Novel Mutations Involving NF-κB and B-Cell Signaling Pathways in Primary Cutaneous Large B-Cell Lymphoma, Leg-Type and Comparison with Sézary Syndrome

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Multiple genomic mutations, especially those involving the NF-κB pathway, have been characterized in primary cutaneous large B-cell lymphoma, leg type. However, its genomic profiling remains limited given its rarity. In a recent study, Mareschal et al. performed next-generation sequencing and whole-exome sequencing, identifying new driver genes while also confirming the role of myeloid differentiation primary response gene 88 in the molecular pathogenesis of the disease.

Primary cutaneous large B-cell lymphoma, leg-type (PCLBCL-LT) is the most aggressive form of primary cutaneous B-cell lymphoma. It is characterized by progressive nodules and tumors initially localized to one anatomic region, most commonly the legs (Lima, 2015). Extracutaneous dissemination is also very common and usually involves spread to regional lymph nodes (Lima, 2015). Although Grange et al. showed a 5-year survival rate as high as 75% with rituximab plus polychemotherapy, the current 5-year survival rate reported by other groups remains less than 60% (Grange et al., 2014; Nicolay and Wobser, 2016). Patients’ old age and associated comorbidities often limit the effectiveness of immunchemotherapy. Therefore, it is imperative to characterize the specific chromosomal abnormalities and driver mutations of PCLBCL-LT to understand its molecular pathogenesis and identify potential therapeutic targets. A recent study by Mareschal et al. (2017) further explores PCLBCL-LT at the genomic level through whole exome sequencing and provides both confirmation of previous findings and novel genomic markers that are potential therapeutic targets.

Diagnosis of PCLBCL-LT
PCLBCL-LT is now a new distinct entity under cutaneous B-cell lymphoma along with primary cutaneous marginal zone lymphoma and primary cutaneous follicle center lymphoma. It occurs at an average age of 76 and mostly in elderly women (Grange et al., 2014). The clinical manifestations vary from small, skin-colored cutaneous nodules to deep violaceous tumors, localized to one or both legs in 90% of cases (Nicolay and Wobser, 2016). Histologically, PCLBCL-LT shows diffuse and dense infiltrates of centroblasts and immunoblasts that usually spare the epidermis but extend into the dermis and subcutaneous tissue (Wilcox, 2016). Moreover, MIB-1 staining shows high proliferative activity with more than 80% of infiltrating cells being malignant (Nicolay and Wobser, 2016). Immunohistochemically, primary diffuse large B-cell lymphoma, leg-type is characterized by the following phenotypes: (i) expression of CD20 and CD79a, which are B-cell surface proteins; (ii) lack of CD5 and CD10, which differentiates it from secondary leukemic infiltrates; (iii) negativity for Epstein-Barr virus, which differentiates it from Epstein-Barr virus-associated primary diffuse large B-cell lymphoma; (iv) strong expression of Bcl-2, MUM-1/IRF, and FOX-P1, which differentiates it from primary cutaneous follicle center lymphoma, and (v) uniform expression of the IgM heavy chain (Nicolay and Wobser, 2016) (Figure 1). Further workup, including laboratory tests, imaging studies, and bone marrow biopsy, should be conducted to rule out other entities.

New molecular markers and confirmation of myeloid differentiation primary response gene 88 (MYD88) mutations
In this study, Mareschal et al. analyzed 20 PCLBCL-LT cases with next-generation sequencing using a lymphopanel specifically designed for DLBCL. They were also the first to perform whole exome sequencing for this disease, analyzing 12 pairs of tumor versus control DNA samples (peripheral blood mononuclear cells) and resequencing three new genes not previously studied in PCLBCL-LT: transducin beta like 1 X-linked receptor 1 (TBL1XR1), kelch like family member 6, and IKAROS family zinc finger 3. TBL1XR1 is one of the new genes Mareschal et al. found to be commonly mutated in PCLBCL-LT. In their study, 33% of cases displayed nonsynonymous single-nucleotide variants in exons encoding tryptophan-aspartic acid dipeptide (WD)6 and WD7 domains. This finding is interesting because TBL1XR1 has also been found to be a commonly mutated gene in primary central nervous system lymphoma (19%) and primary cutaneous marginal zone lymphoma (18%), and it has also been reported in activated B-cell-type DBLCL and Sézary syndrome (Jung et al., 2017). Mutations in TBL1XR1 facilitate its binding to nuclear receptor corepressor, which increases the clearance of nuclear receptor corepressor from the toll-like receptor/MYD88 target genes, which in turn activates the NF-κB and c-Jun pathways.
pathways (Jung et al., 2017). Specifically, Jung et al. recently demonstrated that mutations in WD40 domains influence binding of TBL1XR1 to nuclear receptor corepressor, and most TBL1XR1 mutations in various cancers have been detected in WD40. However, Mareschal et al. show that TBL1XR1 mutations in PCLBCL-LT mostly occur in WD6 and WD7 domains. Because Mareschal et al. emphasize the role of TBL1XR1 and its possible synergy with MYD88 mutations, TBL1XR1 mutations concentrated in different WD domains represent a topic of future investigation.

Other notable findings by Mareschal et al. include confirmation of the MYD88 mutation of the L265P variant. Their current study demonstrated a 78% mutation rate, which is comparable to a 69% mutation rate in their previous study and a 75% mutation rate in other groups’ studies (Pham-Ledard et al., 2012; Wilcox, 2016). This unusually high mutation rate is clinically significant, especially because their group also previously showed that the MYD88 mutations are directly associated with a lower disease-specific survival rate (Pham-Ledard et al., 2014).

