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Étude clinique, histologique, phénotypique et moléculaire d'une série de 64 cas de lymphomes B primitifs cutanés à grandes cellules

Sarah Menguy

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**Thèse pour l'obtention du
DIPLOME D'ÉTAT DE DOCTEUR EN MÉDECINE**

Présentée et soutenue publiquement le 4 septembre 2018 par

Sarah MENGUY

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**Étude clinique, histologique, phénotypique et moléculaire d'une série de 64 cas de
lymphomes B primitifs cutanés à grandes cellules**

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A mon président du jury,

Monsieur le Professeur Jean-Philippe MERLIO

Professeur des universités, praticien hospitalier

Service de Biologie des Tumeurs

Hôpital Haut-Lévêque, CHU de Bordeaux, Pessac

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Madame le Professeur Béatrice VERGIER

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Hôpital Haut-Levêque, CHU de Bordeaux, Pessac

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Professeur des universités, praticien hospitalier

Service d'anatomie et cytologie pathologiques

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Professeur des universités, praticien hospitalier

Service de dermatologie

Hôpital Saint André, CHU de Bordeaux

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Professeur des universités, praticien hospitalier

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Hôpital Saint André, CHU de Bordeaux

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ABREVIATIONS

ABC : Activated B-cell

ADN : Acide désoxyribonucléique

ARN : Acide ribonucléique

BCL6 : B-cell lymphoma 6

BCL2 : B-cell lymphoma 2

BCR : B-cell receptor

BTK : Bruton Tyrosin Kinase

CARD11 : Caspase recruitment domain-containing protein 11

CD : Classe de différenciation

CDKN2A : Cyclin Dependent Inhibitor 2A

EBV : Epstein Barr Virus

EORTC : European Organisation for Research and Treatment of Cancer

FOXP1 : Forkhead Box P1

GC : Centre germinatif

GEP : Profil d'expression génique

GFELC : Groupe Français d'Etude des Lymphomes cutanés

HES : Hematoxyline éosine safran

IFN : Interféron

IHC : Immunohistochimie

IL: Interleukine

IRAK : Interleukin-receptor-associated kinase

IRF4 : Interferon regulatory factor 4

JAK : Janus Kinase

LBPC : Lymphome B primitif cutané

LBPC-GC : Lymphome B primitif cutané à grandes cellules

LBCF : Lymphome B cutané centro-folliculaire

LBCF-GC : Lymphome B cutané centro-folliculaire à grandes cellules

LBTJ : Lymphome B cutané diffus à grandes cellules, de type jambe

LBDGC : Lymphome B diffus à grandes cellules

MLPA : Amplification ligature-dépendante multiplexe de sonde

MUM1 : Multiple myeloma oncogene 1

MYD : Myeloid differentiation

NF-KB : Nuclear factor Kappa B

NGS : Next-generation sequencing

Non-GC : Non centre germinatif

RT-MLPA : Transcriptase inverse d'amplification ligature-dépendante multiplexe de sonde

TLR : Toll like receptor

TNF : Tumor necrosis factor

WHO : World Health Organisation

TABLEAU ET FIGURES

Tableau 1 : Classifications des lymphomes primitifs cutanés B

Figure 1 : Images cliniques de lymphome B diffus à grandes cellules, de type jambe (LBTJ) (Service de Dermatologie, CHU Bordeaux)

Figure 2 : Images cliniques de lymphome B cutané centro-folliculaire (LBCF) (Service de Dermatologie, CHU de Bordeaux)

Figure 3 : Algorithme diagnostique des lymphomes B primitifs cutanés à grandes cellules (LBPC-GC) (Laban E. et al. *Annales de Pathologie*. 2015)

Figure 4 : Cinq différents algorithmes pour établir le statut centre germinatif (GC) et non-GC

Figure 5 : Voies de signalisation impliquées dans le lymphome B diffus à grandes cellules (LBDGC) avec notamment l'implication de la mutation *MYD88* (Roschewski M. et al. *Nat. Rev. Clin. Oncol.* 2014)

