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Follicular lymphomas with plasmacytic differentiation include two subtypes

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Follicular lymphomas with plasmacytic differentiation were described more than two decades ago. However, the possibility that some of these reported cases are marginal zone lymphomas or composite lymphomas must be considered. In addition, it is also uncertain whether follicular lymphomas with plasmacytic differentiation have any unique cytogenetic or other features. Therefore, fluorescence immunophenotypic and interphase cytogenetic analysis of 14 well-characterized follicular lymphomas with plasmacytic differentiation was performed using a CD138 antibody to identify the plasma cells and with *BCL2*, *BCL6*, *IGH@* and *MALT1* break-apart probes and a chromosome 12 centromeric probe. CD10 was expressed in 12/14 cases, BCL6 in 12/12 cases and BCL2 in 12/14 cases. At least one cytogenetic abnormality was identified in 12/14 cases. The same abnormality was present in both the plasmacytic (CD138+) and non-plasmacytic (CD138−) component in all 10 evaluable cases. *BCL2* rearrangements were present in seven cases (5 *IGH@* rearranged, 1 *IGH@*-not rearranged, 1 *IGH@*-not evaluable), *BCL6* rearrangement in two (1 also with *BCL2*/*IGH@* rearrangement), +12 in 1, +*MALT1* without +18 in 1, *IGH@* rearrangement without other abnormalities in 1 and *IGH@* rearranged or partially deleted in 1 case. No cases showed +*BCL6* (3q27) or a *MALT1* rearrangement. All six cases with an isolated *BCL2* rearrangement had predominantly interfollicular plasmacytic cells whereas, 6/7 cases without the translocation had concentrations of intrafollicular or perifollicular plasmacytic cells ($P<0.005$), as did the case with *BCL2* and *BCL6* translocations. These results support the existence of bona fide follicular lymphomas with plasmacytic differentiation and support the clonal relationship of the neoplastic lymphoid and plasma cells in at least most of these cases. The differential distribution of the plasma cells, specifically in relation to the presence or absence of an isolated *BCL2* rearrangement suggests that the latter cases may be distinctive, sharing some features with marginal zone lymphomas.
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Follicular lymphomas are neoplasms of follicular/germinal center cells; however, it is recognized that, like normal follicular center cells, the neoplastic cells may show post-follicular maturation into memory B-cells and plasma cells. Marginal zone differentiation in follicular lymphomas is well documented[1–9] and, based on the morphological features of the interfollicular neoplastic cells and their downregulation of CD10 and BCL6 in some cases,[10,11] even more cases probably have non-monocytoid differentiation to post-follicular memory type B cells. The clonal relationship of the follicular lymphoma cells to those showing marginal zone differentiation has been well documented in at least some cases.[1,4,8,9] In addition, follicular lymphomas with marginal zone differentiation have been reported to be distinctive, with an increased

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frequency of the type of cytogenetic abnormalities that have been seen in nodal marginal zone lymphomas, particularly +3 or +3q, although they lack the type of translocations more specifically associated with extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphoma).[1,12]

The other major cell type derived from follicular center cells is plasma cells.[13,14] Follicular lymphomas with plasmacytic differentiation have also been reported;[15–20] however, much of this literature is from a time before marginal zone lymphomas were widely recognized. As marginal zone lymphomas with plasmacytic differentiation and follicular colonization can easily be confused with follicular lymphomas, the possibility that some of the originally reported cases are not truly follicular lymphomas must be considered. Furthermore, while congruence of light chain expression in the lymphoid cells and plasma cells has been documented,[15–17,19,20] more definitive studies to determine the clonal relationship of the plasma cells to the lymphoid cells of the follicular lymphomas are lacking. In addition, it is unknown whether follicular lymphomas with plasmacytic differentiation are cytogenetically distinct from other follicular lymphomas most of which show *BCL2* translocations.[2] Trisomy 3, for example, has been associated with plasmacytic differentiation in small B-cell lymphomas, but not in those of follicular type.[21] Other unanswered questions include whether, like follicular lymphomas with marginal zone differentiation, those with plasmacytic differentiation share any cytogenetic abnormalities with marginal zone lymphomas, and if so whether the abnormalities are in both the lymphoid and plasmacytic cells or only in one subpopulation.

For these reasons, fluorescence immunophenotyping and interphase cytogenetics as a tool for investigation of neoplasms (FICTION)/Immuno FISH, with an immunohistochemical stain to identify plasma cells (CD138) combined with cytogenetic FISH probes, was used to investigate 14 follicular lymphomas that showed plasmacytic differentiation. The plasma cells and lymphoid cells were separately analyzed. Probes were used for genes that are often rearranged in follicular lymphoma (*BCL2, IGH@*), for a gene rearranged in a minority of follicular lymphomas that might have distinctive features (*BCL6*)[2,22] and for abnormalities that have been associated with marginal zone lymphomas of varied types (*BCL6* that also helps identify potential cases with +3, *MALT1* looking for rearrangements or potential +18, and CEP12 to look for +12). The latter probes were chosen because an important differential diagnosis for follicular lymphomas is marginal zone lymphomas with follicular colonization, and both can show plasmacytic differentiation. Finally, the results were correlated with the pathologic and immunophenotypic features of the cases.

Materials and methods

Case Selection and Morphological/ Immunophenotypical Review

Fourteen cases that fulfilled the WHO classification criteria for a follicular lymphoma and that also had plasmacytic differentiation based on CD138-positive light chain-restricted plasmacytic cells were identified from the Division of Hematopathology at UPMC-Presbyterian Hospital, The National Institutes of Health and Stanford University Medical Center. Hematoxylin and eosin stained sections, all available flow cytometric immunophenotypic data and the pathology reports were reviewed by two authors (JFG, SHS). The growth pattern, cytology, presence of Dutcher bodies and degree of plasmacytic differentiation were assessed on hematoxylin- and eosin-stained sections. All available immunohistochemical stains were reviewed, including stains for CD20, kappa, lambda, CD10, BCL2, BCL6 and, in more selected cases, IRF4/MUM1. In three cases the CD10, BCL2 and BCL6 stains were no longer available and were not re-reviewed. Additional immunohistochemical stains for CD10 and BCL6 were performed because of missing or suboptimal stains in which the material was available using the Ventana BenchMark XT (Tuscon, AZ, USA) and CD10, mouse monoclonal, prediluted (Cell Marque, Rocklin, CA, USA) and BCL6, mouse monoclonal, 1:25 (Dako, Carpenteria, CA, USA), respectively. The distribution of the plasma cells was assessed from the CD138 or light chain immunohistochemical stains. Independent of the other findings, cases were divided into those with prominent intrafollicular or perifollicular light chain-restricted plasma cell populations and those with a predominant interfollicular distribution.

Fluorescence Immunophenotyping and Interphase Cytogenetics as a Tool for Investigation of Neoplasms (FICTION)

Paraffin section immunohistochemical staining for CD138 was performed on deparaffinized formalin-fixed sections using the TSA Kit no. 7 (Invitrogen, Carlsbad, CA, USA) with horseradish peroxidase goat anti-mouse IgG and Alexa Fluor 350 tyramide. Briefly, deparaffinized slides were placed in a prewarmed (90–95°C) retrieval solution for 40 min. The solution was then allowed to cool for 20 min. The slides were removed from the solution and rinsed twice for 5 min each in 1 × PBS/0.1% Tween 20 at room temperature. CD138 antibody (Cell Marque, Rocklin, CA, USA) was then applied at a dilution of 1:20. The slides were then coverslipped and incubated overnight in a dark chamber at 4°C. The slides were then washed and blocked according to the manufacturer's instructions. Horseradish peroxidase-conjugated secondary antibody (100 µl) was added to the tissue section. The slide was

coverslipped and incubated in a humidified chamber at room temperature in the dark for 30 min. The coverslip was then removed and the slide was rinsed. Alexa Fluor 350 Tyramide mixture (100 µl) was added to the slide, which was coverslipped and incubated in a humidified chamber at room temperature for 10 min. The slides were then washed once. One hundred and fifty microliters of 1:5000 Proteinase K/50 mM Tris-HCL pH 7.6 was added and the slides were incubated in the dark for 20 min at room temperature. The slides were then washed once and soaked in 1% formaldehyde for 30 min in the dark at room temperature. The slides were again washed once and dehydrated by immersion for 2 min each in 80, 95 and 100% ethanol.

After the CD138 staining was completed, the slides were prepared for hybridization in a dark room with 15 min of pretreatment with 2 × SSC (pH 7.0) at 37°C and dehydration in 70, 85 and 100% cold ethanol for 2 min each followed by air drying. DNA denaturation was performed at 72°C for 5 min. The dehydration steps were then repeated. Break-apart probes for *BCL2, BCL6, IGH@,*and *MALT1* and a centromeric probe for chromosome 12 (Vysis, Downers Grove, IL, USA) were prepared according to the manufacturer's insert. The slides were then warmed until dry. Hybridization was performed at 37°C using 10 µl of probe mix, the slides coverslipped and sealed with Duco cement (ITW Devcon, Danvers, MA, USA). The slides were incubated overnight in the dark. After incubation the coverslips were removed and the slides were washed in pre-warmed (72°C) Wash no. 1 (0.4 × SSC/0.3% NP-40) for 2 min with gentle agitation for the first few seconds. The slides were then washed in room temperature Wash no. 2 (0.4 × SSC/ 0.1% NP-40) for 1 min with gentle agitation for the first few seconds. The slides were then allowed to air dry in darkness. The slides were coverslipped and sealed with Duco cement. In one case with both *BCL2* and *BCL6* rearrangements, a dual color dual fusion *IGH@/ BCL2* probe was used.

Slides were viewed and photographed with a × 100 Oil plan fluor objective using a Nikon Optiphot-2 and Quips Genetic Workstation equipped with Chroma, the Technology 83 000 filter set with single band excitors for Texas Red/Rhodamine, FITC, DAPI (UV 360 nm) and a dual filter for Spectrum Orange/FITC, and a triple filter for Spectrum Orange, FITC, and DAPI. Cases were scored both in the CD138-positive and CD138-negative cells except only the CD138-positive cells were scored for the *MALT1* probe and in six cases for the *IGH@* probe. Approximately 200 of each cell type were scored except in some cases with insufficient numbers of CD138-positive cells. Cells were analyzed both for rearrangements with split signals and for polysomies with extra fused signals. A cutoff of 10% was used as the threshold for positivity for all tested probes.

Results

Clinicopathologic Features

The 14 patients (eight male; six female) were adults (38–82 years). Eleven cases were nodal, one involved the kidney, one the skin and one the parotid gland. Eight of the follicular lymphomas were grade 1–2, 5 grade 3A and 1 was grade 3B with diffuse large B-cell lymphoma (Table 1). Five cases had plasmacytic differentiation with classic plasma cells (Figure 1a), seven cases had plasmacytoid differentiation (Figure 1b) and two cases had plasmacytic cells only recognizable on CD138 immunohistochemical-stained sections. The plasmacytic cells were at least focally numerous in seven cases, somewhat less frequent in two and scanty in five

Table 1 Clinicopathological features

Case	*Age*	*Sex*	*Site*	*Grade*	*Plasma cell location*	*Morphology of plasmacytic cells*	*Marginal zone differentiation*
1	66	M	Axillary LN	3A	Interfollicular	IHC only[b]	No
2	82	F	Kidney	1–2	Interfollicular	Plasmacytic	No
3	79	F	Parotid	3B	Interfollicular	Plasmacytoid	No
4	43	F	Inguinal LN	1–2	Interfollicular	Plasmacytoid	Yes
5	41	F	Groin LN	1–2	Interfollicular	IHC only[b]	Yes
6	65	M	Skin	1–2	Interfollicular	Plasmacytic	Yes
7	56	M	Cervical LN	1–2	Intrafollicular	Plasmacytic	No
8	38	M	Neck LN	3A	Intrafollicular	Plasmacytic	Yes
9	63	F	Axillary LN	3A	Intrafollicular	Plasmacytoid	No
10	59	M	Inguinal LN	1–2	Interfollicular[a]	Plasmacytoid	No
11	80	F	Cervical LN	3A	Perifollicular	Plasmacytoid	No
12	67	M	Neck LN	3A	Intrafollicular	Plasmacytoid	No
13	72	M	Axillary LN	1–2	Intrafollicular[a]	Plasmacytic	No
14	51	M	Inguinal LN	1–2	Intrafollicular	Plasmacytoid	No

LN, lymph node;
[a]plasma cells in many areas;
[b]plasma cells identified on immunohistochemical staining only.

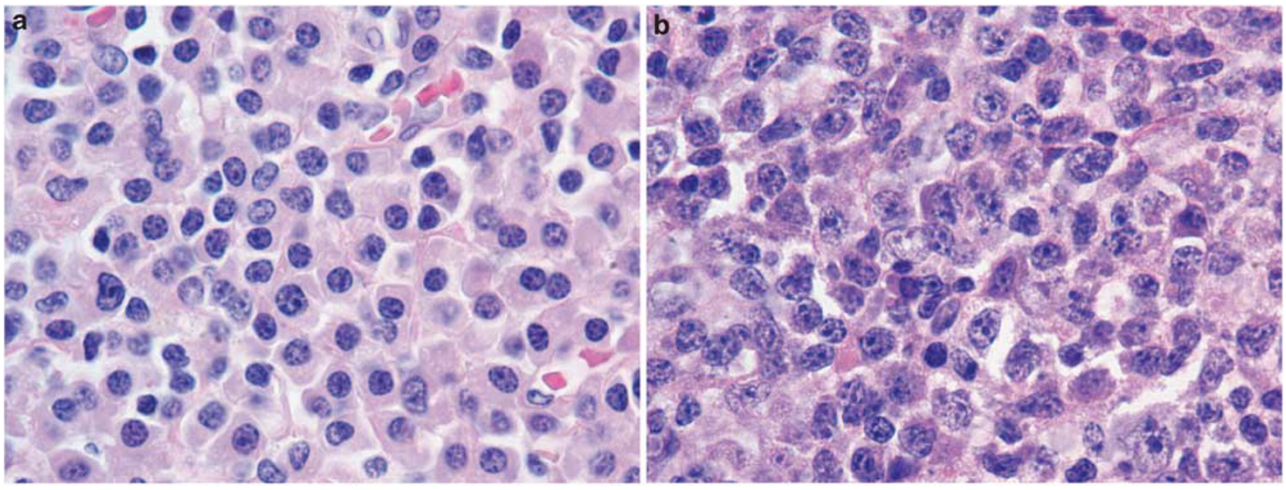

Figure 1 Note the frank plasmacytic differentiation with classic plasma cells (**a**, case 2). In contrast, here there is plasmacytoid differentiation with eccentric nuclei and amphophilic cytoplasm, but few if any classic plasma cells (**b**, case 9) (hematoxylin and eosin).

cases. In one case, an initial iliac crest biopsy originally diagnosed as a plasma cell neoplasm, showed only numerous plasma cells. A subsequent lymph node biopsy showed the follicular lymphoma with plasmacytic differentiation. Dutcher bodies were identified in the hematoxylin- and eosin-stained sections in 11/14 cases. The neoplastic lymphoid cells included many classical centrocytes in five cases (cases 2, 4, 6, 7 and 14), represented what appeared to be less angulated or clefted centrocytes in five cases (cases 1, 5, 9, 10, 12) and were predominantly centroblastic in three cases (cases 3, 8, 11). One case had admixed centrocytes, plasmacytoid lymphocytes, plasma cells and transformed cells without a predominance of one morphologic cell type. Four cases also showed marginal zone ('monocytoid') differentiation. None of the cases had prominent eosinophils.

CD10 was expressed in 12/14 cases, BCL6 in 12/12 cases and BCL2 in 12/14 cases. All cases had monotypic plasma cells by immunohistochemical staining for kappa (10 cases) or lambda (four cases) light chains. The three cases with diagnostic concurrent flow cytometric immunophenotypic studies showed the same light chain class restriction on the lymphoid cells. Although not restricted to a single anatomic compartment, the plasma cells were primarily present in interfollicular areas in seven cases (Figure 2a). Six cases had prominent intrafollicular concentrations of the plasma cells (Figure 2b) and in one case a prominent perifollicular accentuation was observed (Figure 2c).

