MYD88 somatic mutation is a diagnostic criterion in primary cutaneous large B-cell lymphoma

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Short title: *MYD88* mutation in Cutaneous Large B-cell Lymphoma

Abbreviations:
ABC: activated B-cell
DLBCL: diffuse large B-cell lymphoma
GC: germinal center
NF-κB: nuclear factor-kappa B
PCBCL: primary cutaneous B-cell lymphoma
PCFCL: primary cutaneous follicle center lymphoma
PCLBCL-LT: primary cutaneous large B-cell lymphoma, leg-type
PCMZL: primary cutaneous marginal zone lymphoma

To the editor,

Primary cutaneous B-cell lymphoma (PCBCL) have been divided into the 3 following groups: primary cutaneous marginal zone lymphoma, primary cutaneous follicle center lymphoma (PCFCL) and primary cutaneous diffuse large B-cell lymphoma leg-type (PCDLBCL-LT) (Willemze et al., 2005). While the latter category displays an aggressive behaviour requiring a combination of rituximab and polychemotherapy (Grange et al., 2014), first line therapy of the two former categories which have an indolent course consists in local therapy especially in patients with solitary or localized lesions (Senff et al., 2008). However, patients with PCFCL presenting on the leg have a worse prognosis and may require a systemic therapy (Senff et al., 2007). Moreover, differential diagnosis between PCDLBCL-LT and PCFCL may be challenging especially when PCFCL exhibit large cell morphology. Hierarchical determination of CD10, BCL6 and MUM1 expression according to the Hans' algorithm (Hans et al., 2004) is not a reliable tool for cutaneous B-cell lymphoma diagnosis as CD10 expression may be lost in PCFCL and BCL6 may be expressed as frequently in PCDLBCL-LT than in PCFCL (Dewar et al., 2015). Moreover, such algorithm does not include BCL2 immunostaining which is a major diagnostic and prognostic factor for primary cutaneous large B-cell lymphoma (PCLBCL) (Grange et al., 2004).

MYD88, a gene encoding for an adaptor protein of both Toll-like receptors and Interleukin-1 receptors was recently shown to exhibit driver oncogenic mutations in nodal activated B-cell diffuse large cell lymphoma (ABC DLBCL) resulting in NF-kB signaling pathway activation (Ngo et al., 2010). We previously reported the high prevalence of *MYD88*^{L265P} mutation in above 60% of the 58 PCDLBCL-LT studied

cases using a robust and highly sensitive allele specific PCR technique (Pham-Ledard et al., 2014), while others described a lower rate at 40% in ten patients (Koens et al., 2014). Whether such assessment could discriminate PCFCL with large cell morphology from PCDLBCL-LT has not been evaluated. Indeed, only 3 PCFCL with large cell morphology cases were included in our initial study and exhibited a wild type *MYD88* status using a Sanger sequencing technique (Pham-Ledard et al., 2012).

Therefore, we retrieved 21 typical PCFCL cases with large cell morphology (samples with at least 80% large tumor cells) from the French cutaneous lymphoma study group database collected from 2005 to 2015. These cases were compared to 25 cases of PCDLBCL-LT recorded from 2004 to 2015 including 4 cases already reported (Pham-Ledard et al., 2014). DNA was extracted from formalin-fixed paraffin-embedded material and analyzed by real-time PCR analysis with Taqman allele specific probes as reported (Pham-Ledard et al., 2014).

All patients had primary cutaneous lymphoma and per definition initial staging including CT-scan was negative. Both clinical and histopathological features are summarized in Table 1. The gender-ratio (male/female) was 2.5 for PCFCL, large cell and 0.4 for PCDLBCL-LT. Patients with PCDLBCL-LT were older than patients with PCFCL, large cell. Localization on the lower limb was found in 80% of PCDLBCL-LT and 9.5% of PCFCL cases. While BCL2 and MUM1 expression by more than 50% of tumor cells was a recurrent feature of PCLBCL-LT (100%), PCFCL, large cell type cases were typically considered negative for BCL2 (81%) and MUM1 (100%) when using a cut-off criteria of 50% for BCL2 and 80% for MUM1. However, such phenotypic criteria are not always reached and a partial overlap of individual marker expression may be observed between PCFCL with a diffuse growth pattern