Mareschal et al. compare their findings of molecular markers with those of different cutaneous B-cell lymphomas, namely activated B-cell DBLCL, primary central nervous system lymphoma, germinal center B-cell like DBLCL, and primary mediastinal large B-cell lymphoma. These comparisons show that PCLBCL-LT actually shares many characteristics with primary central nervous system lymphoma, including a high mutation rates in MYD88 L265P (up to 92% in primary central nervous system lymphoma), cluster of differentiation 79B, and TBL1XR1.

Comparison with Sézary syndrome
Interestingly, we found a few overlapping molecular markers between Mareschal et al.’s study of PCLBCL-LT and our previous genetic analysis of Sézary syndrome, the leukemic form of mycosis fungoides (Wang et al., 2015). First, both PCLBCL-LT and Sézary syndrome exhibit activation of the NF-κB pathway, although they appear to utilize different signaling scaffold proteins. Caspase recruitment domain family member 11, mutated in 15% of cases of Sézary syndrome, is a critical regulator of both T- and B-cell activation and NF-κB activation (Wang et al., 2015). Although this mutation is rarely found in PCLBCL-LT, MYD88 mutations, reported in 78% of PCLBCL-LT by Mareschal et al., appear to be the major driver of the NF-κB pathway in PCLBCL-LT (Figure 2).

Second, both Sézary syndrome and PCLBCL-LT are characterized by a high frequency of deletions in TNF-alpha-induced protein 3, a negative regulator of the NF-κB pathway. Sézary syndrome has been shown to exhibit frequent deletions of the gene in up to 46% of cases (Braun et al., 2011). Similarly, Mareschal et al. also detected TNF-alpha-induced protein 3 deletions in approximately 25% of cases, making it one of the most common gene deletions in PCLBCL-LT.

Lastly, both conditions exhibit a very high frequency of 9p21 deletions (cyclin-dependent kinase inhibitor 2A

**Clinical Implications**

- The aggressive nature of primary cutaneous large B-cell lymphoma, leg-type requires prompt diagnosis versus more indolent variants.
- Myeloid differentiation primary response gene 88 L265P is a significant genetic marker that reinforces the diagnosis of primary cutaneous large B-cell lymphoma, leg-type.
- Genomic markers may lead to targeted therapy for patients who fail conventional immunochemotherapy.

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**Clinical**
- Women in their 70’s
- Nodules, plaques, or tumors on the legs (unilateral/ bilateral)

**Histology**
- Diffuse dermal infiltrates of centroblasts and immunoblasts
- Large and round nuclei
- MIB-1 staining: >80% lymphoma cells

**Immunohistochemistry**
- CD19+, CD20+, CD22+, CD79a+, BCL2+, and MUM1+
- CD5-, CD10-, CD138-, and Cyclin D1-

**Genetic**
- Mutations: MYD88, CD79B, PIM1, TBL1XR1, CREBBP, MYC, IRF4, HIST1H2AC
- Deletions: CDKN2A, PRDM1/BLIMP1, TNFAIP3/A20

**Figure 1.** Pathogenetic and clinical features of PCLBCL-LT in different stages. PCLBCL-LT, primary cutaneous large B-cell lymphoma, leg-type.
and/or cyclin-dependent kinase inhibitor 2B. We previously found focal deletions of cyclin-dependent kinase inhibitor 2A in 58% of our patients with Sézary syndrome (Wang et al., 2015). Mareschal et al. also note loss of 9p21 in 75% of patients—the most frequent copy number variations identified. Cyclin-dependent kinase inhibitor 2A encodes inhibitor of kinase 4, which inhibits cell proliferation, and ADP ribosylation factor, which stabilizes tumor protein p53 (Lima, 2015). Interestingly, tumor protein p53 mutations were not detected in PCLBCL-LT, although they were the most frequently mutated genes in Sézary syndrome, found in 10 of 11 patients in the discovery cohort in our study (Wang et al., 2015).

Therapeutic targets
Because of the rarity of PCLBCL-LT, no large clinical studies evaluating drugs for PCLBCL-LT have been performed. Currently, rituximab with cyclophosphamide, doxorubicin hydrochloride, oncovin, and prednisone is the first-line treatment. However, many targeted therapies have been piloted, including (i) lenalidomide, targeting IRF-4 and SPIB; (ii) ibrutinib, targeting Bruton tyrosine kinase; (iii) nivolulam, targeting PD-1; and (iv) bortezomib, targeting the NF-kB proteasome (Nicolay and Wobser, 2016).

Future directions
There have been consistent efforts to understand the molecular differences between PCLBCL-LT and indolent cutaneous B-cell lymphomas. Studies to date often have been limited to fewer than 30 patients each due to the rarity of this disease. Mareschal et al. performed both whole exome sequencing in 12 patients and lymphopanel analysis in 20 patients with PCLBCL-LT. This cohort was part of a relatively large study made possible by collaboration between multiple centers and investigators over many years. This recent study further extends our understanding of the genomics of PCLBCL-LT through discovery of two potential driver genes, TBL1XR1 and GNA13, and confirmation of frequent mutations in MYD88. Future prospective studies with old and new markers should be conducted in larger cohorts of patients, with the hope of developing more effective targeted therapy.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES