectrum of staining intensity (Table 3 and 4).

Small B Cell Lymphomas
Class II Beta-tubulin

Class II Beta-tubulin was expressed in all cases of follicular lymphoma (n=20), extra-nodal marginal zone lymphoma (n=12), nodal marginal zone lymphoma (n=3), mantle cell lymphoma (n=12) and chronic lymphocytic leukemia/small lymphocytic lymphoma (n=7) (Table 3).

Class III Beta-tubulin

Class III Beta-tubulin was expressed in 11/12 cases (92%) of mantle cell lymphoma and in 8/20 cases (40%) of follicular lymphoma. It was negative or weakly labelled in extra nodal and nodal marginal zone lymphomas and chronic lymphocytic leukemia/small lymphocytic lymphoma (Table 4).

T Cell/ Nk Cell Lymphomas
Class II Beta-tubulin

Class II Beta tubulin was expressed in all T cell lymphomas including peripheral T-cell lymphoma (n=8), NK/T cell lymphoma (n=5), angioimmunoblastic T-cell lymphoma (n=4), subcutaneous panniculitis-like T-cell

Table 3. Class Iibeta-Tubulin Expression in Neoplastic Lymphoid Tissues

	BetaII expression	Expression group			
	n (%)	Group 1	Group 2	Group 3	Group 4
Precursor lymphoid neoplasms`					
B lymphocytic lymphoma(n=9)	9 (100%)				9 (100%)
T lymphocytic lymphoma(n=6)	6 (100%)				6 (100%)
Mature B cell neoplasms					
DLBCL(n=171)	169 (99)	0 (0%)	5 (3%)	57 (33%)	107 (63%)
Follicular lymphoma(n=20)	20 (100%)	0 (0%)	4 (20%)	7 (35%)	9 (45%)
Extranodal marginal zone lymphoma(n=12)	12 (100%)	0 (0%)	0 (0%)	8 (67%)	4 (33%)
Nodal marginal zone lymphoma(n=3)	3 (100%)	0 (0%)	1 (33%)	0 (0%)	2 (67%)
Mantel cell lymphoma(n=12)	12 (100%)	0 (0%)	0 (0%)	3 (25%)	9 (75%)
Chronic lymphocytic leukemia/ SLL(n=7)	7 (100%)	1 (14%)	1 (14%)	4 (58%)	1 (14%)
Burkitt lymphoma(n=6)	6 (100%)	0 (0%)	0 (0%)	0 (0%)	6 (100%)
Mature T and NK cell neoplasms					
Peripheral T cell lymphoma(n=8)	8 (100%)	0 (0%)	0 (0%)	6 (75%)	2 (25%)
Extranodal NK/T cell lymphoma, nasal type(n=5)	5 (100%)	0 (0%)	0 (0%)	2 (40%)	3 (60%)
Angioimmunoblastic T cell lymphoma(n=4)	4 (100%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)
Subcutaneous panniculitis-like T cell lymphoma(n=3)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	3 (100%)
Anaplastic large cell lymphoma, ALK negative(n=2)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Classical Hodgkin lymphoma	36	0 (0%)	1 (3%)	2 (6%)	33 (91%)
Mixed cellularity(n=16)	16 (100%)				
Nodular sclerosis(n=15)	15 (100%)				
Lymphocyte depleted(n=1)	1 (100%)				
-Unclassified(n=4)	4 (100%)				

lymphoma (n=4) and ALK-negative anaplastic large cell lymphoma (n=2) (Table 3).

Class III Beta-tubulin

Class III Beta tubulin was expressed in 1/8 (13%) of peripheral T-cell lymphomas, 1/5 (20%) of NK/T cell lymphomas, 3/4 (75%) of angioimmunoblastic T-cell lymphoma, 3/3 (100%) of subcutaneous panniculitis-like T-cell lymphoma and 1/2 (50%) ALK-negative anaplastic large cell lymphoma (Table 4).

Lymphoblastic Lymphoma

Class II Beta-tubulin was expressed in all 15 cases of lymphoblastic lymphoma (9 B-ALL and 6 T-ALL) (Table 3).

Class III Beta-tubulin was expressed in 2/9 (22%) of B ALL and none of the six cases of T-ALL (Table 4).

Classical Hodgkin Lymphoma

Class II Beta-tubulin

Class II Beta-tubulin was expressed in all 36 classical Hodgkin lymphomas. It labels Hodgkin and Reed-Sternberg cells (HRS cells) as well background lymphocytes but the staining intensity was stronger in the HRS cells (Table 3).

Class III Beta-tubulin

Interestingly, almost all cases (34/36, 94%) of Hodgkin lymphoma were also positive for Class III Beta-tubulin showing a spectrum of positivity (Table 4). The labelling was seen in the cytoplasm of HRS cells with para-nuclear dot-like accentuation while the background lymphoid cells were negative (Fig.2B). This consistently positive pattern is not seen in the other lymphoma types with Class III Beta-tubulin.

Discussion

The present study revealed that classII Beta-tubulin has wide spread expression in all cellular compartments of the lymphoid tissue including the germinal centre, the mantle zone and interfollicular regions. In addition, in the interfollicular region, two population of positivity was observed i.e., strongly positive scattered large cells and more weakly stained smaller lymphocytes. It would be of interest to characterise further the nature of these strongly positive large cells in this region as it may be related to the interfollicular large B cells(Marafioti et al., 2003)and DLBCL with unusual pattern(Haycocks and Zhao, 2010).The expression of class III Beta- tubulin in lymphoid tissues was more unique. It was found to be strictly restricted to the large cells in the germinal centres exhibiting cytoplasmic and membrane associated labelling whilst cells in other compartments are negative. These observations highlight the potential use of class III Beta-tubulin as an additional marker for germinal centre

Table 4.Class Iii Beta-Tubulin Expression in Neoplastic Lymphoid Tissues

	BetaIII expression	Expression group			
	n (%)	Group 1	Group 2	Group 3	Group 4
Precursor lymphoid neoplasms					
B lymphocytic lymphoma(n=9)	2 (20%)		1 (10%)	1 (10%)	
T lymphocytic lymphoma(n=6)	0 (0%)				
Mature B cell neoplasms					
DLBCL(n=171)	58 (34%)	20 (11%)	27 (16%)	8 (5%)	3 (2%)
Follicular lymphoma (n=20)	8 (40%)	2 (10%)	4 (20%)	1 (5%)	1 (5%)
Extranodal marginal zone lymphoma(n=12)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nodal marginal zone lymphoma(n=3)	1 (33%)	0 (0%)	1 (33%)	0 (0%)	0 (0%)
Mantel cell lymphoma(n=12)	11 (92%)	5 (42%)	2 (17%)	3 (25%)	1 (8%)
Chronic lymphocytic leukemia/ SLL(n=7)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Burkitt lymphoma(n=6)	6 (100%)	0 (0%)	3 (50%)	2 (33%)	1 (17%)
Mature T and NK cell neoplasms					
Peripheral T cell lymphoma(n=8)	1 (13%)	0 (0%)	0 (0%)	0 (0%)	1 (13%)
Extranodal NK/T cell lymphoma, nasal type(n=5)	1 (20%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)
Angioimmunoblastic T cell lymphoma(n=4)	3 (75%)	1 (25%)	1 (25%)	1 (25%)	0 (0%)
Subcutaneous panniculitis-like T cell lymphoma(n=3)	3 (100%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)
Anaplastic large cell lymphoma, ALK negative(n=2)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)
Classical Hodgkin lymphoma	36	4 (11%)	11 (31%)	5 (14%)	14 (38%)
Mixed cellularity(n=16)	16 (100%)				
Nodular sclerosis(n=15)	14 (93%)				
Lymphocyte depleted(n=1)	1 (100%)				
Unclassified(n=4)	3 (75%)				

cells.The germinal centre restricted expression was also noted by Yoon et al(Yoon et al., 2010)however we did not observe follicular dendritic cell meshwork labelling that they reported.

We also observed that class II and III Beta-tubulins were strongly expressed in the endothelial cells of blood vessels as previously reported(Sève et al., 2005) making it useful as internal control in the immunohistochemical studies of these proteins.

The expression of class II Beta tubulin in lymphoid neoplasms has not been studied previously with the exception of classical Hodgkin lymphoma (Choi et al., 2012)where it was suggested that this protein could potentially act as a marker in detecting the HRS cells. However, with a larger series of lymphoma cases, the present study was able to demonstrate that Class II Beta-tubulin is non-selective and is ubiquitously expressed in all categories of lymphoma, rendering it impractical as a differentiating marker for lymphoid neoplasms.

The observation that class III Beta-tubulin was germinal centre-associated is reflected in the lymphomas that are positive for this marker wherethe tumours are predominantly germinal centre origin (8/20 of follicular lymphomas, 6/6 of Burkitt lymphomas and 58/171 of DLBCL). Although Yoon et al., (2010) also showed that 75% of Burkitt lymphomas and 30%of GCB type DLBCLwere positive, they did not find any class III Beta-tubulin-positive follicular lymphomas. Another novel finding is the consistent expression of class III Beta-tubulin in mantle cell lymphomas which was not observed previously (Yoon et al., 2010). In addition, angioimmunoblastic T-cell lymphomas, which are germinal centre associated (Piccaluga et al., 2007) were also positive for class III.

It is interesting to note that most cases of DLBCL are negative for class III Beta-tubulin and when positive, the expression is heterogenous, exhibiting variation in labelling between individual cells within the same tumour. This heterogeneity was also observed by Yoon et al., (2010). It would be of interest to characterise the positive and negative cells within individual case in relation to their proliferation index, expression of anti-apoptotic marker such as BCL2 and other markers of prognostic importance (ideally by double immunostaining techniques) knowing that class III Beta-tubulin upregulation is associated with resistance to Vincaalkaloids or taxanes (Choi et al., 2012).

Of interest is the expression of Class III Beta-tubulin in classical Hodgkin lymphoma. In contrast to the previous report (Choi et al., 2012)our results revealed that Class III Beta-tubulin is consistently expressed in the HRS cells, albeit with some degree of heterogeneity. Although the staining was heterogeneous, it was not difficult to identify the positive cells given that the surrounding lymphocytes were negative. The findings of the present study suggest that Class III Beta tubulin could be potentially used as an additional marker for Hodgkin and Reed Sternberg cells

in classical Hodgkin lymphoma together with CD30, CD15 and Pax5.

The findings that there were more Non-GCB DLBCL cases positive for Class III Beta-tubulin does not fit into our theory that positive tumours are usually germinal centre associated neither is it in keeping with previous report (Yoon et al., 2010).One possible explanation is the number of cases that could be classified as GCB and non-GCB (37/58) were small. Furthermore we know that in our own population (Rahman et al., 2013), most DLBCL cases are non-GCB subtype further skewing the distribution of the two subgroups.

It can be concluded that the unrestricted Class II Beta-tubulin expression renders it impractical as a marker of differentiation in lymphoma. In contrast, Class III Beta-tubulin expression is confined primarily to the germinal centre cells of normal lymphoid tissues and is more frequently expressed in aggressive lymphoma (DLBCL, BL and MCL).Further investigations are warranted to clarify the predictive role of Class III Beta-tubulin in lymphoid neoplasms. In classical Hodgkin lymphoma, Class III Beta-tubulin shows selective expression in the neoplastic cells. Therefore, we postulate that Class III Beta-tubulin might be potentially useful as an additional marker for Hodgkin and Reed Sternberg cells.

Conflict of interest statement

The authors whose names are listed above certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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