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From Cell Biology to Tissue Engineering

Expression of the activation markers Blimp1, Foxp1 and pStat3 in extranodal diffuse large B-cell lymphomas

Georgios Petrakis[1,2], Ioannis Kostopoulos[2], Ioannis Venizelos[3], Maria Lambropoulou[1], Kyriakos Vouras[4], Sofia Vakalopoulou[5], Eudokia Mandala[6], Constantinos Tsatalas[7] and Nicolas Papadopoulos[1]

[1]Histology – Embryology Department, Medical School, Democritus University of Thrace, Alexandroupolis, [2]Pathology Department, Medical School, Aristotle University of Thessaloniki, [3]Pathology Department of Hippokration Hospital of Thessaloniki, [4]School of Mathematics, Faculty of Sciences, [5]Hematology Department, Hippokration Hospital of Thessaloniki, Medical School, [6]Fourth Department of Medicine, Hippokration Hospital, Thessaloniki, Medical School, Aristotle University of Thessaloniki, Thessaloniki and [7]Hematology Department, Medical School, Democritus University of Thrace, Alexandroupolis, Greece

Summary. Different studies have suggested that the expression of biomarkers related to lymphoid cell activation may provide information on the behavior of DLBCL. Most studies have concentrated on nodal or a mixture of nodal and extranodal lymphomas. The differential expression and potential clinical impact of these markers in a homogeneous group of extranodal DLBCLs are not well defined. In this study, we investigated the expression of three activation markers, Blimp1, Foxp1 and pStat3, in a cohort of 35 extranodal DLBCLs homogeneously treated with R-CHOP. Immunohistochemical stains were evaluated using an immunoreactivity score on representative paraffin sections. Blimp1 was positive in 55% (19/35), Foxp1 in 60% (21/35), and pStat3 in 69% (24/35) of our cases. We did not observe any statistical differences in the expression of these markers in GCB and non-GCB tumors or in gastrointestinal and non-gastrointestinal tumors. Blimp1 expression was negatively correlated with overall survival (OS) (p=0.001) in the whole series and in the non-GCB group (Muris algorithm) (p=0.002). Foxp1 positivity and pStat3 positivity had no impact on the outcome of the patients in the global cohort, but they were associated with a better survival in the non-GCB subgroup (p=0.033, p=0.044 respectively). Multivariate analysis showed that Blimp1 expression but not COO was an independent negative prognostic factor for OS (HR=17.5, 95%, CI=2.2-141.1, p=0.007). Our results suggest that these markers are differentially expressed and have different impacts on outcome in extranodal DLBCLs compared to nodal tumors, emphasizing the need to evaluate separately these and probably other markers in these subsets of tumors.

Key words: Blimp1, Foxp1, pStat3, DLBCL, Extranodal DLBCL

Introduction

Primary lymphomas are defined as Extranodal, when the major tumor mass is located at an extranodal site. The incidence of non-Hodgkin Lymphomas (NHL) has increased in the last decades, with almost 25% of them arising in extranodal sites. The most common site of these lymphomas is the gastrointestinal tract, with Diffuse Large B-cell Lymphoma (DLBCL) being the most frequent subtype (Jaffe et al., 2011). Interestingly, extranodal DLBCLs may show differences in the immunophenotype and genetic alterations, when compared to nodal counterparts and may have site-specific differences in prognosis. In addition, the subtypes of DLBCL according to the cell of origin (COO) seem to vary between nodal and extranodal sites and also in different extranodal locations (Lu et al., 2007; Kim et al., 2011; Lin et al., 2011; Martin-Arruti et al., 2012).

Offprint requests to: Georgios Petrakis, Histology-Embriology Department, Medical School, Democritus University of Thrace, 68100 Alexandoupolis, Greece. e-mail: georgiospetrakismd@gmail.com

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Abbreviations. DLBCL, Diffuse large B-cell lymphoma; GCB, germinal center B-cell-like; non-GCB, non-germinal center B-cell-like; R-CHOP, Rituximab-Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone; COO, Cell of Origin

The subclassification of DLBCL according to their COO is one of the most important characteristics related to the heterogeneous behavior of these tumors. The COO was initially defined by gene expression profiling dividing these tumors into the favorable germinal center B-cell-like (GCB) and the unfavorable activated B-cell-like (ABC) subgroups (Rosenwald et al., 2002). The introduction of Rituximab in the chemotherapy regimens has lessened the differences in the outcome of these two groups, but they are still relevant for the prognosis and may also influence future treatment management decisions (Fu et al., 2008; Nyman et al., 2009; Ott et al., 2010; Gutiérrez-García et al., 2011; Meyer et al., 2011; Visco et al., 2012; Culpin et al., 2013). Since gene expression profiling is not used in routine clinical practice, different algorithms have been designed, based on the differential expression of several markers detected by immunohistochemistry (IHC) (Hans et al., 2004, Muris et al., 2006, Visco et al., 2012). Although all of them have been shown to have a good relationship between the IHC subgroups and the GEP classification, the distribution of cases in the two subgroups of GCB and non-GCB varies among the algorithms (Martin-Arruti et al., 2012; Visco et al., 2012; Culpin et al., 2013).

In addition to the COO, different studies have suggested that the expression of biomarkers related to cell activation may provide information on the behavior of DLBCL (Garcia et al., 2006; Mandelbaum et al., 2010; Martin-Arruti et al., 2012; Van Keimpema et al., 2015). However, these studies have provided conflicting results that may be due to the heterogeneous composition of the patients studied, mixing nodal and extranodal cases together or including cases treated before and after the immunochemotherapy era. The potential clinical impact of these markers in a homogeneous group of extranodal DLBCLs treated with immunochemotherapy has not been well studied.

In this study, we aim to investigate the clinical significance of the expression of several activation markers in a cohort of extranodal DLBCLs treated with R-CHOP. We studied Blimp1, Foxp1 and pStat3 immunohistochemical expression as markers engaged in major pathways related to lymphoma cell activation and differentiation in DLBCLs (Lam et al., 2008; Sweetenham 2011; Yu et al., 2011; Wu et al., 2011; Hu et al., 2012; Gupta et al., 2012; O'Shea et al., 2013, Huang et al., 2013, Van Keimpema et al., 2015). We also investigated the expression of these activation markers in the context of the two subgroups of DLBCL according to their COO defined by three different immunohistochemical algorithms (Hans, Visco-Young (V&Y) and Muris) (Hans et al., 2004, Muris et al., 2006, Visco et al., 2012).

Table 1. Clinical characteristics of 35 patients with extranodal DLBCL.

	Extranodal DLBCL
Median age (range) in years	59 (17-79)
Males/Females	19 (54%)/16 (46%)
Stage I	20 (58%)
Stage II	2 (6%)
Stage III	4 (10%)
Stage IV	9 (26%)
B symptoms (%)	10 (29%)
	IPI score and IPI groups
Low	9 (29%)
Low-Intermediate	7 (23%)
High-Intermediate	11 (35%)
High	4 (13%)
Relapses	14 (40%)

Materials and methods

Patients

Thirty five patients with extranodal DLBCL diagnosed at the Pathology Departments of the Aristotle University Medical School of Thessaloniki and of the Hippokration General Hospital of Thessaloniki were included in the study. The patients were 19 males (54%) and 16 females (46%) with a median age of 59 years (range 17-79). The median duration of the follow up was 65 months, ranging from 1 to 162 months. The clinical characteristics of the patients are shown in detail in Table 1.