Figure 6 : Voies de signalisation impliquées dans les LBDGC et thérapies ciblées en lien (Roschewski M. et al. *Nat. Rev. Clin. Oncol.* 2014)

Figure 7 : Algorithme diagnostique des lymphomes B primitifs cutanés à grandes cellules (LBPC-GC)

LBTJ : lymphome B diffus à grandes cellules, de type jambe

LBCF-GC : lymphome B cutané centro-folliculaire à grandes cellules

GC : centre germinatif

RÉSUMÉ

Devant un lymphome B primitif cutané à grandes cellules (LBPC-GC), le diagnostic différentiel « lymphome B diffus à grandes cellules de type jambe (LBTJ) » versus « lymphome B centro-folliculaire (LBCF) à prédominance de grandes cellules » a un impact pronostique et thérapeutique fort mais parfois difficile. La WHO 2017 propose des critères diagnostiques morphologiques et phénotypiques de classification que nous avons évalués sur 64 cas de LBPC-GC (>80% de grandes cellules tumorales) issus du Groupe Français d'Etude des Lymphomes Cutanés. Et nous avons montré l'impact pronostique de cette classification. Par ailleurs, nous avons testé les algorithmes développés dans les lymphomes B diffus à grandes cellules ganglionnaires prédisant la cellule d'origine afin de déterminer leur utilité diagnostique et leur valeur pronostique dans les LBPC-GC. Par ailleurs, nous avons recherché la mutation *MYD88* dans cette série afin d'évaluer son intérêt diagnostique. Enfin, par comparaison avec les lymphomes B diffus à grandes cellules ganglionnaires, nous avons recherché le statut double hit et le profil double expresseur dans un sous-groupe de cette série. Selon la morphologie et le phénotype, nous avons classé 32 lymphomes en LBTJ et 25 en LBCF à prédominance de grandes cellules. Sept cas n'ont pas pu être classés. Les critères morphologiques étaient peu reproductibles contrairement au phénotype. L'expression de MUM1, BCL2, FOXP1, MYC et IgM étaient en faveur du diagnostic de LBTJ de façon significative, mais BCL2 était fréquemment exprimé dans les LBCF (28%). L'expression de CD10, BCL6 et la présence d'un réseau folliculaire dendritique CD21+ étaient significatifs pour le diagnostic de LBCF, mais BCL6 était fréquemment exprimé dans les LBTJ (78%). L'index de prolifération Ki67, élevé, et la P63 n'étaient pas des marqueurs discriminants. Selon les algorithmes de Hans et Hans modifié, les 25 LBCF avaient un profil centre germinatif (GC) et les 32 LBTJ un profil non-GC. La survie globale était moins bonne pour les LBTJ comparée aux LBCF avec une survie intermédiaire pour les 7 cas inclassés

($p=0.0002$). Dans les LBTJ, l'expression de MYC était associée aux récurrences cutanées ($p=0.014$). La survie globale était moins bonne pour les cas non-GC ($p=0.0002$). La mutation *MYD88* était présente dans 22 cas de LBTJ, absente dans tous les LBCF. La présence de mutation *MYD88* avait un impact négatif sur la survie des LBPC-GC ($p=0.03$). Dans un sous-groupe de 44 cas de LBPC-GC, nous avons montré que le statut double hit était un événement très rare (un cas), celui-ci correspondait à un LBCF-GC et le patient au bout de 29 mois de suivi était en vie en rémission complète. En revanche, le profil double expresseur était fréquent (57%) et était significativement associé au diagnostic de LBTJ : 19 sur 23 cas (89%). L'expression de BCL2 seul, MYC seul et la double expression MYC et BCL2 étaient associées à une moins bonne survie dans les LBPC-GC, et dans le sous-groupe des LBTJ, les patients avec un profil double expresseur avaient une maladie plus agressive.

