EXTENDED REPORT

Plasmacellular differentiation in extranodal marginal zone B cell lymphomas of the ocular adnexa: an analysis of the neoplastic plasma cell phenotype and its prognostic significance in 136 cases

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Aim: To determine (a) the expression of plasma cell related antigens in extranodal marginal zone B cell lymphomas (EMZL) of the ocular adnexa; and (b) the prognostic value of plasmacellular differentiation in these tumours.

Methods: A consecutive case series of 136 ocular adnexal EMZL obtained from three ocular pathology centres over 20 years was analysed retrospectively. An extensive immunohistochemical panel, including the plasma cell related antigens VS38c, CD38, CD138, multiple myeloma oncogene-1-protein (MUM1/IRF4), and CREB binding protein (CBP) was performed. EMZL were defined as "plasmacellular differentiated" on the basis of morphological features, evidence of cytoplasmic immunoglobulin, negativity for BSAP/PAX5, and expression of at least one of the investigated plasma cell related antigens. Controls included normal or hyperplastic lymphatic tissues. Detailed clinical data were collected for most patients, and compared with the results of immunohistochemistry. The end points considered for statistical analysis were development of local tumour recurrence, development of systemic disease, and lymphoma related death.

Results: 57 (42%) of the 136 ocular adnexal EMZL showed a plasmacellular differentiation; 45 of these plasmacytoid cases were primary tumours. In contrast with most admixed normal plasma cells, which displayed co-expression of MUM1/IRF4, Vs38c, CD38, CD138, and CBP, the plasmacellular differentiated EMZL tumour cells demonstrated co-expression of all five plasma cell related antigens in only six of 57 (11%) plasmacellular differentiated ocular adnexal EMZL. The most commonly expressed plasma cell related antigen was MUM1/IRF4, immunoreactivity being seen in 56/57 (98%) plasmacellular differentiated EMZL examined. Although the association of plasmacellular differentiation in primary ocular adnexal EMZL and disseminated disease was statistically significant on univariate analysis ($p = 0.042$), this was weaker on multivariate analysis.

Conclusion: Plasmacellular differentiated tumour cells in EMZL demonstrate an aberrant immune profile for plasma cell related antigens when compared with normal plasma cells. On multivariate analysis, plasmacellular differentiation in ocular adnexal EMZL was not significantly associated with local recurrence, the development of systemic disease, or with lymphoma related death.

The ocular adnexal lymphomas (OAL) represent malignant lymphoid neoplasms which develop as primary or secondary tumour manifestations in the orbit, the lacrimal gland, the conjunctiva, and eyelid. The majority of OAL are B cell non-Hodgkin's lymphomas (NHL), with the most common subtype being the extranodal marginal zone B cell lymphoma (EMZL),^{1–7} according to the new World Health Organization (WHO) lymphoma classification.⁸

EMZL develop in a variety of locations normally possessing minimal lymphoid tissue, the most common site being the stomach.⁹ Regardless of location, these tumours have similar clinical, pathological, and molecular features.^{8,9} Usually, EMZL are low grade NHL with indolent clinical courses, although high grade transformations have been described,¹⁰ including in the ocular adnexa.^{4,5,11,12} Morphologically, EMZL are characterised by a mixture of centrocyte-like, monocytoid, and plasmacellular differentiated tumour cells with occasional blasts in the marginal zone surrounding reactive follicles. The degree of plasmacellular differentiation can be extensive, and may result in difficulties in distinguishing lymphoplasmacytic lymphoma/immunocytoma from EMZL in small biopsies.^{2,7,13,14}

The clinical significance of plasmacellular differentiation of tumour cells in small cell B-NHL in general is unclear. Patients with "plasmacytoid" B cell chronic lymphocytic leukaemia (B-CLL) are proposed to have a better prognosis.^{15–17} In this study, we determined which ocular adnexal EMZL could be defined as "plasmacellular differentiated" on the basis of morphology and of their immunophenotype (including evidence of cytoplasmic immunoglobulin (Ig) with light chain (IgL) restriction, negativity for B cell specific activator protein (BSAP, also known as PAX5), as well as expression of the plasma cell related antigens VS38c, CD38, CD138, multiple myeloma oncogene-1-protein (MUM1, also known as IRF4), and of the CREB binding protein (CBP)). The results were compared with clinical