All cases were classified according to the 2008 WHO classification and staged according to the Ann Arbor staging system. The International Prognostic Index (IPI) was calculated and used for the clinical correlations. We divided patients in two different prognostic groups, namely High Risk (HR) (IPI high and high/intermediate scores) and Low Risk (LR) (IPI low and low intermediate scores). All the patients received chemotherapy with R-CHOP (Rituximab-Cyclophosphamide/ Doxorubicin/ Vincristine/ Prednisone). Patients staged I-IIA received 4 cycles of chemotherapy ± radiotherapy and those staged IIB-IV received 6 cycles.

All investigations related to the present study have been conducted according to the principles expressed in the Declaration of Helsinki and under approval of the ethics committee of the Medical School, Democritus University of Thrace.

Immunohistochemistry (IHC)

All IHC was performed on 4 μm sections of whole tissue biopsies mounted on Superfrost slides using the Dako Envision Flex System autostainer exploiting 3,3'-diaminobenzidine (DAB) as the chromagen and using mouse anti-human antibodies. Specifically, we used CD10 (56C6, Novocastra, ER1, pH 6, dilution 1/30), BCL6 (PG-B6p, Dako, ER2, pH 9, dilution 1/30), BCL2 (100/D5, Novocastra, ER2, pH 9, dilution 1/50), IRF4/MUM1 (Mum1p, Dako, ER2, pH 9, dilution 1/30), Ki-67 (MIB-1, Dako, ER2, pH 9, dilution 1/70), Blimp1

(ab2, PRS3991, Sigma-Aldrich, pre-treatment in pH 9, dilution 1/500), Foxp1 (SP133, Spring, pre-treatment in pH 9, dilution 1/100), pStat3 (Y705, EP2147Y, ab76315, Abcam, pre-treatment in pH 9, dilution 1/150) as per Kit. These protocols are thoroughly described in this link www.dako.com/dist/download.pdf?objectid=114786005. Tonsil sections were used as controls. Antigen expression for each marker was evaluated and graded by independent experienced pathologists, who were blinded to the clinical data. Any disagreement between the pathologists was resolved by joint reevaluation of the cases, and consensus was reached on a multi-head microscope. Positivity threshold of >50% was used for BCL2 (cytoplasmic stain) and >30% for CD10 (membranous stain), BCL6 (nuclear stain) and MUM1 (nuclear stain).

Blimp1, Foxp1 and pStat3 expression was semi-quantified using an Immunoreactivity score (IRS) that took into consideration both the percentage of the positive cells and the intensity of the staining. The IRS was calculated by multiplying the percentage of positive cells (PP) by the staining intensity (SI) obtaining a total score between 0 and 12. PP was graded as follows: 0=negative, 1=up to 10% positive cells, 2=11 to 50%, 3=51 to 80% and 4=more than 80% positive cells. SI was categorised in 4 groups namely 0=negative, 1, 2, 3=weakly, moderately and strongly positive respectively (Nagata et al., 2004). Blimp1 (nuclear stain), Foxp1 (nuclear stain) and pStat3 (nuclear stain) were considered as positive when IRS≥2. Representative cases stained for Blimp1, Foxp1 and pStat3 are shown in Fig. 1.

DLBCL cell of origin - Immunohistochemical algorithms

All cases were classified as GCB or non-GCB type according to three well-established algorithms with high concordance to molecular subtyping of DLBCLs, namely Hans (CD10, BCL6, MUM1), Visco & Young (V&Y) (CD10, Foxp1, BCL6), and Muris (BCL2, CD10, MUM1), using the percentage thresholds for positivity stated by these authors (Hans et al., 2004, Muris et al., 2006; Yu et al., 2011; Visco et al., 2012; Culpin et al., 2013).

Statistical Analysis

SPSS 20 software program was used to perform statistical analysis by a statistician (KV). Patients with positive and negative immunohistochemical results for each marker were compared with the Kaplan-Meier survival analysis. $P<0.05$ was used for statistical significance. The Log-rank and Breslow tests were used for the comparison between survival curves. Cox-regression analysis was used to estimate the hazard ratio (HR) with 95% confidence interval (CI) to determine independent prognostic factors for survival and to correct the confounding effect of differences in prognostic factors. Categorical data were compared using Fisher's exact test for a two-sided p-value, whereas