Cytogenetic FICTION Findings

At least one cytogenetic abnormality was detected in 12/14 cases. *BCL2* rearrangement was found in both the CD138-positive plasma cells and the CD138-negative presumed lymphoid cells in 7/14 cases (Table 2) (Figure 3). Definite *IGH@* gene rearrangement was detected in the CD138-positive and CD138-negative cells in 6/13 evaluable cases with an additional case showing either *IGH@* gene rearrangement or partial deletion with loss of the signal corresponding to the centromeric region of the probe. Four of the seven cases with *IGH@* abnormalities also had a *BCL2* rearrangement, one had both *BCL2* and *BCL6* rearrangements and, in two, a potential partner gene was not identified. There was no correlation between grade and *BCL2* rearrangement. Four of the five cases with classic centrocytes had *BCL2* rearrangements. The fifth case with classic centrocytes had an isolated *BCL6* rearrangement in both the CD138-positive and CD138-negative cells. *MALT1* rearrangement was not detected in 13 of 13 tested cases; however, the cells in one case had an extra signal for *MALT1*, but lacked an extra signal for the centromere of chromosome 18 suggesting partial trisomy of chromosome 18. Trisomy 12 was detected in the CD138-positive and CD138-negative cells in one case.

Correlation of Anatomic Plasma Cell Distribution with Cytogenetic FICTION Results

All six cases with an isolated *BCL2* rearrangement had predominantly interfollicular plasmacytic cells, whereas 6/7 cases without a *BCL2* rearrangement had concentrations of intrafollicular or perifollicular plasma cells ($P<0.005$). The single case with both *BCL2* and *BCL6* rearrangements had a predominantly intrafollicular pattern.

Discussion

Follicular/germinal centers have a critical role in normal B-cell development.[13,14] B cells enter follicular centers where they transform into centroblasts.

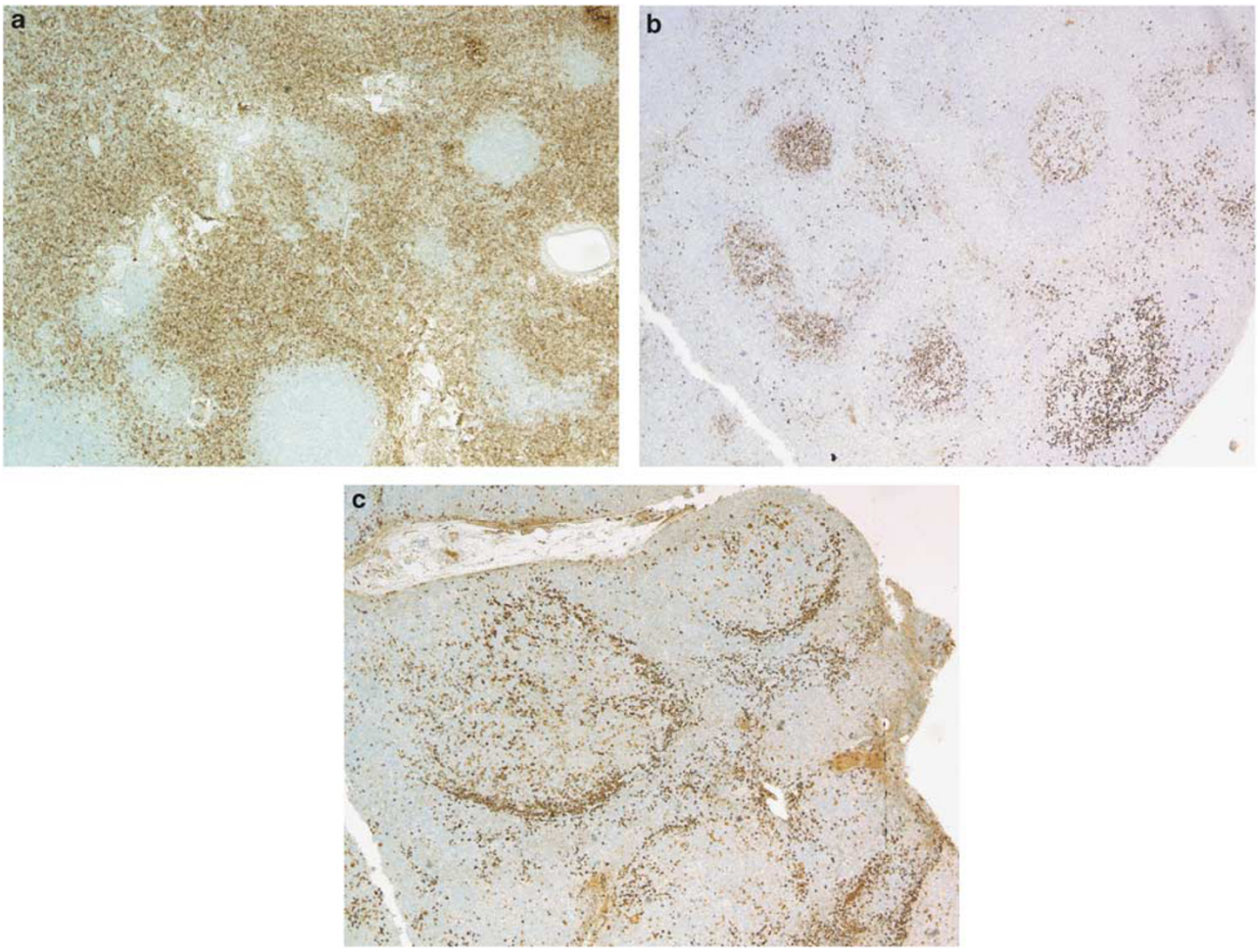

Figure 2 Primary patterns of plasma cell distribution (**a**) CD138 stain demonstrates predominantly interfollicular plasma cells (case 2). (**b**) CD138 stain shows prominent intrafollicular concentrations of plasma cells. Interfollicular plasma cells are also present (case 8). (**c**) Kappa light chain stain shows perifollicular accentuation of plasma cells that are also present in the follicles as well as scattered in the interfollicular regions (case 11). (Immunohistochemistry with hematoxylin counterstain).