Abbreviations: APAAP, alkaline phosphatase anti-alkaline phosphatase; B-CLL, B cell chronic lymphocytic leukaemia; BSAP, B cell specific activator protein; CBP, CREB binding protein; CREB, cyclic AMP responsive element binding protein; EMZL, extranodal marginal zone B cell lymphoma; Ig, immunoglobulin; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; MUM1/IRF4, multiple myeloma oncogene-1-protein; NHL, non-Hodgkin's lymphomas; OAL, ocular adnexal lymphomas; RLH, reactively hyperplastic lymphoid tissue

data, in particular, with the development of local tumour recurrence, of systemic disease, and of lymphoma related death to establish whether plasmacellular differentiation of ocular adnexal EMZL has any value in the prediction of these events.

METHODS

Tissue samples

A series of 136 consecutive cases of ocular adnexal EMZL were collected and slides retrospectively reviewed from the pathology departments of Charité-Medical Faculty Berlin, Campus Benjamin Franklin, Berlin, Germany, and of Western Infirmary, Glasgow, UK, as well as the Department of Ophthalmology, University Hospital Freiburg, Germany. The cases, which were collected between the 1980 and 2000, were assessed on the basis of morphology and immunophenotype, and classified according to the new "World Health Organization (WHO) Classification of Tumors of Haematopoietic and Lymphoid Tissues".⁸ Only those cases with biopsies of sufficient size, adequate morphology, appropriate fixation (4% buffered formaldehyde), and clinical information were analysed. In addition, 10 cases of normal or reactively hyperplastic lymphoid tissue (RLH)—for example, hyperplastic tonsil and lymphoplasmacellular infiltrates within lacrimal glands, were evaluated.

Immunohistology

Immunohistochemical investigations were performed using monoclonal and polyclonal antibodies reactive in paraffin sections. A pressure cooker antigen retrieval method was performed before immunohistochemical staining.¹⁸ The staining consisted of a first stage incubation with the following primary monoclonal antibodies against the following antigens: CD79a; CD20; BSAP/PAX5; CD5; CD10; CD21; CD23; CD43; BCL2; cyclin D1; BCL6; VS38c; CD138; MUM1/IRF4; CBP; MIB-1 (Ki-67 antigen); as well as anti-pancytokeratin. Polyclonal antibodies were used for the CD3 antigen, and for immunoglobulin light and heavy chains (IgL and IgH, respectively). The antibodies were obtained from Dako (Glostrup, Denmark), Santa Cruz (CA, USA), Serotec (UK), Transduction and Novocastra (UK) (table 1), except for MUM1/IRF4, which was provided by Dr B Falini (Perugia, Italy). Bound antibodies were visualised using an indirect immunoperoxidase method¹⁹ for the antibodies to IgL and IgH, whereas the alkaline phosphatase anti-alkaline phosphatase (APAAP) method was used for the remainder.²⁰ Double staining of some tumours was performed: the first antigen was detected by an avidin-biotin-peroxidase technique using diaminobenzidine/hydrogen peroxide as substrate, and the second antigen, by the APAAP procedure using naphthol AS-MX plus Fast Red TR (Sigma-Aldrich) as substrate. Incubation omitting the specific antibody was used as a control of the technique. The number of cells demonstrating clear nuclear positivity for MIB1 was determined as described previously.²¹

Table 1 B cell and plasma cell associated antibodies used with corresponding clones and commercial source		
Antibody	Clone	Company
CD79a	JCB117	Dako, Denmark
CD20	L26	Dako, Denmark
BSAP/PAX5	24	Transduction Laboratories, Lexington, KY, USA
VS38c	Vs38c	Dako, Denmark
CD138	B-B4	Serotec, UK
CD38	SPC32	Novocastra, UK
CBP	C-1	Santa Cruz Biotechnology, CA, USA

Definition of plasmacellular differentiation of EMZL

Ocular adnexal EMZL were defined as plasmacellular differentiated when 10% or more of the tumour cells displayed morphological features similar to plasma cells, such as eccentric nuclei, violet coloured or basophilic cytoplasm, the presence of Dutcher bodies, and/or Russell bodies, as defined in the literature (fig 1).²² Confirmation was achieved as proposed by other authors²³ using immunohistochemical investigations for evidence of cytoplasmic (c) Ig and IgL restriction, negativity for BSAP/PAX5, and expression of at least one of the investigated plasma cell related antigens (VS38c, CD38, CD138, MUM1/IRF4, and CBP). Following a hypothesis that plasmacellular differentiated EMZL of the ocular adnexa which demonstrated immunoreactivity for a larger number (if not all) of the five investigated plasma cell related antigens possibly being better differentiated tumours than those which displayed positivity for only a few antigens, we repeated the statistical evaluations for all chosen end points by increasing the number of plasmacellular antigen expressed (for example, 2, 3, or 4) required in the inclusion criteria.

Normal plasma cells entrapped within the lymphoma were excluded from the evaluation. The distinction between such plasma cells and neoplastic cells with plasmacellular differentiation was based on features such as the maturity of cell morphology and the presence or absence of atypia (fig 1).

The staining of MUM1/IRF4 was localised predominantly to the nucleus, although weak cytoplasmic staining was also present in most cases with nuclear positivity. Strong staining in more than 20% of plasmacellular differentiated tumour cells was scored as positive, as defined by Tsuboi *et al.*²⁴ Strong staining localised to the nucleus was considered positive for CBP; that localised to the plasma membrane was considered positive for CD138 and CD38; and that to the cytoplasm was considered positive for Vs38c.

Medical history

Detailed clinical information was gathered in all patients; those cases where significant data were not traceable, were excluded from the study. In order to obtain a follow up period of at least 2 years in patients still alive at the final examination, cases of ocular adnexal EMZL diagnosed after July 2000 were excluded. "Stage at presentation" was defined according to the modified Ann-Arbor classification scheme at the time of first diagnosis. Patients who had bilateral OAL without systemic manifestations were considered to have stage IE disease. "Primary disease" was defined as primary involvement of the ocular adnexa, corresponding to stage IE. "Secondary disease" was defined as a lymphomatous infiltration of the ocular adnexa by an identical lymphoma of another primary site.

Statistical analysis

Three clinically relevant end points were considered: (a) the development of local tumour recurrence; (b) the development of systemic disease; and (c) death related to lymphoma (time to event). For the three end point analysis, we also categorised the percentage of tumour cell expression for the proliferation marker MIB1 as previously described.²¹ The association of categorical variables was assessed by cross tabulation, exact Pearson χ^2 tests, and multiple logistic regression. Corresponding relative risks and odds ratios (multivariable) with 95% confidence intervals were calculated. The equality of "time to event" distributions was investigated by Kaplan-Meier estimates of "survival curves," log rank tests, and (multiple) Cox regression. Corresponding hazard ratios (univariable and multivariable) with 95% confidence intervals were calculated. p Values (two tailed) lower than alpha = 5% were considered statistically significant

Figure 1 Illustration of normal and neoplastic plasma cells. (A) Normal plasma cells in lacrimal glands with their characteristic morphological appearance with eccentric nuclei with a "clock face" or "cartwheel" pattern distribution of chromatin, abundant cytoplasm, and distinct perinuclear holes. (B) Extranodal marginal zone B cells with a plasmacellular differentiation demonstrated violet coloured or basophilic cytoplasm, the presence of Dutcher bodies, and/or Russell bodies (see also fig 4B) as well as atypical nuclear features.

Figure 2 Expression of the five investigated plasma cell related antigens in reactive lymphoid tissue. Subepithelial reactive plasma cells in tonsils were positive for (A) Vs38c (cytoplasmic), (B) MUM1/IRF4 (nuclear), (C) CD138 (membranous), (D) CD38 (membranous), and (E) CBP (cytoplasmic), with polytypical expression for the light immunoglobulin chains kappa (F) and lambda (G). The tonsillar epithelium also demonstrates positivity for CD138 (C); while the germinal centre cells display mild to moderate expression of CD38 (D). Original magnification, ×1000.

Figure 3 (A) EMZL without significant plasmacellular differentiation consisting of a heterogeneous population of centrocyte-like cells (arrowhead), monocytoid cells (medium arrow) as well as occasional blasts (long arrow) (Giemsa, original magnification $\times 400$). The tumour cells are positive for (B) CD20 and (C) demonstrate an aberrant expression of the T cell antigen CD43. (D) Occasional extrafollicular blasts are positive for BCL-6. (APAAP, original magnification $\times 400$).

though not adjusted for multiple testing. All statistical analyses were performed using the software package SPSS for Windows, Release 11.0.1.

RESULTS

Clinical features and anatomical distribution

Most patients with ocular adnexal EMZL were female (F = 100; M = 36; F:M 2.7:1), with a median age of 64 years (range 19–91 years) at first diagnosis. The ocular adnexal EMZL were distributed as follows: orbit (n = 47+7—that is, 47 stage IE and seven stage II, III, or IV) with lacrimal gland involvement in 8+2 cases; conjunctiva (n = 41 +7) and lid (n = 21+3). Synchronous bilateral EMZL were seen in 14+2 patients. (In 43 cases, clinical information on this aspect was missing; however, subsequent treatment would indicate that these ocular adnexal EMZL must have been unilateral.) Most ocular adnexal EMZL (117 of 136 cases, 86%) represented *primary* OAL (that is, stage I, IE lymphoma). Nineteen (14%) of the EMZL patients had disseminated disease at the initial examination (that is, stage II, III, or IV), with the ocular adnexal tumours in 10 (53%) of these cases representing *secondary* manifestations of systemic disease. In some patients with ocular adnexal EMZL, concurrent disease (representing stage IIE and above) was observed in other extranodal locations.

Histology and immunohistology

Normal or reactive hyperplastic tissues

The palatine tonsils and the interstitial tissue of the lacrimal glands contained an infiltrate of small bland mixed T and B lymphocytes with reactive lymphoid follicles. In the mantle zones, weak positivity for MUM1/IRF4 and CBP but not for Vs38c, CD38, or CD138 was observed (fig 2). Most germinal centre cells demonstrated weak to moderate positivity for CD38 (membranous; mainly centroblasts), but were negative for MUM1/IRF4, Vs38c, CD138, and CBP (fig 2). Several germinal centre cells in the light zone displayed strong nuclear and moderate cytoplasmic positivity for MUM1/IRF4 and Vs38c, respectively. Some marginal zone B cells showed

weak nuclear immunoreactivity for MUM1/IRF4 and CBP, but were negative for Vs38c, CD38, and CD138.

Admixed plasma cells were seen in the subepithelial areas in the tonsils, in the interstitial tissue of the lacrimal glands, and occasionally in the reactive germinal centre. These cells demonstrated mature cytomorphology with basophilic cytoplasm, eccentric nuclei, clumped “clock faces” like chromatin and perinuclear halos. Virtually all plasma cells, which were negative for BSAP, demonstrated strong co-expression of CD79a, MUM1/IRF4-protein (nuclear strong, cytoplasmic weak), Vs38c (cytoplasmic), CD38 (membranous), CD138 (membranous), and CBP (nuclear strong, cytoplasmic weak), as well as polyclonal staining for IgH and IgL (fig 2). In some cases, occasional plasma cells failed to express CD138 and CBP. Varying immunoreactivity for Vs38c, CD138 (fig 2), and CBP but not for MUM1/IRF4 or CD38 was seen in the adjacent epithelium of the tonsils and lacrimal acini.

Extranodal marginal zone B cell lymphoma

Briefly, the EMZL consisted of small cells resembling centrocytes, monocytoid B cells, or small lymphocytes, with

Table 2 Immunophenotype of normal plasma cells as well as of non-plasmacytoid and plasmacytoid cells in EMZL	
Normal plasma cells	CD79a+, CD20–, BSAP/PAX5–, polyclonal IgL MUM1+, CD138+, CD38+, Vs38c+, CBP+*
Non-plasmacytoid cells in EMZL	CD79a+, CD20+, BSAP/PAX5+, CD43+*, monoclonal IgH+ (usually IgM+) MUM1–*, CD138–, CD38–, Vs38c–, CBP– Cyclin D1–, CD5–, CD23–, CD10–, BCL6– CD79+, CD20–, BSAP/PAX5–, CD43+, BCL2+, monoclonal IgL+ (kappa+>lambd+), monoclonal IgH+ (usually IgM+)
Plasmacytoid cells in EMZL	MUM1+>CD138+>CD38+>Vs38c+>CBP+ cyclin D1–, CD5–, CD23–, CD10–, BCL6–
+ = positive; – = negative; *in most cases; IgL, immunoglobulin light chain; IgH, immunoglobulin heavy chain.	

occasional blasts surrounding reactive B cell follicles (fig 3). Lymphoepithelial lesions were seen in some conjunctival specimens and in the orbital cases with lacrimal gland involvement. In most cases, the tumour cells expressed CD20, BSAP, CD79a, CD43, and BCL2 with absence of staining for CD10 and CD23 (fig 3) (table 2). Furthermore, scattered extrafollicular blasts stained for BCL6 or MUM1/IRF4 protein (fig 3). The MIB1 growth fraction ranged from 2–50%, median 10%. In cases where an increased number of blasts were present in the marginal zone, an increased percentage of

tumour cells (>10%) positive for either BCL6 or MUM1/IRF4, together with an increased growth fraction, was observed.

Fifty seven of 136 (42%) EMZL demonstrated plasmacellular differentiation, fulfilling the criteria as mentioned above (tables 2 and 3); 45 (33%) of these “plasmacytoid” tumours represented stage I, IE EMZL. The tumour cells of the plasmacellular differentiated EMZL were positive for CD79a but negative for CD20 and BSAP (table 2). They demonstrated clg and a monotypic expression for IgL or IgH, as well as a variable positivity for MUM1/IRF4, VS38c, CD38,

Figure 4 (A) EMZL with extensive plasmacellular differentiation (haematoxylin and eosin stain, original magnification $\times 400$). The “plasmacytoid” cells are highlighted in the PAS stain (inset). (B) The plasmacellular differentiated tumour cells are negative for CD20 (arrow) (APAAP, original magnification $\times 400$). (C) A monotypic expression of the tumour cells for the immunoglobulin light chain kappa is seen. The contrasting stain for immunoglobulin light chain lambda is shown in the inset (PAP, original magnification $\times 400$). Immunoreactivity of the plasmacellular differentiated cells in the same EMZL for (D) MUM1/IRF4 (nuclear), (E) Vs38c (membranous), (F) CD138 (membranous), and (G) CD38 (APAAP, original magnification $\times 400$). (H) Double staining for MUM1/IRF4 (PAP, brown nuclear) and CD38 (APAAP, red membranous), demonstrating only a small population of CD38/MUM1 positive cells (arrows), indicating the loss of CD38 expression by many of the MUM1 positive plasmacellular differentiated B cells. (original magnification, $\times 400$).

Table 3 Immunophenotype of the tumour cells in the plasmacellular differentiated ocular adnexal EMZL (BSAP negative, kappa, or lambda positive)

Immunophenotype*	EMZL	
	Stage I, IE	Stage II, III, IV
MUM1+/Vs38c+/CD138+/CD38+/CBP–	20	5
MUM1+/Vs38c–/CD138+/CD38+/CBP–	11	2
MUM1+/Vs38c+/CD138+/CD38–/CBP+	6	
MUM1+/Vs38c+/CD138+/CD38–/CBP–	4	
MUM1+/Vs38c+/CD138–/CD38+/CBP+	1	2
MUM1+/Vs38c+/CD138–/CD38+/CBP–	1	1
MUM1+/Vs38c–/CD138+/CD38–/CBP–	1	1
MUM1–/Vs38c+/CD138+/CD38+/CBP–	1	
MUM1+/Vs38c–/CD138+/CD38+/CBP+		1
Total	45	12

*The remaining plasma cells related antigen combinations were not represented in this sample of 136 lymphomas and are, therefore, not listed.

CD138, and CBP (fig 3) (tables 2 and 3). The most consistently expressed plasma cell related antigen of the plasmacytoid tumour cells was MUM1/IRF4, its immunoreactivity being seen in 56/57 (98%) plasmacellular differentiated EMZL examined (fig 4). The other plasma cell related markers showed a variable expression with CD138 protein positivity in 52/57 (91%) cases, CD38 in 51/57 (89%), Vs38c in 41/57 (72%), and CBP in 10/57 (18%) cases. Co-expression of all plasma cell related antigens by the “plasmacytoid” EMZL tumour cells was observed in only six of the 136 (4%, table 3).

Treatment

The differing treatments of the 136 ocular adnexal EMZL were dependent on the stage of disease at time of diagnosis and included surgical excision, radiotherapy, chemotherapy, or various combinations of these. The most common form of therapy for stage I, IE EMZL patients was radiotherapy (89/117 = 76%), with a dosage varying between 4 Gy and 50 Gy. The follow up period of the patient collective was 2–239 months, median 49 months (4.1 years). In total, 32/136 (24%) patients had died at last follow up, either as a result of lymphoma (n = 18) or non-lymphoma related causes (n = 14).

Statistical analysis

Either an ipsilateral or contralateral local recurrence occurred in 27 (23%) of the 117 patients with ocular adnexal EMZL stage IE. The median time period between initial and recurrent tumour was 20 months. A systemic manifestation occurred in 38/117 (32%) patients with stage IE ocular adnexal EMZL. The time period for the systemic disease manifestation to be clinically detected after diagnosis of ocular adnexal EMZL ranged from 2 to 125 months (median, 5 months).

Of the 136 EMZL patients, 18 (13%) had succumbed to their disease at last follow up. Of these 18 patients, 10 (56%) were diagnosed with localised disease at first diagnosis—that is, 8.5% of all patients in the group diagnosed with stage IE EMZL died as a result of lymphoma related causes.

The evaluation of the three end points and their relation to the degree of plasmacellular differentiation is summarised in table 4 and figure 5. When applying the above mentioned definition of plasmacellular differentiation of tumour cells, a statistical significance could be demonstrated for the development of systemic disease on univariate analysis ($p = 0.042$). On multivariate analysis, however, this significance became weaker when adjusted for other important prognostic parameters previously described,^{1 2 21} such as age at diagnosis (in years, $p = 0.286$, odds ratio with 95% confidence interval: 1.02 (0.98 to 1.07)) and an increased

growth fraction of the tumour cells (determined using MIB1, $>10\%$, $p < 0.001$, OR with 95% CI: 41.30 (12.50 to 136.40)) (table 4).

A statistical significance could not be demonstrated using univariate or multivariate analysis between plasmacellular differentiation of the tumour cells and either local tumour recurrence or lymphoma related death. For the latter, the parameters which reached statistical significance on multivariate analysis were (a) age at diagnosis (in years, $p = 0.058$, hazard ratio with 95% confidence interval: 1.07 (1.00 to 1.15)) and (b) an increased growth fraction ($p = 0.006$, HR with 95% CI: 6.01 (1.65 to 21.80)). As described above, alternative definitions of plasmacellular differentiation were investigated—that is, simultaneous negativity for BSAP, positivity for kappa or lambda and positivity of at least one, two, three, or four of the markers MUM1, CD138, VS38C, CD38, and CBP. Even when the tumour cells expressed more than one plasma cell related antigen, there was no significant alteration in the results for any of the end points (table 4).