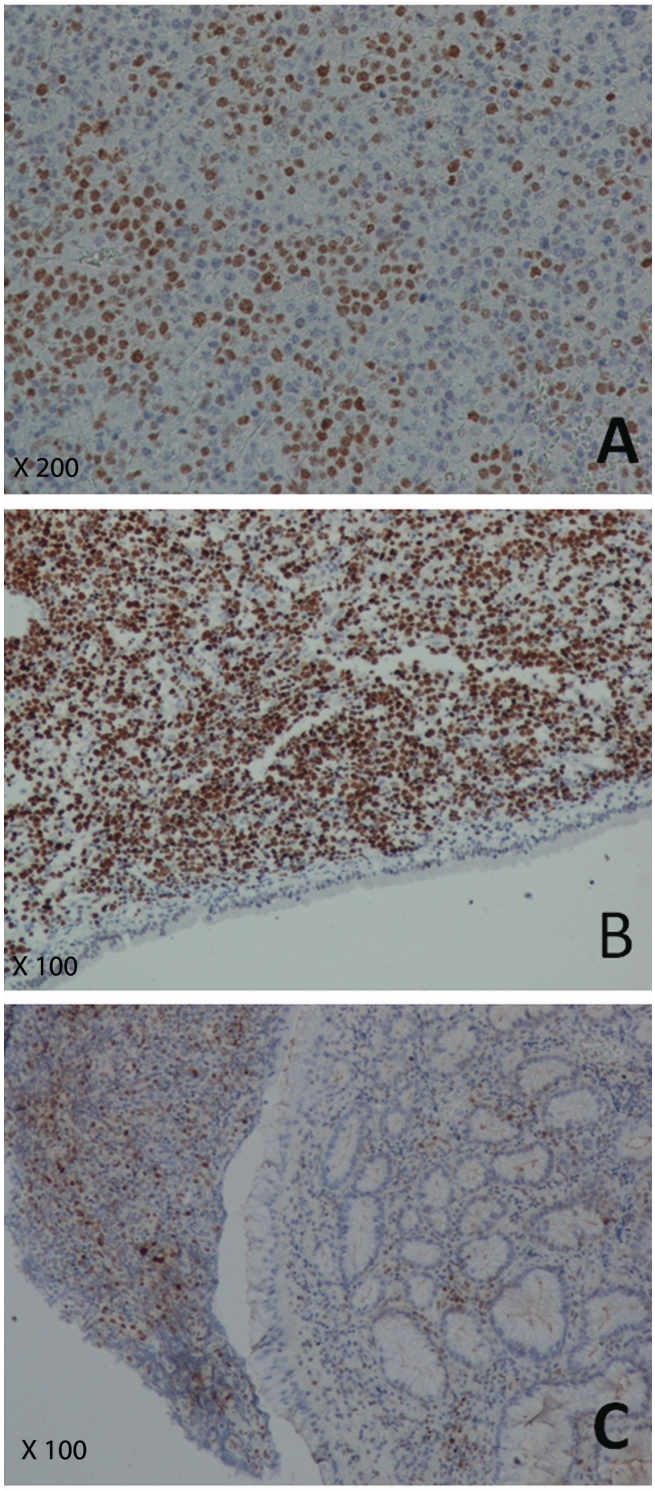


Fig. 1. Immunohistochemical expression in our extranodal DLBCLs of: Blimp1 (**A**), Foxp1 (**B**), pStat3 (**C**). We used a semi-quantitative Immunoreactivity score (IRS) taking into consideration both the percentage of the positive cells (PP) and the intensity of the staining (SI). PP was graded as follows: 0=negative, 1=up to 10% positive cells, 2=11 to 50%, 3=51 to 80% and 4=more than 80% positive cells. SI was categorised in 4 groups, namely 0=negative, 1, 2, 3=weakly, moderately and strongly positive respectively. The IRS was calculated by multiplying the PP by the SI obtaining a total score between 0 and 12. Nuclear stain with IRS≥2 was considered as positive.

for ordinal data, nonparametric tests were used. Chi-square tests were used to compare percentages in cross tabulations.

Results

Cell of origin

The results of the three algorithms showed a relative high concordance among them (Table 2). The COO categorization was concordant in 71% of the cases between Hans and V&Y algorithm, 76% between Hans and Muris, and 71% between Muris and V&Y. Most of the cases in the three algorithms were non-GCB irrespectively of the algorithm used. However, the number of cases classified as GCB and non-GCB were slightly different among the three algorithms. According to Hans 80% of the cases was non-GCB, whereas they were 67% according to V&Y and 57% according to Muris. Similarly, the number of GCB and non-GCB cases assigned to the different IPI subgroups was also different (Table 2).

Eleven patients (31%) died during follow up, with only four of them (40%) in the HR IPI group. According to the IHC algorithm, 91% (Hans), 55% (V&Y) and 82% (Muris) of the deceased patients were in the non-GCB subgroup. Among the three algorithms, Muris was the only one that showed statistically significant differences in survival between the GCB and non-GCB DLBCL, with a better outcome for patients with GCB-DLBCL (Breslow test) (Fig. 2). No significant differences were seen with the other two algorithms. Therefore, we used only the Muris subclassification of DLBCLs for subsequent analysis.

Blimp1, Foxp1 and pStat3 expression

The immunohistochemical results of the three biomarkers are summarized in Table 3. Blimp1 was positive in 55% (19/35) of our cases with an

Table 2. GCB and non-GCB DLBCL cases assigned to IPI scores and groups according to different algorithms.

IPI group	Hans*		V&Y**		Muris***	
	GCB	Non-GCB	GCB	Non-GCB	GCB	Non-GCB
Low#	3	12	3	13	5	10
High##	3	12	7	8	6	9
Total	6 (20%)	24 (80%)	10 (32%)	21 (68%)	11 (43%)	19 (57%)

#: Low IPI group includes Low and Low-Intermediate IPI scores; ##: High IPI group includes High and Intermediate-High IPI scores; *: In the Hans column, there are 30/35 cases with the missing 5 consisting of 4 with no available IPI and 1 with no COO (CD10 negative, missing BCL6 and MUM1); **: In the V&Y column, the missing 4 cases are the ones with no IPI data; ***: In the Muris column, there are 30/35 cases with the missing 5 consisting of 4 with no available IPI and 1 with no COO (MUM1 missing).

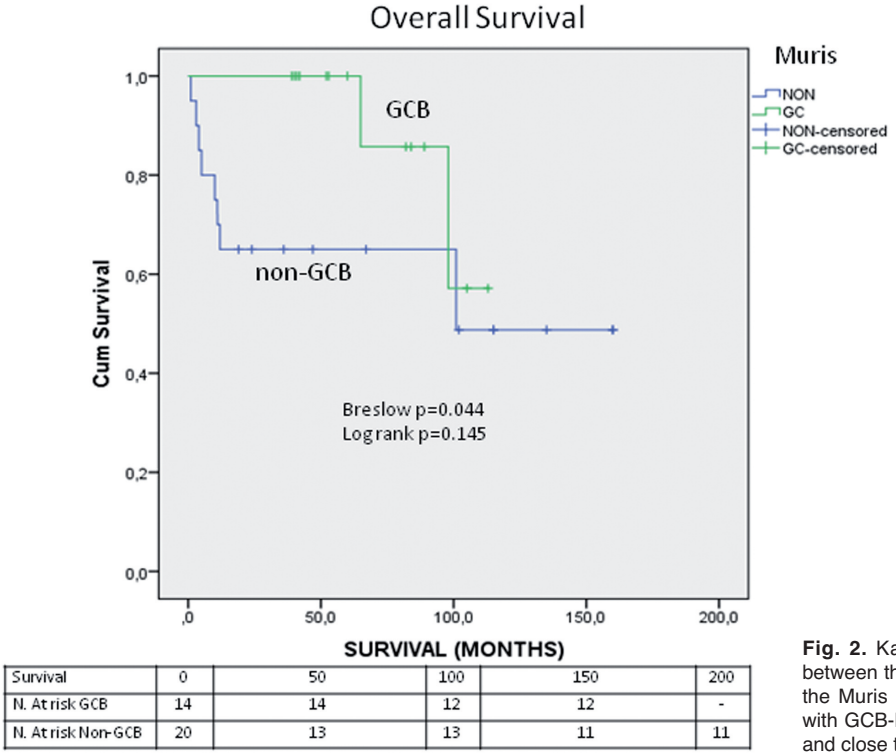


Fig. 2. Kaplan-Meier curves regarding overall survival between the patients with GCB and non-GCB DLBCL. Only the Muris algorithm showed a better outcome for patients with GCB-DLBCL with statistical significance (Breslow test) and close to statistical significance (Log rank test).

immunoreactivity score (IRS) ranging from 0 to 6 (average 1.5). Foxp1 was positive in 60% (21/35) of our cases with an IRS ranging from 0 to 12 (average 3.6). pStat3 was positive in 69% (24/35) of our cases with IRS ranging from 0 to 9 (average 3.1) (Fig. 1). No statistically significant differences in the expression between GCB