Table 2 Cytogenetic FICTION findings; percentage of plasma cells (PC) and lymphocytes (L) with detected abnormalities

Case	*BCL2 Rearrangement*		*IgH@ Rearrangement*		*BCL6 Rearrangement*		*MALT1 Rearrangement*		*Cep12 Trisomy*	
	PC	*L*	*PC*	*L*	*PC*	*L*	*PC*	*L*	*PC*	*L*
1	20%	62%	50%	50%	N	N	N	NT	N	N
2	24%	72%	68%	NT	N	N	N	NT	N	N
3	78%	57%	83%	NT	N	N	N	NT	N	N
4	50%	52%	54%	53%	N	N	N	NT	N	N
5	16%	54%	F	NT	F	N	F	NT	N	N
6	60%	54%	N	N	N	N	N	NT	N	N
7	N	N	N	N	69%	91%	N	NT	N	N
8	N	N	85%	23%	N	N	N	NT	N	N
9	N	N	86%	NT	F	N	N	NT	N	N
10	N	N	N	NT	N	N	N	NT	46%	34%
11	N	N	N	N	N	N	N, 64%[a]	NT	N	N
12	N	N	N	NT	N	N	N	NT	N	N
13	N	N	F	N	N	N	N	NT	N	N
14	94%	30%	100%	32%	67%	21%	NT	NT	NT	NT

N, negative; F, failed; NT, not tested.

[a]no MALT1 rearrangement, but three copies of MALT1 without full trisomy by cep18 probe.

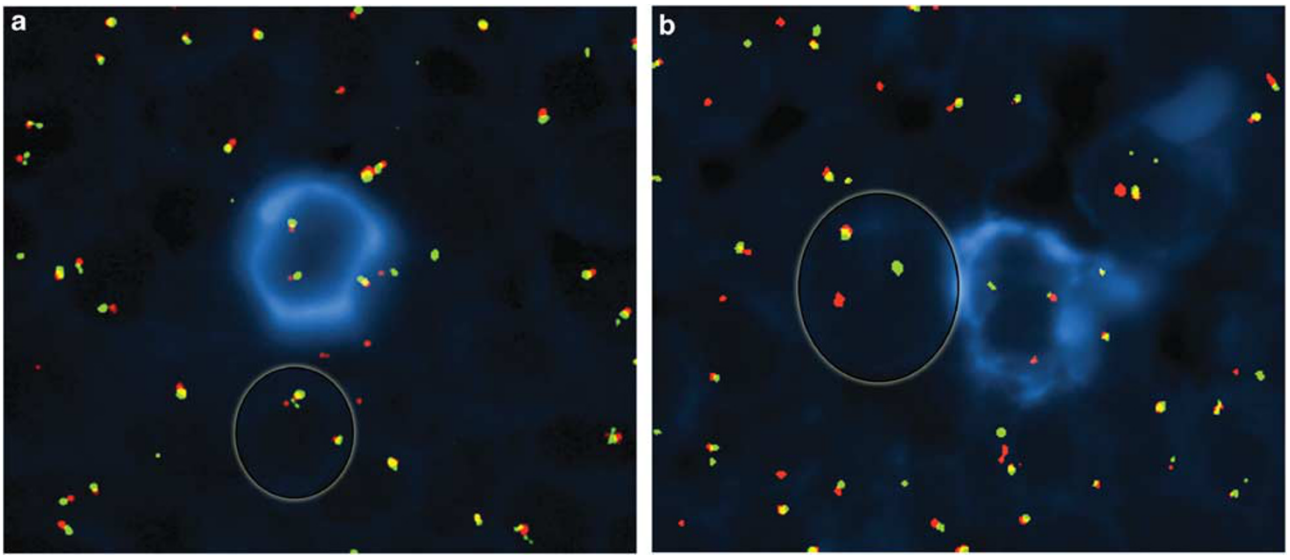

Figure 3 FICTION analysis with CD138 immunohistochemical stain (blue) and *BCL2* break apart probe. A representative CD138 negative cell is circled in each image. (**a**) Note two fusion signals in a case with *BCL2* in wild type configuration, both in the CD138-positive and CD138-negative cells (case 9). (**b**) In this case with *BCL2* rearrangement, there is one fusion signal, plus separate red and green signals in both the CD138-positive and CD138-negative cells (case 4).

Somatic hypermutation, selection and heavy chain class switching all occur in the follicular centers and are accompanied by a morphologic transition to centrocytes. Although many of the follicular center cells undergo apoptotic cell death, those that survive develop into either memory type B cells or plasma cells. Some centrocytes may also return to a transformed/centroblastic state.[14] Memory B cells, recognized in part by an absence of the BCL6 and CD10 follicular center cell-associated antigens, recirculate and also populate the follicular marginal zone. Plasmacytic differentiation can occur in follicles.[14,23] However, other plasmacytic differentiation from memory type B cells takes place in extrafollicular regions. Naive B-cells may also develop into short-lived plasma cells. In lymph nodes with follicular hyperplasia, plasma cells are frequently found in the interfollicular regions, especially in the medullary cords, and, less frequently within the follicles.[24]

Many lymphomas are considered to represent blocks in the lymphoid maturational pathway; however, others show evidence of ongoing lymphocyte development. Marginal zone differentiation in follicular lymphomas is one such example. Furthermore, even in the absence of overt marginal zone differentiation with 'monocytoid' type cells, when neoplastic follicular center cells leave the follicles, they often show morphological and phenotypical features associated with differentiation to memory type B-cells.[10] It is well-documented that the marginal zone type cells in many follicular lymphomas with marginal zone differentiation are clonally related to the follicular lymphoma cells.[1,3,7,8,18]

It is also of interest that marginal zone differentiation in follicular lymphomas has been associated with specific chromosomal abnormalities including trisomy 3, an abnormality also associated with marginal zone lymphomas.[1,12,25] Trisomy 3 or 3q has also been reported to be associated with plasmacytoid differentiation in chronic B-cell lymphoproliferative disorders.[21] In addition, in a previous study of ocular MALT lymphomas, all of the cases with plasmacytic differentiation showed +3 and/or +18q (*MALT1*).[26]

Plasmacytic differentiation is a second common type of maturation seen in a variety of B-cell lymphomas. It is present in all lymphoplasmacytic lymphomas (by definition), many marginal zone lymphomas (particularly those in the skin and thyroid), occasional chronic lymphocytic leukemia/small lymphocytic lymphomas, rare mantle cell lymphomas and some transformed B-cell lymphomas.[27] The plasma cells are often extrafollicular although intrafollicular plasma cells characterize 'type 3' follicular colonization as described in some marginal zone lymphomas.[28] Neoplastic plasma cells in splenic marginal zone lymphomas are also often in the central portion of the white pulp nodules.[29] In at least some circumstances, the presence of significant plasmacytic differentiation in a lymphoma may have clinical implications such as an increased incidence of a paraprotein[30] or association with a higher stage.[26] Biological correlates of plasmacytic differentiation have also been identified in MALT lymphomas such as absence of the t[11;18] or a distinctive gene expression profile in pulmonary MALT lymphomas.[31,32]

Plasma cells used to be a feature considered supportive of follicular hyperplasia rather than a follicular lymphoma; however, their presence in many follicular lymphomas has been recognized for a long time. Most frequently they are found in the interfollicular regions.[24] Intrafollicular plasma cells

were noted in only 12% of cases in one series; however, whether or not they were a part of the lymphoma was not documented.[24] In a later study of 189 follicular lymphomas, large numbers of plasma cells were found in 9% of cases including 10 with polytypic plasma cells and seven with monotypic plasmacytic cells.[33] Where evaluable, the monotypic plasma cells had a light chain consistent with that found in the follicular lymphoma cells. In contrast to an interfollicular distribution in the cases with polytypic plasma cells, 6/7 cases with monotypic plasma cells had both interfollicular, and easily identifiable intrafollicular plasma cells. The cases were reported to show varying proportions of mature plasma cells, immature plasma cells and plasmacytoid cleaved cells. Both intra- and interfollicular monotypic plasma cells also have been described in other reported follicular lymphomas with, at least two cases noted to show an accentuation of plasma cells at the periphery of the follicles.[15–17] Clinically, some cases reported as follicular lymphoma with plasmacytic differentiation have been associated with paraproteins and, at least one also had leukemic involvement suggesting a full maturational mimic of normal B-cell differentiation.[15–17] One series suggested a more aggressive course.[15] These reported follicular lymphomas with plasmacytic differentiation are distinguished from follicular lymphomas with other types of immunoglobulin accumulation such as those that form signet ring cells.[34]

Most reports describing plasmacytic differentiation in follicular lymphomas, however, are from the period before the concepts of marginal zone lymphomas and follicular colonization were broadly recognized and accepted. Furthermore, the relationship of the plasma cells to the more typical follicular lymphoma cells, if investigated at all, was largely based solely on common light chain expression. Moreover, whether the cases had any distinctive cytogenetic abnormalities was not investigated. Therefore, we undertook this study to determine the relationship of the CD138-positive plasmacytic cells to the non-plasmacytic follicular lymphoma cells and to determine if follicular lymphomas with plasmacytic differentiation have distinctive cytogenetic, phenotypic or additional morphologic features. Fourteen follicular lymphomas, diagnosed using the current WHO criteria, were investigated using FICTION and a spectrum of FISH probes. This is the largest series of such cases in the literature.

Twelve of the 14 cases had at least one cytogenetic FISH abnormality. The abnormalities, where evaluated, were always shared between the plasmacytic and non-plasmacytic cells, supporting that these cases are follicular lymphomas with plasmacytic differentiation rather than composite lymphomas. Consistent with previous studies, the plasmacytic cells did not have a uniform morphologic appearance demonstrating that, even within this group of cases, the degree of plasmacytic differentiation varied. The majority of cases lacked easily recognized mature plasma cells with the most common appearance that of plasmacytoid cells often without classic plasmacytic nuclei. In a small number of cases, plasmacytic differentiation was only identified once immunohistochemical stains were reviewed. The number of plasma cells also varied greatly, being numerous and sheet-like in some cases, and more scattered in others. In one case, the bone marrow involvement was indistinguishable from a plasma cell neoplasm. The distribution of plasma cells also varied. Half of the cases had a predominantly interfollicular distribution, with the others having a prominent intrafollicular or, in one case, perifollicular plasmacytic population.

Review of the non-plasmacytic cells suggested that while some cases had numerous classic centrocytes, many cases showed less easily categorizable relatively small lymphoid cells. These presumptive centrocytes often had rounder, less angulated or clefted nuclei or were sometimes hard to recognize as centrocytes at all. Four cases, three of which had predominantly interfollicular plasma cells, showed other post-follicular marginal zone-type differentiation. Thus particularly in small biopsies, these cases may be difficult to recognize either as a follicular lymphoma or as having plasmacytic differentiation. The cases included in this series did show what may be a somewhat higher than expected proportion of grade 3 follicular lymphomas; however, the numbers are too small to draw any definite conclusions. The presence of numerous centroblasts in some cases could also cause confusion with large B-cell lymphomas with plasmacytic differentiation. Although prominent eosinophilia in B-cell lymphomas is reported to be associated with plasmacytic differentiation, often in nodal marginal zone lymphomas,[35] none of the cases in this series demonstrated this finding.