presenting on the leg and PCDLBCL-LT (Kempf et al., 2014). Indeed, MUM1 expression was observed in less than 80% of tumor cells but above 50% in five cases of PCLBCL-LT (20%). In addition, partial MUM1 expression ranging from 10% to 40% of tumor cells was observed in seven PCFCL, large cell type cases (33%), as reported by others (Hoefnagel et al., 2006). Finally, four PCFCL, large cell type samples (19%) exhibited a strong BCL2 immunostaining in more than 50% of tumor cells, only one of them occurring at the leg site. These four cases were however all MUM1 negative. A *MYD88*^{L265P} mutation was detected in 19 out of 25 (76%) PCDLBCL-LT cases with a slight difference between those developing at the leg site (16 out of 20, 80%) and those developing at other sites (3 out of 5, 60%) (Table 1). Interestingly, the *MYD88*^{L265P} mutation was never detected in the 21 PCFCL cases. Specificity and Positive Predictive Value for *MYD88* status were determined both at 100% while Negative Predictive Value was at 78 %.

This study not only confirms the original and high prevalence of the *MYD88*^{L265P} mutation in PCLBCL-LT but also underscores that such determination may be relevant to differentiate this entity from PCFCL cases with large cell morphology. According to prognosis difference between the two diseases and international guidelines (Senff et al., 2007; Senff et al., 2008), the two groups of patients were treated differently hampering evaluation of the impact of *MYD88* status on prognosis. We already reported that *MYD88*^{L265P} mutation is an independent bad prognosis factor in patients with PCLBCL-LT whatever the treatment they have received (Pham-Ledard et al., 2014). Such feature was also recently reported in patients with nodal or systemic DLBCL carrying the *MYD88*^{L265P} mutation (Rovira et al., 2016). Despite improvement in the prognosis of patients with PCLBCL-LT since the introduction of rituximab in front-line therapy in association with polychemotherapy (Grange et al.,

2014), a significant proportion of these patients will relapse and often disseminate to extra-cutaneous sites. Therefore determination of *MYD88* status along with other mutations could also be relevant to identify patients which could respond to specific therapy targeting either the BCR, JAK/STAT or NF-kB signaling pathways (Wilson et al., 2015). Altogether, the present study supports that *MYD88* status is a necessary biomarker in patients with primary cutaneous large B-cell lymphomas both for diagnosis, prognosis and management purpose.

Table 1:

	PCFCL-LC	PCLBCL-LT
Patients	21	25
Age, median, y	62.3	83.3
Male: n (%)	15 (71.4)	7 (28)
Female: n (%)	6 (28.6)	18 (72)
Location: n (%)		
Leg: n (%)	2 (9.5)	20 (80)
Elsewhere n (%)	19 (90.5)	5 (20)
Phenotype: n (%)		
BCL2 expression by >50% cells	4 (19)	25 (100)
MUM1 expression by>80% cells	0	20 (80)
***MYD88* status: n (%)**		
MYD88 L265P mutation	0	19 (76)
MYD88 $^{\text{wild type}}$	21(100)	6 (24)

PCFCL-LC: primary cutaneous follicle center lymphoma with large cell morphology

PCLBCL-LT: primary cutaneous large B-cell lymphoma, leg-type

Table 1. Clinical findings at diagnosis, immunohistochemistry data concerning BCL2 and MUM1 and results of MYD88 mutation status in two groups: PCFCL with large cell morphology and PCLBCL-LT

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Legends

Figure 1. Two examples of primary cutaneous large B cell lymphoma subtypes included in this study:

Left panel (a, b, c and d), primary cutaneous follicle center lymphoma with large cell morphology: (a) Diffuse infiltration of the dermis by lymphomatous cells, Hematoxylin, eosin and safran (HE&S) x100. (b) large tumor cells with centroblastic morphology and small nucleoli, HE&S x400. (c) Negativity of BCL2 immunostaining x400. (d) Negativity of MUM1 immunostaining x400.

Right panel (e, f, g and h), primary cutaneous large B-cell lymphoma, leg-type: (e) Diffuse infiltration of the dermis by lymphomatous cells HE&S x100. (f) Large tumor cells with immunoblastic morphology and prominent nucleolus, HE&S x400. (g) Strong BCL2 immunostaining x400 (h). Positivity of MUM1 immunostaining x400.

Scale bar for a and e = 0.5 mm. Scale bar for b, c, d, f, g and h = 0.05 mm.

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