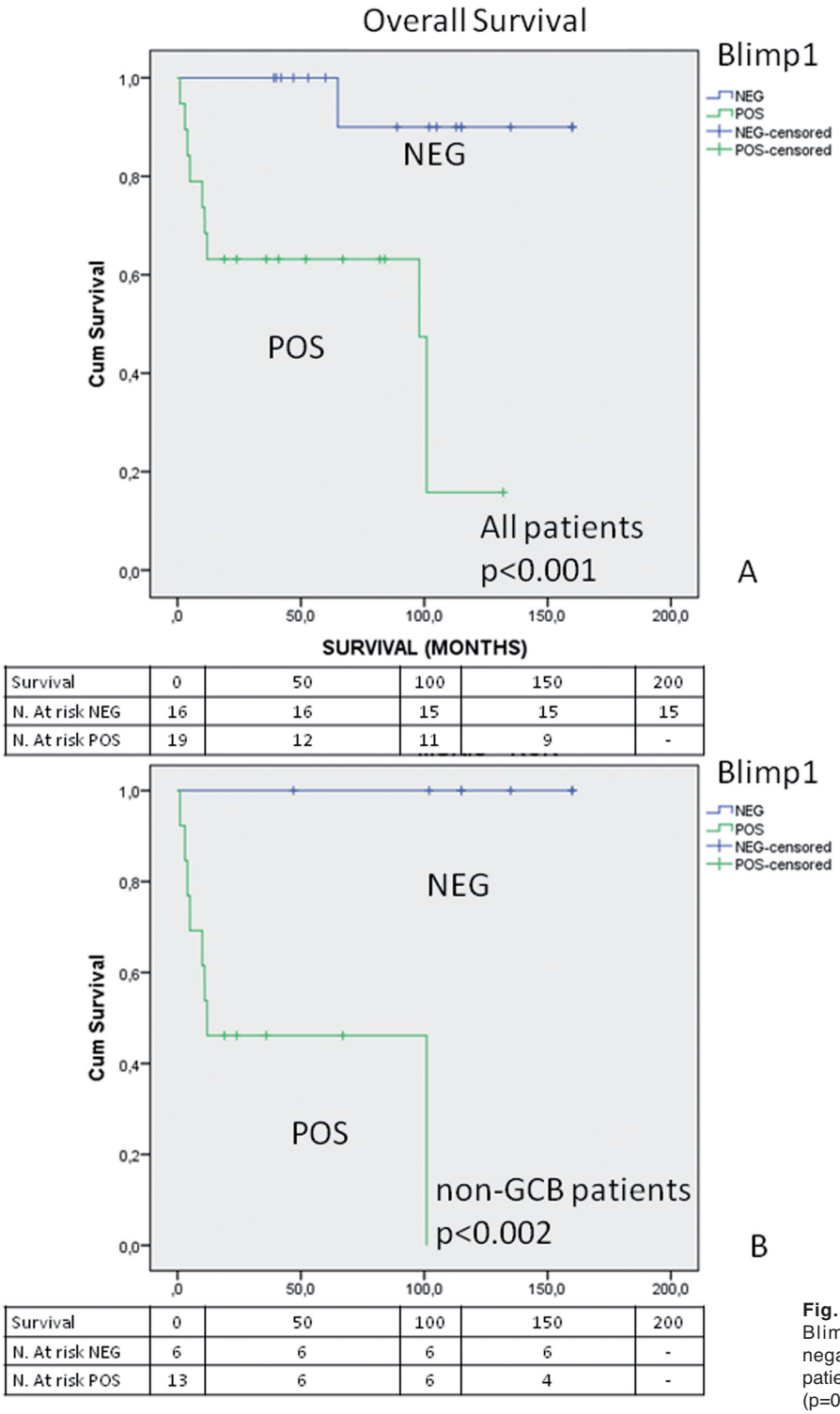
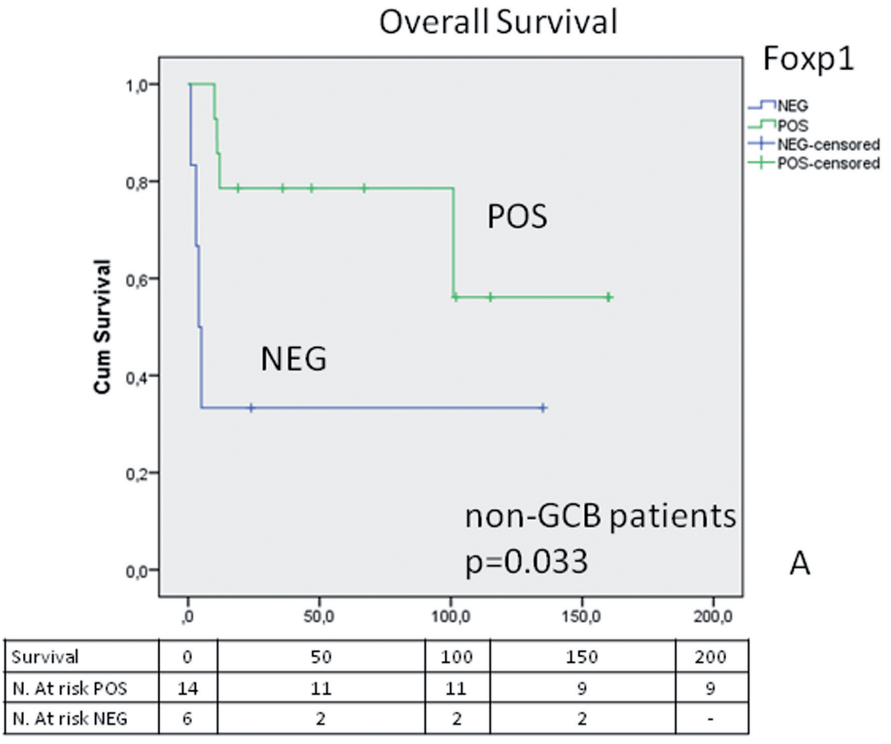


Fig. 3. Kaplan-Meier survival curves showing that Blimp1 expression in extranodal DLBCLs was negatively correlated with overall survival in **(A)** all patients (p=0.001) and **(B)** in the non-GCB subgroup (p=0.002).

and non-GCB tumors or between locations (gastrointestinal, respiratory tract and other) were observed with any of these markers. All our cases of the respiratory tract showed positivity for pStat3 (Table 3).