DISCUSSION

Plasma cells are derived from B cells during immune responses^{25 26} and can be essentially divided into short and long lived plasma cells. Short lived plasma cells, with a life span of a few days, are generated following the

Figure 5 Kaplan-Meier estimate of survival curve for stage I, IE patients with (solid line) and without (broken line) plasmacellular differentiation defined as simultaneous negativity for BSAP, positivity for kappa or lambda and positivity of at least one of the markers MUM1, CD138, VS38C, CD38, and CBP (p value from log rank test: 0.404; hazard ratio with 95% confidence interval: 1.69 (0.49 to 5.84); tick marks correspond to censored observations).

transformation of IgM+/IgD+ “naive mature” B cells into extrafollicular B blasts after antigen contact. In contrast, long lived plasma cells, with a life span of possibly years, are derived either from germinal centre cells or from memory B cells, which have undergone the germinal centre reaction. Normally, long lived plasma cells are to be found only in peripheral lymphoid organs, the bone marrow, and at sites of immune responses, and are not detected in the peripheral blood. In their mature form, these cells have a typical morphological appearance, and are immunophenotypically characterised by positivity for CD79a, CD138, CD38, and Vs38c by negativity for CD20 and BSAP.^{27–28}

Whereas CD79a is expressed during most stages of B cell differentiation, both CD38 and CD138 are present on pre-B cells, lost during differentiation, and are re-expressed at the plasma cell stage in normal cells.^{29–30} Vs38c is also expressed at this terminal stage of B cell differentiation, when CD20 and BSAP antigens are lost. Recently, plasma cells have also been described as expressing MUM1/IRF4,³¹ known to be active in the control of plasma cell differentiation. The co-activator of the cyclic AMP responsive element binding protein (CREB)—namely the CREB binding protein (designated CBP), was included in the present study as we have observed a strong cytoplasmic expression of this transcriptional co-activator in plasma cells in our investigations of normal and neoplastic lymphatic tissues (unpublished results). At what point CBP is expressed during normal B cell differentiation is yet to be established.

In the current investigation, 136 ocular adnexal EMZL were analysed for their degree of plasmacellular differentiation on the basis of morphology, evidence of cIg, BSAP negativity, and the expression of five plasma cell related antigens. The results were subsequently correlated with three clinically relevant end points. Despite plasmacellular differentiation being described in up to 30% of EMZL,²² its clinical significance has not been subject to thorough investigation in any anatomical location to date. Furthermore, although some of the plasma cell related antigens investigated in the current study have been the subject of examination using both FACS and immunohistochemical analysis in haematopoietic malignancies,^{15–24–31–36} there are only limited (or no) analyses of the prognostic value of these markers.

Similar to previous studies,^{24–31} virtually all plasma cells in normal or hyperplastic lymphoid tissues demonstrated co-expression of Vs38c, CD138, and MUM1/IRF4. Many but not all mature peripheral plasma cells also displayed strong membranous and cytoplasmic reactivity for CD38 and CBP, respectively; 57/136 (42%) EMZL examined demonstrated features of plasmacellular differentiation. In contrast with their non-plasmacellular differentiated counterparts, the “plasmacytoid” EMZL tumour cells were negative for the B cell marker BSAP, and demonstrated Ig light chain restriction as well as expression of at least one of MUM1/IRF4, VS38c,

CD38, CD138, or CBP. In contrast with normal plasma cells, the neoplastic tumour cells of only six plasmacellular differentiated ocular adnexal EMZL demonstrated immunoreactivity for all five plasma cell related antigens (table 3).