En conclusion nous avons montré l'applicabilité de la classification WHO 2017 des LBPC-GC dans la grande majorité des cas. Nous avons confirmé la différence de pronostic entre les LBTJ et les LBCF-GC, soulignant l'importance du diagnostic différentiel. En plus de sa valeur pronostique, l'algorithme de Hans modifié, économique par l'utilisation de 2 anticorps (CD10 et MUM1), semble suffisant pour différencier les LBCF des LBTJ. Nous avons démontré l'intérêt diagnostique de la recherche de la mutation *MYD88* par sa fréquence élevée dans les LBTJ et son absence dans les LBCF-GC. Au vu de la rareté des cas double hit, la recherche de ce statut dans les LBPC-GC paraît inutile. En revanche, la recherche du profil double expresseur met en évidence des sous-groupes au pronostic défavorable et démontre son intérêt à la fois diagnostique et pronostique. Les cas inclassés soulèvent la question de conserver la catégorie provisoire "autre" des LBPC-GC, retirée de la classification WHO 2008. Au vu de l'hétérogénéité clinique, histologique et phénotypique de ce sous-groupe, de nouveaux outils moléculaires seraient nécessaires pour mieux classer ces cas et guider leur prise en charge thérapeutique.

1. GENERALITES

1.1. Les lymphomes primitifs cutanés

Les lymphomes sont des proliférations lymphoïdes clonales malignes de phénotype lymphocytaire B ou T. Les lymphomes peuvent être ganglionnaires ou extra-ganglionnaires. Les lymphomes primitifs cutanés sont parmi les lymphomes extra-ganglionnaires les plus fréquents, après les lymphomes du tube digestif. Ce sont des lymphomes localisés à la peau, sans signe d'atteinte extra-cutanée lors du bilan d'extension au diagnostic. Ils sont à distinguer des lymphomes ganglionnaires avec localisations secondaires cutanées.

1.2. Classification des lymphomes B primitifs cutanés

Les lymphomes B primitifs cutanés (LBPC) représentent 25% de l'ensemble des lymphomes cutanés. Selon la classification WHO/EORTC (World Health Organization / European Organization for Research and Treatment of Cancer) de 2005, les LBPC sont classés¹ :

Lymphome primitif cutané B de la zone marginale

Lymphome primitif cutané B centro-folliculaire

Lymphome primitif cutané B diffus à grandes cellules, de type jambe

Lymphome primitif cutané B diffus à grandes cellules, autres

Lymphome B intravasculaire

Cette classification a été révisée en 2008² puis 2016³ et la nouvelle classification des LBPC selon la WHO 2017 les catégorisent⁴ :

Lymphome B de la zone marginale extra-nodal

Lymphome primitif cutané B centro-folliculaire

Lymphome primitif cutané B diffus à grandes cellules, de type jambe

Tableau 1 : Classifications des lymphomes primitifs cutanés B

Classification WHO/EORTC 2005	Classification WHO 2017
Lymphome primitif cutané B de la zone marginale	Lymphome B de la zone marginale extra-nodal
Lymphome primitif cutané B centro-folliculaire	Lymphome primitif cutané B centro-folliculaire
Lymphome primitif cutané B diffus à grandes cellules, de type jambe	Lymphome primitif cutané B diffus à grandes cellules, de type jambe
Lymphome primitif cutané B diffus à grandes cellules, autres	
Lymphome B intravasculaire	

1.3. Les lymphomes primitifs cutanés B diffus à grandes cellules, de type jambe

1.3.1. Présentation clinique

Les lymphomes primitifs cutanés B diffus à grandes cellules, de type jambe (LBTJ) sont les plus rares des LBPC (20%). Cliniquement, ils surviennent chez des patients âgés, avec une prédominance féminine. Les lésions cutanées sont le plus souvent localisées aux membres inférieurs, même s'il ne s'agit pas d'un critère exclusif. Ils se manifestent typiquement par des tumeurs cutanées, érythémato-violacées, de croissances rapides, uniques ou multiples, parfois ulcérées. La classification TNM adaptée aux lymphomes cutanés (hors mycosis fongoïde et syndrome de Sézary) permet d'évaluer la sévérité clinique.^{5,6}

Figure 1 : Images cliniques de LBTJ (Service de Dermatologie, CHU Bordeaux)



1.3.2. Histologie et phénotype

Les LBTJ se caractérisent histologiquement par une prolifération dermique +/- hypodermique d'architecture diffuse non épidermotrope de grandes cellules type immunoblastiques +/- centroblastiques.^{7,8} Les immunoblastes sont décrits comme de grandes cellules non clivées, au noyau rond avec un seul gros nucléole central. Les centroblastes sont décrits comme de grandes cellules non clivées, au noyau rond avec plusieurs nucléoles en périphérie du noyau.