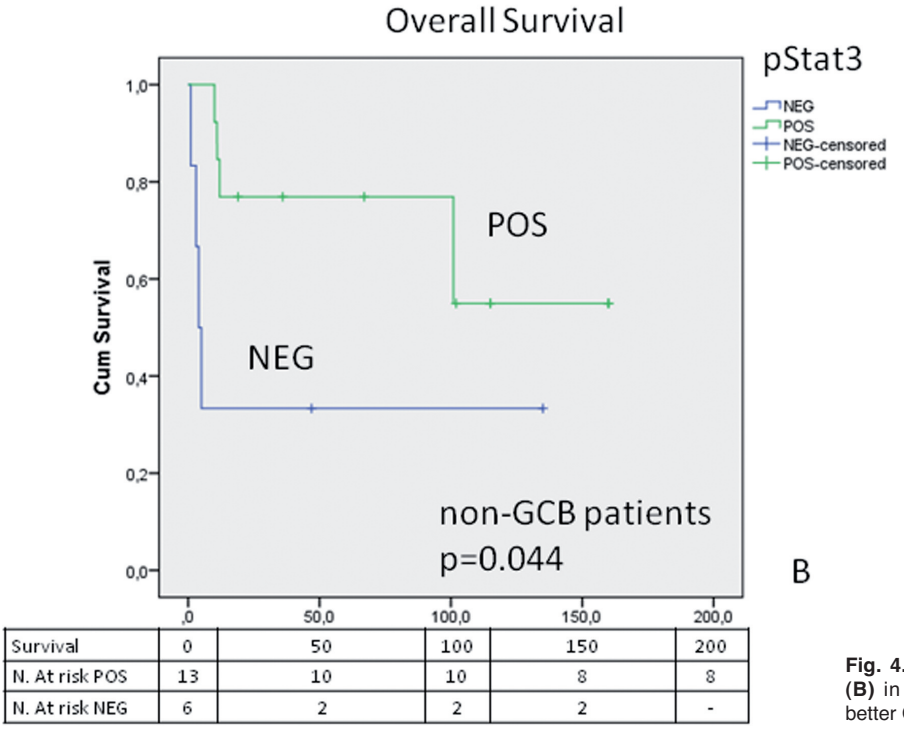
Outcome analysis

Blimp1 expression was negatively correlated with OS (p=0.001) (Fig. 3A). In addition, Blimp1 expression



Survival	0	50	100	150	200
N. At risk POS	14	11	11	9	9
N. At risk NEG	6	2	2	2	-

A



Survival	0	50	100	150	200
N. At risk POS	13	10	10	8	8
N. At risk NEG	6	2	2	2	-

B

Fig. 4. Foxp1 expression **(A)** and pStat3 expression **(B)** in the non-GCB subgroup was correlated with better OS (p=0.033, p=0.044 respectively).

Table 3. Number of positive cases for each marker according to subgroups GCB vs non-GCB and location in the gastrointestinal tract, respiratory tract and other.

Positivity	N	Cell of origin*		Extranodal site		
		GCB (n=14)	Non-GCB (n=20)	GI tract (n=16)	R tract (n=9)	Other (n=10)
Blimp1	19 (55%)	5 (36%)	14 (70%)	8 (42%)	3 (33%)	5 (50%)
Foxp1	21 (60%)	6 (43%)	15 (75%)	7 (33%)	6 (66%)	6 (60%)
pStat3	24 (69%)	10 (71%)	14 (70%)	8 (33%)	9 (100%)	6 (60%)

*: according to Muris algorithm. One case could not be classified. GI: gastrointestinal, R: respiratory.

in the non-GCB (Muris) patients was also negatively associated with OS (p=0.002) (Fig. 3B). The Cox regression analysis showed that Blimp1 expression but not COO was an independent negative prognostic factor for OS (Hazard Ratio=17.5, 95%, CI=2.2-141.1, p=0.007).

Foxp1 positivity was associated with more frequent relapses (p=0.048), but it had no impact on the outcome of the patients in the global cohort. However, Foxp1 positive non-GCB DLBCL had a better outcome than the negative ones (p=0.033) (Fig. 4A).

The level of expression of pStat3 was associated with the risk of relapse (Odds Ratio=10.6, 95%CI=1.2-97.6, p=0.037). There was no statistical difference in OS between pStat3 positive and negative cases in the global series but pStat3 positive cases in the non-GCB subgroup had better OS than the negative patients (p=0.044) (Fig. 4B).

Cox regression analysis, including Blimp1, pStat3 and Foxp1 expression, showed that Blimp1 expression was an independent negative prognostic factor for OS (Hazard Ratio=1.7, 95% CI=1.1-2.7, p=0.022).

Discussion

In this study, we have performed a combined analysis of three lymphoid activation markers in a homogenous cohort of extranodal DLBCLs treated with R-CHOP. These markers were expressed in 50-70% of the tumors with a tendency to be more common in the non-GCB subgroup. The expression of these markers seems to have different impacts on the outcome of the patients.

Blimp-1 or PRDM1 (PR Domain Zinc Finger Protein 1) is a transcriptional repressor that is essential for the terminal differentiation of B-cells into plasma cells (Mandelbaum et al., 2010). The precise mechanism by which Blimp1 contributes to lymphoma development has not yet been fully elucidated. Nevertheless, BLIMP1 is frequently inactivated in a variety of lymphomas, including DLBCLs, Natural Killer cell lymphomas and anaplastic large T-cell lymphoma. *PRDM1/BLIMP1* inactivating mutations have been found exclusively in the non-GCB subgroup and a transcriptional repressor role in a subset of GCB cells. In addition, Blimp1 plays a critical role for most terminal effector cell differentiation in T-cells (Pasqualucci et al., 2006; Mandelbaum et al., 2010; Boi et al., 2013, 2014). In our cases, Blimp1 was expressed more frequently in the non-GCB cases (70%) than in the GCB cases (36%) (Table 3). Few studies have explored the possible relationship of Blimp1 expression with outcome in DLBCL. In nodal tumors, Blimp1 expression has been associated with treatment-resistance (Saez et al., 2009) and shorter failure-free survival (FFS) (Garcia et al., 2006). One study showed that Blimp1 positive gastric DLBCL had shorter OS and FFS (Martin-Arruti et al., 2012). Our study expands these observations showing that Blimp1 expression was significantly correlated with shorter OS in the whole series and also in the unfavorable non-GCB subgroup. Multivariate analysis showed that Blimp1 expression but not COO was an independent negative prognostic factor for OS. Also, we found that double positivity of Blimp1 and MUM1 showed increased death risk and Blimp1 expression retained its negative prognostic impact when coexpressed with BCL2 and even BCL6. Moreover, Blimp1 expression was an independent negative prognostic factor, when compared with Foxp1 and pStat3 expression.

Foxp1 (Forkhead box protein P1) is a winged helix transcription factor of the *FOXP* subfamily. The interesting ability of Foxp1 to repress or activate genes leads to its function as an oncogene in some tumors (e.g. hepatocellular carcinomas) (Zhang et al., 2012) or tumor suppressor in others (e.g. breast carcinomas) (Fox et al., 2004; Koon et al., 2007; Yu et al., 2011; Katoh et al., 2013). It is considered an essential transcriptional factor for B-cell development and critical for plasma cell differentiation, since it represses master plasma cell transcriptional regulators, like *BLIMP1, IRF4* and *XBP1* (Van Keimpema et al., 2015). Some studies have shown that Foxp1 may be useful as a biomarker for molecular subtyping of DLBCL, for evaluation of prognosis, and as a target for new therapeutic strategies (Koon et al., 2007; Wong et al., 2014; Tzankov et al., 2015). However, its particular value in nodal and extranodal lymphomas is still controversial, probably due to the heterogeneity of treatments (pre- and post-rituximab era), cut-off values used in the scoring system, or mixture of nodal and extranodal locations (Barrans et al., 2004; Hans et al., 2004; Banham et al., 2005; Choi et al., 2009; Nyman et al., 2009a,b; Hoeller et al., 2010; Yu et al., 2011; Wong et al., 2014; Tzankov et al 2015). In our cohort of extranodal DLBCLs, Foxp1 was expressed more frequently in the non-GCB cases (75%), as previously described for nodal DLBCLs (Table 3). Also, its coexpression with BCL2 was more frequent in the non-GCB cases (70%), as also shown by Barrans et al (Barrans et al. 2004). Foxp1 expression was correlated with more relapses, but we did not observe any difference in OS in the whole series, although it was associated with a significantly better OS in the non-GCB subgroup, alone and when coexpressed with BCL2.

Since Foxp1 behaves differently in various tumors (Katoh et al., 2013), these results could be explained by the previous suggestion that Foxp1 may use different mechanisms in GCB and non-GCB subgroups (Banham et al., 2005; Hu et al., 2012).

Stat3 is a transcription factor of the Signal Transducers and Activators of Transcription (*STAT*) family. Stat3 is activated by phosphorylation (pStat3) and translocated to the nucleus where it regulates several target genes such as *MYC* (Ding et al., 2008; Ok et al., 2014). The expression of pStat3 has been observed in 30-40% of DLBCLs with a tendency to be more frequently expressed in non-GCB than in GCB subgroups. Some studies have shown no relation with outcome whereas others revealed an association of high pStat3 with poor outcome (Lam et al., 2008; Wu et al., 2011; Gupta et al., 2012; Huang et al., 2013; Ok et al., 2014; Paik et al., 2014; Batlle-Lopez et al., 2016). These conflicting results were observed in cohorts of cases treated both in the pre- and post- immunochemotherapy era. All these studies have been performed on nodal DLBCLs or in series in which the topographic location of the tumor has not been specified. pStat3 has not been studied in extranodal DLBCLs. In our study, we observed pStat3 expression in 70% of the cases with a similar distribution in GCB and non-GCB subgroups. Although we did not observe a relationship with outcome in the whole series, high pStat3 expression was associated with better OS in the non-GCB subgroup.

In this study we observed a high number of pStat3 positive extranodal DLBCLs (70%) (reaching 100% in the respiratory tract) compared with the lower number of positive cases previously described in nodal cases (30-40%) (Lam et al., 2008; Wu et al., 2011; Gupta et al., 2012; Huang et al., 2013; Ok et al., 2014; Paik et al., 2014; Batlle-Lopez et al., 2016). This observation is intriguing and may be reminiscent of the constitutive activation of the *JAK/STAT* pathway in primary mediastinal large B-cell lymphoma, also an extranodal large cell lymphoma with relatively better outcome than conventional nodal DLBCLs (Hao et al., 2014). These observations suggest that activation of *JAK/STAT* pathway may play an important role in extranodal lymphomas.

In summary, we studied the expression of three activation markers Blimp1, Foxp1 and pStat3 in a cohort of pure extranodal DLBCLs treated with R-CHOP. Our results indicate that these markers are differentially expressed and had different impacts on outcome in extranodal DLBCLs compared to nodal tumors. These results emphasize the need to evaluate separately the clinical and biological significance of these, and probably other, markers in nodal and extranodal DLBCL. As previously suggested, the same markers may have different functions in different subsets of tumors. Since there are limitations in our study regarding the number of patients, further expanded series are needed to verify our findings. The use of these markers in clinical practice may require a better understanding of their respective role in specific subset of tumors.

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Authors' Contribution. All authors revised and approved the final manuscript. GP designed the study, collected the data, participated in the diagnosis of part of the cases, reviewed all slides, participated in the immunohistochemical scoring of all cases, interpreted the data and wrote the manuscript. IK diagnosed most of the cases, participated in the immunohistochemical scoring of his cases, interpreted the data, supervised the project, provided critical suggestions, evaluated and edited the manuscript. IV supplied part of the material, diagnosed part of the cases, participated in the immunohistochemical scoring of his cases, provided critical suggestions and reviewed the manuscript. ML supervised the study and critically reviewed the manuscript. KV analyzed statistically the results. SV supplied part of the clinical data and critically reviewed the manuscript. EM supplied part of the clinical data and critically reviewed the manuscript. CT supplied most part of the clinical data, provided critical suggestions, evaluated and edited the manuscript. NP assigned, supervised and financed the study, critically reviewed, evaluated and edited the manuscript.

All authors declare no conflict of interest.

References

Banham A.H., Connors J.M., Brown P.J., Cordell J.L., Ott G., Sreenivasan G., Farinha P., Horsman D.E. and Gascoyne R.D. (2005). Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma. Clin. Cancer Res. 11, 1065-1072.

Barrans S.L., Fenton J.A., Banham A., Owen R.G. and Jack A.S. (2004). Strong expression of FOXP1 identifies a distinct subset of diffuse large B-cell lymphoma (DLBCL) patients with poor outcome. Blood 104, 2933-2935.

Batlle-López A., González de Villambrosía S., Mazorra F., Malatxeberria S., Sáez A., Montalban C., Sánchez L., Garcia J.F., González-Barca E., López A., Ruiz-Marcellan M.C., Mollejo M., Grande C., Richards K.L., Hsi E.D., Tzankov A., Visco C., Xu-Monette Z.Y., Cao X., Young K.H., Angel Piris M., Conde E. and Montes-Moreno S. (2016). Stratifying diffuse large B-cell lymphoma patients treated with chemoimmunotherapy: GCB/non-GCB by immunohistochemistry is still a robust and feasible marker. Oncotarget. 7, 18036-18049.

Boi M., Rinaldi A., Kwee I., Bonetti P., Todaro M., Tabbò F., Piva R., Rancoita P.M., Matolcsy A., Timar B., Tousseyn T., Rodríguez-Pinilla S.M., Piris M.A., Beà S., Campo E., Bhagat G., Swerdlow S.H., Rosenwald A., Ponzoni M., Young K.H., Piccaluga P.P., Dummer R., Pileri S., Zucca E., Inghirami G. and Bertoni F. (2013). PRDM1/BLIMP1 is commonly inactivated in anaplastic large T-cell lymphoma. Blood 122, 2683-2693.

Boi M., Zucca E., Inghirami G., Bertoni F. (2014). PRDM1/BLIMP1: a tumor suppressor gene in B and T cell lymphomas. Leuk. Lymphoma 56, 1-12.

Choi W.W., Weisenburger D.D., Greiner T.C., Piris M.A., Banham A.H., Delabie J., Braziel R.M., Geng H., Iqbal J., Lenz G., Vose J.M., Hans C.P., Fu K., Smith L.M., Li M., Liu Z., Gascoyne R.D., Rosenwald A., Ott G., Rimsza L.M., Campo E., Jaffe E.S., Jaye D.L., Staudt L.M. and Chan W.C. (2009). A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin. Cancer Res. 15, 5494-5502.

Culpin R.E., Sieniawski M., Angus B., Menon G.K., Proctor S.J., Milne P., McCabe K. and Mainou-Fowler T. (2013). Prognostic significance of immunohistochemistry-based markers and algorithms in immunochemotherapy-treated diffuse large B cell lymphoma patients. Histopathology 63, 788-801.

Ding B.B., Yu J.J., Yu R.Y., Mendez L.M., Shaknovich R., Zhang Y., Cattoretti G. and Ye B.H. (2008). Constitutively activated STAT3 promotes cell proliferation and survival in the activated B-cell subtype of diffuse large B-cell lymphomas. Blood 111, 1515-1523.

Fox S.B., Brown P., Han C., Ashe S., Leek R.D., Harris A.L. and Banham A.H. (2004). Expression of the forkhead transcription factor FOXP1 is associated with estrogen receptor alpha and improved survival in primary human breast carcinomas. Clin. Cancer Res. 10, 3521-3527.

Fu K., Weisenburger D.D., Choi W.W.L., Perry K.D., Smith L.M., Shi X., Hans C.P., Greiner T.C., Bierman P.J., Bociek R.G., Armitage J.O., Chan W.C. and Vose J.M. (2008). Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell like subtypes of diffuse large B-cell lymphoma. J. Clin. Oncol. 26, 4587-4594.

Garcia J.F., Roncador G., García J.F., Sánz A.I., Maestre L., Lucas E., Montes-Moreno S., Fernandez Victoria R., Martinez-Torrecuadrara J.L., Marafioti T., Mason D.Y. and Piris M.A. (2006). PRDM1/BLIMP-1 expression in multiple B and T-cell lymphoma. Haematologica 91, 467-474.

Gupta M., Maurer M.J., Wellik L.E., Law M.E., Han J.J., Ozsan N., Micallef I.N., Dogan A. and Witzig T.E. (2012). Expression of Myc, but not pSTAT3, is an adverse prognostic factor for diffuse large B-cell Lymphoma treated with Epratuzumab/R-CHOP. Blood 120, 4400-4406.

Gutiérrez-García G., Cardesa-Salzmann T., Climent F., González-Barca E., Mercadal S., Mate J.L., Sancho J.M., Arenillas L., Serrano S., Escoda L., Martínez S., Valera A., Martínez A., Jares P., Pinyol M., García-Herrera A., Martínez-Trillos A., Giné E., Villamor N., Campo E., Colomo L., López-Guillermo A. and Grup per l'Estudi dels Limfomes de Catalunya I Balears (GELCAB) (2011). Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. Blood 117, 4836-4843.

Hans C.P., Weisenburger D.D., Greiner T.C., Gascoyne R.D., Delabie J., Ott G., Müller-Hermelink H.K., Campo E., Braziel R.M., Jaffe E.S., Pan Z., Farinha P., Smith L.M., Falini B., Banham A.H., Rosenwald A., Staudt L.M., Connors J.M., Armitage J.O. and Chan W.C. (2004). Confirmation of the Molecular classification of diffuse large B cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103, 275-282.

Hao Y., Chapuy B., Monti S., Sun H.H., Rodig S.J. and Shipp M.A. (2014). Selective JAK2 inhibition specifically decreases Hodgkin lymphoma and mediastinal large B-cell lymphoma growth in vitro and in vivo. Clin. Cancer Res. 20, 2674-2683.

Hoeller S., Schneider A., Haralambieva E., Dirnhofer S. and Tzankov A. (2010). FOXP1 protein overexpression is associated with inferior outcome in nodal diffuse large B-cell lymphomas with nongerminal centre phenotype, independent of gains and structural aberrations at 3p14.1. Histopathology 57, 73-80.

Huang X., Meng B., Iqbal J., Ding B.B., Perry A.M., Cao W., Smith L.M., Bi C., Jiang C., Greiner T.C., Weisenburger D.D., Rimsza L., Rosenwald A., Ott G., Delabie J., Campo E., Braziel R.M., Gascoyne R.D., Cook J.R., Tubbs R.R., Jaffe E.S., Armitage J.O., Vose J.M., Staudt L.M., McKeithan T.W., Chan W.C., Ye B.H. and Fu K. (2013). Activation of the STAT3 signaling pathway is associated with poor survival in diffuse large B-cell lymphoma treated with R-CHOP. J. Clin. Oncol. 31, 4520-4528.

Hu C.R., Wang J.H., Wang R., Sun Q. and Chen L.B. (2012). Both FOXP1 and p65 expression are adverse risk factors in diffuse large B-cell lymphoma: a retrospective study in China. Acta Histochem. 115, 137-143.

Jaffe E.S., Harris N.L., Vardiman J., Campo E. and Arber D. (2011). Diagnosis of Lymphoma in Extranodal Sites Other than Skin, In: Hematopathology, Elsevier Saunders, pp. 991-1020.

Katoh M., Igarashi M., Fukuda H., Nakagama H. and Katoh M. (2013). Cancer genetics and genomics of human FOX family genes. Cancer Lett. 328, 198-206.

Kim M.K., Bae S.H., Bae Y.K., Kum Y.S., Ryoo H.M., Cho H.S., Lee K.H., Koh S.A., Lee H.Y., Yun S.Y., Choi J.H., and Hyun M.S. (2011). Biological characterization of nodal versus extranodal presentation of diffuse large B-Cell lymphoma using immunohistochemistry. Clin Lymphoma Myeloma Leuk. 11, 403-408.

Koon H.B., Ippolito G.C., Banham A.H and Tucker P.W (2007). FOXP1: a potential therapeutic target in cancer, Expert Opin. Ther. Targets 11, 955-965.

Lam L.T., Wright G., Davis R.E., Lenz G., Farinha P., Dang L., Chan J.W., Rosenwald A., Gascoyne R.D. and Staudt L.M. (2008). Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-{kappa}B pathways in subtypes of diffuse large B-cell lymphoma. Blood 111, 3701-3713.

Lin L., Min M., Bi C.F., Wang X.Q., Luo T.Y., Zhao S., Zhang W.Y. and Liu W.P. (2011). Primary gastrointestinal diffuse large B-cell lymphoma: an immunohistochemical and prognostic study of 90 cases. Zhonghua Bing Li Xue Za Zhi 40, 220-226.

Lu J.B., Li X.Q., Zhang P.H., Zhou X.Y., Zhang T.M., Li X.M. and Zhu X.Z. (2007). Nodal versus extranodal diffuse large B-cell lymphoma: comparison of clinicopathologic features, immunophenotype and prognosis. Zhonghua Bing Li Xue Za Zhi 36, 470-473.

Mandelbaum J., Bhagat G., Tang H., Mo T., Brahmachary M., Shen Q., Chadburn A., Rajewsky K., Tarakhovsky A., Pasqualucci L. and Dalla-Favera R. (2010). BLIMP1 is a tumor suppressor gene frequently disrupted in activated B cell-like diffuse large B cell lymphoma. Cancer Cell 18, 568-579.

Martin-Arruti M., Vaquero M., Díaz de Otazu R., Zabalza I., Ballesteros J., Roncador G. and García-Orad A. (2012). Bcl-2 and BLIMP-1 expression predict worse prognosis in gastric diffuse large B cell lymphoma (DLCBL) while other markers for nodal DLBCL are not useful. Histopathology 60, 785-792.

Meyer P.N., Fu K., Greiner T.C., Smith L.M., Delabie J., Gascoyne R.D., Ott G., Rosenwald A., Braziel R.M., Campo E., Vose J.M., Lenz G., Staudt L.M., Chan W.C. and Weisenburger D.D. (2011). Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J. Clin. Oncol. 29, 200-207.

Muris J.J., Meijer C.J., Vos W., van Krieken J.H., Jiwa N.M.,

Ossenkoppele G.J. and Oudejans J.J. (2006). Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. J. Pathol. 208,714-723.

Nagata Y., Lan K.H., Zhou X., Tan M., Esteva F.J., Sahin A.A., Klos K.S., Li P., Monia B.P., Nguyen N.T., Hortobagyi G.N., Hung M.C. and Yu D. (2004). PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6, 117-127.

Nyman H., Jerkeman M., Karjalainen-Lindsberg M.L., Banham A.H. and Leppa S. (2009a). Prognostic impact of activated B-cell focused classification in diffuse large B-cell lymphoma patients treated with R-CHOP. Mod. Pathol. 22, 1094-1101.

Nyman H., Jerkeman M., Karjalainen-Lindsberg M.L., Banham A.H., Enblad G. and Leppä S. (2009b). Bcl-2 but not FOXP1, is an adverse risk factor in immunochemotherapy-treated non-germinal center diffuse large B-cell lymphomas. Eur. J. Haematol. 82, 364-372.

Ok C.Y., Chen J., Xu-Monette Z.Y., Tzankov A., Manyam G.C., Li L., Visco C., Montes-Moreno S., Dybkær K., Chiu A., Orazi A., Zu Y., Bhagat G., Richards K.L., Hsi E.D., Choi W.W., van Krieken J.H., Huh J., Zhao X., Ponzoni M., Ferreri A.J., Bertoni F., Farnen J.P., Møller M.B., Piris M.A., Winter J.N., Medeiros L.J. and Young K.H. (2014). Clinical implications of phosphorylated STAT3 expression in De Novo diffuse large B-cell lymphoma. Clin. Cancer Res. 20, 5113-5123.

O'Shea J.J., Holland S.M. and Staudt L.M. (2013). JAKs and STATs in immunity, immunodeficiency and cancer. N. Engl. J. Med. 368, 161-170.

Ott G., Ziepert M., Klapper W., Horn H., Szczepanowski M., Bernd H.W., Thorns C., Feller A.C., Lenze D., Hummel M., Stein H., Müller-Hermelink H.K., Frank M., Hansmann M.L., Barth T.F., Möller P., Cogliatti S., Pfreundschuh M., Schmitz N., Trümper L., Loeffler M. and Rosenwald A. (2010). Immunoblastic morphology but not the immunohistochemical gcb/nongcb classifier predicts outcome in diffuse large B-cell lymphoma in the ricover-60 trial of the DSHNHL. Blood 116, 4916-4925.

Paik J.H., Nam S.J., Kim T.M., Heo D.S., Kim C.W. and Jeon Y.K. (2014). Overexpression of sphingosine-1-phosphate receptor 1 and phospho-signal transducer and activator of transcription 3 is associated with poor prognosis in rituximab-treated diffuse large B-cell lymphomas. BMC Cancer 14, 911.

Pasqualucci L., Compagno M., Houldsworth J., Monti S., Grunn A., Nandula S.V., Aster J.C., Murty V.V., Shipp M.A. and Dalla-Favera R. (2006). Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. J. Exp. Med. 203, 311-317.

Rosenwald A., Wright G., Chan W.C., Connors J.M., Campo E., Fisher R.I., Gascoyne R.D., Muller-Hermelink H.K., Smeland E.B., Giltnane J.M., Hurt E.M., Zhao H., Averett L., Yang L., Wilson W.H., Jaffe E.S., Simon R., Klausner R.D., Powell J., Duffey P.L., Longo D.L., Greiner T.C., Weisenburger D.D., Sanger W.G., Dave B.J., Lynch J.C., Vose J., Armitage J.O., Montserrat E., López-Guillermo A., Grogan T.M., Miller T.P., LeBlanc M., Ott G., Kvaloy S., Delabie J., Holte H., Krajci P., Stokke T., Staudt L.M and Lymphoma/Leukemia Molecular Profiling Project. (2002). The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N. Engl. J. Med. 346, 1937-1947.

Saez A.I., García-Cosío M., Sáez A.J., Hernández J.M., Sánchez-Verde L., Alvarez D., de la Cueva P., Arranz R., Conde E., Grande C., Rodríguez J., Caballero D. and Piris M.A. (2009). Identification of biological markers of sensitivity to high-clinical-risk-adapted therapy for patients with diffuse large B-cell lymphoma. Leuk. Lymphoma 50, 571-581.

Sweetenham J.W. (2011). Molecular signatures in the diagnosis and management of diffuse large B-cell lymphoma. Curr. Opin. Hematol. 18, 288-292.

Tzankov A., Leu N., Muenst S., Juskevicius D., Klingbiel D., Mamot C. and Dirnhofer S. (2015). Multiparameter analysis of homogeneously R-CHOP-treated diffuse large B cell lymphomas identifies CD5 and FOXP1 as relevant prognostic biomarkers: report of the prospective SAKK 38/07 study. J. Hematol. Oncol. 14, 70.

Van Keimpema M., Grüneberg L.J., Mokry M., van Boxtel R., van Zelm M.C., Coffer P., Pals S.T. and Spaargaren M. (2015). The forkhead transcription factor FOXP1 represses human plasma cell differentiation. Blood 126, 2098-2109.

Visco C., Li Y., Xu-Monette Z.Y., Miranda R.N., Green T.M., Li Y., Tzankov A., Wen W., Liu W.M., Kahl B.S., d'Amore E.S., Montes-Moreno S., Dybkær K., Chiu A., Tam W., Orazi A., Zu Y., Bhagat G., Winter J.N., Wang H.Y., O'Neill S., Dunphy C.H., Hsi E.D., Zhao X.F., Go R.S., Choi W.W., Zhou F., Czader M., Tong J., Zhao X., van Krieken J.H., Huang Q., Ai W., Etzell J., Ponzoni M., Ferreri A.J., Piris M.A., Møller M.B., Bueso-Ramos C.E., Medeiros L.J., Wu L. and Young K.H. (2012). Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Leukemia 26, 2103-2113.

Wong K.K., Gascoyne D.M., Brown P.J., Soilleux E.J., Snell C., Chen H., Lyne L., Lawrie C.H., Gascoyne R.D., Pedersen L.M., Møller M.B., Pulford K., Murphy D., Green T.M. and Banham A.H. (2014). Reciprocal expression of the endocytic protein HIP1R and its repressor FOXP1 predicts outcome in R-CHOP-treated diffuse large B-cell lymphoma patients. Leukemia 28, 362-372.

Wu Z.L., Song Y.Q., Shi Y.F. and Zhu J. (2011). High nuclear expression of STAT3 is associated with unfavorable prognosis in diffuse large B-cell lymphoma. J. Hematol. Oncol. 4, 31.

Yu B., Zhou X., Li B., Xiao X., Yan S. and Shi D. (2011). FOXP1 expression and its clinicopathologic significance in nodal and extranodal diffuse large B-cell lymphoma. Ann. Hematol. 90, 701-708.

Zhang Y., Zhang S., Wang X., Liu J., Yang L., He S., Chen L. and Huang J. (2012). Prognostic significance of FOXP1 as an oncogene in hepatocellular carcinoma. J. Clin. Pathol. 65, 528-533.

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