MUM1/IRF4 protein was the most stable plasma cell related antigen in the “plasmacytoid” EMZL of the ocular adnexa, being observed in 56/57 (98%) tumours (table 3). That MUM1/IRF4 expression is not exclusively observed in cells with plasmacellular differentiation has been previously reported by other authors.^{24–31–37} However, only those cells with the corresponding cytomorphology, cIg/IgL, and BSAP negativity were considered in the interpretation of these stains. The remaining plasma cell related markers showed considerable variability with CD138 protein expression in 52/57 (91%) cases, CD38 in 51/57 (89%), Vs38c in 41/57 (72%), and CBP in 10/57 (18%) cases (table 3). Therefore, most plasmacellular differentiated tumour cells of ocular adnexal EMZL demonstrate an aberrant plasma cell related antigen profile when compared to normal plasma cells. This aberrant immune profile of “plasmacytoid” EMZL, which we described recently in uveal EMZL,³⁸ may aid the establishment of malignancy in ocular adnexal lymphoproliferative lesions, particularly in those EMZL where the interpretation of light chain expression can be difficult.

Various parameters have been described to be of prognostic value in patients with OAL, including anatomic location;^{39–42} stage at diagnosis;^{1–2–7–41} lymphoma subtyping according to the REAL classification^{1–2–4–6–7–43} and new WHO lymphoma classification;²¹ tumour cell growth fraction and increased blast percentages positive for BCL-6^{1–2–21}; as well as serum lactate dehydrogenase.⁶ The results of the current data suggest, however, that the *clinical* significance of the presence and degree of plasmacellular differentiation in EMZL of the ocular adnexa is not large. When defining plasmacellular differentiation of the tumour cells of the EMZL as described above, the relation between this tumour parameter and the development of systemic disease was statistically significant only on univariate analysis ($p = 0.042$). This significance was lost, however, on multivariate analysis when other factors of previously described importance,^{1–2–21}—namely age >60 years at diagnosis and an increased MIB1 growth fraction of the tumour cells—were also considered.

Following a hypothesis that “plasmacytoid” EMZL of the ocular adnexa which demonstrated immunoreactivity for an increased number of the five investigated plasma cell related antigens (for example, ≥ 3) possibly being better differentiated than those which displayed positivity for only a few of the antigens (for example, < 3), we repeated the statistical evaluations for all chosen end points. However, there was no major alteration in the results for any of the end points investigated on multivariate analysis.

In summary, most plasmacellular differentiated tumour cells in ocular adnexal EMZL demonstrate an aberrant

Table 4 Stage I, IE lymphoma (n = 117) and association of alternative definitions of plasmacellular differentiation and development of local tumour recurrence, systemic disease, and death related to lymphoma

Number of markers positive for definition of plasmacellular differentiation	Development of local tumor recurrence		Development of systemic disease		Death related to lymphoma	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
	RR (95% CI)	OR* (95% CI)	RR (95% CI)	OR* (95% CI)	HR (95% CI)	HR* (95% CI)
≥ 1 or 2†	1.10 (0.56 to 2.15)	0.93 (0.36 to 2.40)	1.77** (1.06 to 2.98)	1.38 (0.44 to 4.38)	1.69 (0.49 to 5.84)	1.10 (0.29 to 4.12)
≥ 3	0.98 (0.49 to 1.94)	0.77 (0.29 to 2.02)	1.65 (0.99 to 2.78)	1.01 (0.31 to 3.30)	1.20 (0.34 to 4.28)	0.70 (0.18 to 2.79)
≥ 4	1.17 (0.55 to 2.46)	1.08 (0.38 to 3.05)	1.73** (1.04 to 2.90)	1.37 (0.38 to 4.94)	1.16 (0.24 to 5.54)	0.98 (0.19 to 5.07)

RR, relative risk (from cross tabulation); OR, odds ratio (from logistic regression); HR, hazard ratio (from Cox regression);

*Adjusted for age at diagnosis and MIB1 ($\leq$, $> 10\%$).

**Statistically significant (p value $< 5\%$).

†These definitions did not yield different results on this dataset.

immune profile for plasma cell related antigens when compared to normal plasma cells. On multivariate analysis, plasmacellular differentiation in ocular adnexal EMZL had no (or very little) value in the prediction of local recurrence, the development of systemic disease, or for lymphoma related death. Strong predictors of (a) systemic tumour dissemination and (b) lymphoma related death included advanced age at diagnosis and an increased MIB-1 growth fraction. None of the investigated parameters in the current study seem to be predictive of local tumour recurrence.

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