The follicular lymphomas reported here had a typical phenotypic profile (CD10 most positive, BCL6 positive and BCL2 mostly positive).[2] The cytogenetic FISH data showed fewer *BCL2* and *IGH@* translocations than might have been expected overall, with too few cases to reliably determine if this might have related to 6/14 cases being grade 3 (5/8 grade1–2 cases and 2/6 grade three cases had *BCL2* rearrangements). Caution is advised, however, as the number of cases is relatively small and the reported proportion of follicular lymphomas with *IGH@/BCL2* translocations does vary. None of the abnormalities typically associated with marginal zone lymphomas were identified such as trisomy 3 (as would be inferred using the *BCL6* 3q27 probe), *MALT1* translocation or trisomy 18. One case did show an extra *MALT1* signal, perhaps similar to our previously reported ocular MALT lymphoma.[26] Trisomy 12, seen in one case here, is one of the more common secondary abnormalities found in follicular lymphomas, although it is usually seen in association with a *BCL2* translocation.[36] *BCL6* translocations, as present in two cases, are reported in 5–15% of follicular lymphomas, particularly in grade 3B cases.[2,22] The

two cases in this series, however, were grade 1–2. As seen in one case here, about 10% of follicular lymphomas have both *BCL2* and *BCL6* translocations with most of the reported cases grade 1 or 2.[37,38]

The presence or absence of a *BCL2* translocation did not clearly correlate with the type of plasmacytic differentiation or with CD10 expression, which was positive in all, but two cases. However, its presence was strongly associated with a predominantly interfollicular plasma cell distribution. The presence of a *BCL2* translocation, a feature of the most typical follicular lymphomas, was therefore associated with a plasma cell distribution mostly like that seen in reactive lymph nodes. Conversely, the absence of a *BCL2* translocation was associated with a prominent intra/perifollicular plasma cell population, a feature typically seen with one form of follicular colonization in marginal zone lymphomas[28] and also seen in a minority of reactive follicles. It is among these cases that the less typical, although non-recurrent cytogenetic FISH abnormalities were found. The one unusual case with both *BCL2/IGH@* and *BCL6* rearrangements also showed a similar intrafollicular plasmacytic cell distribution. Most cases with classical centrocytes also had a *BCL2* translocation.

These findings suggest that there may be two types of follicular lymphomas with clonally related plasmacytic differentiation. One group is composed of classic follicular lymphomas, usually with *BCL2* translocations, that show post-follicular maturation to plasma cells mostly with an interfollicular distribution, consistent with normal post-follicular development. The second group does not demonstrate recurrent cytogenetic abnormalities to suggest placement into another diagnostic category but usually lacks *BCL2* translocations and has a prominent intrafollicular/perifollicular plasma cell distribution. These cases might represent much less classic follicular lymphomas that share some features with marginal zone lymphomas demonstrating extensive follicular colonization.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Goodlad JR, Batstone PJ, Hamilton D, *et al.* Follicular lymphoma with marginal zone differentiation: cytogenetic findings in support of a high-risk variant of follicular lymphoma. Histopathology 2003;42: 292–298.
2 Harris NL, Swerdlow SH, Jaffe ES, *et al.* Follicular lymphoma In: Swerdlow SH, Campo E, Harris NL, *et al.* (eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC: Lyon, 2008, pp 220–226.
3 Nathwani BN, Anderson JR, Armitage JO, *et al.* Clinical significance of follicular lymphoma with monocytoid B cells. Non-Hodgkin's Lymphoma Classification Project. Hum Pathol 1999;30:263–268.
4 Abou-Elella A, Shafer MT, Wan XY, *et al.* Lymphomas with follicular and monocytoid B-cell components. Evidence for a common clonal origin from follicle center cells. Am J Clin Pathol 2000;114:516–522.
5 Hernandez AM, Nathwani BN, Nguyen D, *et al.* Nodal benign and malignant monocytoid B cells with and without follicular lymphomas: a comparative study of follicular colonization, light chain restriction, bcl-2, and t(14;18) in 39 cases. Hum Pathol 1995;26: 625–632.
6 Mollejo M, Menarguez J, Cristobal E, *et al.* Monocytoid B cells. A comparative clinical pathological study of their distribution in different types of low-grade lymphomas. Am J Surg Pathol 1994;18:1131–1139.
7 Schmid U, Cogliatti SB, Diss TC, *et al.* Monocytoid/marginal zone B-cell differentiation in follicle centre cell lymphoma. Histopathology 1996;29:201–208.
8 Yegappan S, Schnitzer B, Hsi ED. Follicular lymphoma with marginal zone differentiation: microdissection demonstrates the t(14;18) in both the follicular and marginal zone components. Mod Pathol 2001; 14:191–196.
9 Robetorye RS, Bohling SD, Medeiros LJ, *et al.* Follicular lymphoma with monocytoid B-cell proliferation: molecular assessment of the clonal relationship between the follicular and monocytoid B-cell components. Lab Invest 2000;80:1593–1599.
10 Dogan A, Du MQ, Aiello A, *et al.* Follicular lymphomas contain a clonally linked but phenotypically distinct neoplastic B-cell population in the interfollicular zone. Blood 1998;91:4708–4714.
11 Dogan A, Bagdi E, Munson P, *et al.* CD10 and BCL-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas. Am J Surg Pathol 2000; 24:846–852.
12 Torlakovic EE, Aamot HV, Heim S. A marginal zone phenotype in follicular lymphoma with t(14;18) is associated with secondary cytogenetic aberrations typical of marginal zone lymphoma. J Pathol 2006; 209:258–264.
13 Jaffe ES, Harris NL, Stein H, *et al.* Introduction and overview of the classification of the lymphoid neoplasms In: Swerdlow SH, Campo E, Harris NL, *et al.* (eds). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC: Lyon, 2008, pp 158–166.
14 Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol 2008;8:22–33.
15 Frizzera G, Anaya JS, Banks PM. Neoplastic plasma cells in follicular lymphomas. Clinical and pathologic findings in six cases. Virchows Arch A Pathol Anat Histopathol 1986;409:149–162.
16 Schmid U, Karow J, Lennert K. Follicular malignant non-Hodgkin's lymphoma with pronounced plasmacytic differentiation: a plasmacytoma-like lymphoma.

Virchows Arch A Pathol Anat Histopathol 1985;405: 473–481.
17 Vago JF, Hurtubise PE, Redden-Borowski MM, *et al.* Follicular center-cell lymphoma with plasmacytic differentiation, monoclonal paraprotein, and peripheral blood involvement. Recapitulation of normal B-cell development. Am J Surg Pathol 1985;9: 764–770.
18 Jourdan F, Molina TJ, Le Tourneau A, *et al.* Florid marginal zone differentiation in follicular lymphoma mimicking marginal zone lymphoma of MALT type in the lung. Histopathology 2006;49:426–429.
19 Kim H-C, Lee Y-S, Choi J-W, *et al.* Follicular lymphoma with monoclonal plasma cell differentiation - a case report. Korean J Pathol 2006;40:151–155.
20 Nemes Z, Thomazy V, Szeifert G. Follicular centre cell lymphoma with alpha heavy chain disease. A histopathological and immunohistological study. Virchows Arch A Pathol Anat Histol 1981;394:119–132.
21 Wong KF, So CC, Chan JC, *et al.* Gain of chromosome 3/3q in B-cell chronic lymphoproliferative disorder is associated with plasmacytoid differentiation with or without IgM overproduction. Cancer Genet Cytogenet 2002;136:82–85.
22 Katzenberger T, Ott G, Klein T, *et al.* Cytogenetic alterations affecting BCL6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B-cell component. Am J Pathol 2004;165: 481–490.
23 van Rooijen N. Direct intrafollicular differentiation of memory B cells into plasma cells. Immunol Today 1990;11:154–157.
24 Nathwani BN, Winberg CD, Diamond LW, *et al.* Morphologic criteria for the differentiation of follicular lymphoma from florid reactive follicular hyperplasia: a study of 80 cases. Cancer 1981;48: 1794–1806.
25 Dierlamm J, Michaux L, Wlodarska I, *et al.* Trisomy 3 in marginal zone B-cell lymphoma: a study based on cytogenetic analysis and fluorescence *in situ* hybridization. Br J Haematol 1996;93:242–249.
26 Ruiz A, Reischl U, Swerdlow SH, *et al.* Extranodal marginal zone B-cell lymphomas of the ocular adnexa: multiparameter analysis of 34 cases including interphase molecular cytogenetics and PCR for Chlamydia Psittaci. Am J Surg Pathol 2007;31:792–802.
27 Swerdlow SH, Campo E, Harris NL, *et al.* WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (eds). IARC: Lyon, 2008.
28 Isaacson PG, Norton AJ. Extranodal Lymphomas. Churchill Livingstone: Edinburgh, 1994, 340 pp.
29 Mollejo M, Menarguez J, Lloret E, *et al.* Splenic marginal zone lymphoma: a distinctive type of low-grade B-cell lymphoma. A clinicopathological study of 13 cases. Am J Surg Pathol 1995;19:1146–1157.
30 Van Huyen JP, Molina T, Delmer A, *et al.* Splenic marginal zone lymphoma with or without plasmacytic differentiation. Am J Surg Pathol 2000;24:1581–1592.
31 Okabe M, Inagaki H, Ohshima K, *et al.* API2-MALT1 fusion defines a distinctive clinicopathologic subtype in pulmonary extranodal marginal zone B-Cell lymphoma of mucosa-associated lymphoid tissue. Am J Pathol 2003;162:1113–1122.
32 Chng WJ, Remstein ED, Fonseca R, *et al.* Gene expression profiling of pulmonary mucosa-associated lymphoid tissue lymphoma identifies new biologic insights with potential diagnostic and therapeutic applications. Blood 2009;113:635–645.
33 Keith TA, Cousar JB, Glick AD, *et al.* Plasmacytic differentiation in follicular center cell (FCC) lymphomas. Am J Clin Pathol 1985;84:283–290.
34 Kim H, Dorfman RF, Rappaport H. Signet ring cell lymphoma. A rare morphologic and functional expression of nodular (follicular) lymphoma. Am J Surg Pathol 1978;2:119–132.
35 Bian S, Jiang L, Vivero A, *et al.* B-Cell lymphomas with marked tissue eosinophilia and plasmacytoid differentiation, a study of 25 cases. Mod Pathol 2007; 20:234A.
36 Cook JR, Shekhter-Levin S, Swerdlow SH. Utility of routine classical cytogenetic studies in the evaluation of suspected lymphomas: results of 279 consecutive lymph node/extranodal tissue biopsies. Am J Clin Pathol 2004;121:826–835.
37 Diaz-Alderete A, Doval A, Camacho F, *et al.* Frequency of BCL2 and BCL6 translocations in follicular lymphoma: relation with histological and clinical features. Leuk Lymphoma 2008;49:95–101.
38 Keller CE, Nandula S, Fisher J, *et al.* The spectrum of B-cell non-Hodgkin's lymphomas with dual IgH-BCL2 and BCL6 translocations. Am J Clin Pathol 2008; 130:193–201.