D'un point de vue phénotypique, les cellules lymphomateuses, de phénotype B CD20+, expriment habituellement BCL2, MUM1, FOXP1, MYC et IgM.^{9,10} BCL6 est souvent exprimé même s'il peut être négatif. CD10 est généralement négatif.¹¹ L'index de prolifération Ki67 est élevé. Le réseau folliculaire dendritique CD21+ est absent.

1.3.3. Traitement et pronostique

Les LBTJ ont un pronostic plus mauvais que les autres types de LBPC. Il a récemment été montré que l'utilisation d'un traitement de première ligne par immuno-chimiothérapie améliorait la survie, y compris chez des sujets très âgés : le taux de survie à 5 ans passait de 46% à 66%.¹² Ainsi en première ligne thérapeutique est proposée l'association du Rituximab et une polychimiothérapie de type CHOP ou COP avec une adaptation des doses pour les patients fragiles. Malgré tout, les rechutes cutanées (52%) et les progressions extra-cutanées restent relativement fréquentes (36%).¹²

1.4. Lymphome B primitif cutané centro-folliculaire

1.4.1. Présentation clinique

Les lymphomes B primitifs cutanés centro-folliculaire (LBCF) représentent environ 50% de LBPC. Il concerne en général des adultes d'âge moyen avec un ratio homme:femme de 1,5:1.^{10,13} Les localisations préférentielles sont le tronc et la tête. Environ 5% des cas ont des lésions cutanées aux membres inférieurs. Le plus souvent les lésions cutanées sont uniques et plus rarement multiples. Les patients présentent des plaques fermes érythémato-violacées, des nodules ou des tumeurs de taille variable. L'état général est conservé. Les lésions peuvent évoluer pendant des mois ou des années.

Figure 2 : Images cliniques de LBCF (Service de Dermatologie, CHU de Bordeaux)



1.4.2. Histologie et Phénotype

Le LBCF est une tumeur composée de cellules lymphomateuses originaires du follicule. Il s'agit d'une prolifération lymphomateuse d'architecture folliculaire (nodulaire), folliculaire et diffuse, ou diffuse. Les cellules lymphomateuses sont des centrocytes (cellules clivées avec peu de cytoplasme et des nucléoles peu visibles) de taille petite ou grande avec un nombre variable de centroblastes. Contrairement aux lymphomes B folliculaires ganglionnaires il n'existe pas de grading selon le pourcentage de centroblastes.

D'un point de vu phénotypique, les cellules lymphomateuses, de phénotype B CD20+, expriment constamment le BCL6. Le CD10 peut être positif en cas d'architecture folliculaire, mais est souvent négatif lorsque l'architecture est diffuse. La majorité des cas n'exprime pas le BCL2, mais ce point est discuté dans la littérature.^{14,15} MUM1 et FOXP1 sont négatifs en général.⁹ Il persiste un réseau folliculaire dendritique CD21+. L'index de prolifération Ki67 est rapporté dans la WHO 2017 comme étant bas.

1.4.3. Lymphome B primitif cutané centro-folliculaire à grandes cellules

Les LBCF sont composés d'un mélange en proportion variable de petites cellules tumorales (centrocytes) et de grandes cellules (grands centrocytes et centroblastes). Mais il existe certains cas dont la population cellulaire tumorale est faite d'une majorité de grandes cellules. Nous avons défini les lymphomes B primitifs cutanés centro-folliculaires à grandes cellules (LBCF-GC) comme des LBCF composés de plus de 80% de grandes cellules tumorales car ce sont eux qui posent réellement un problème de diagnostic différentiel avec les LBTJ. On parle de cellules lymphomateuses de grande taille quand le noyau fait au moins la taille d'un noyau de macrophage ou 2 fois la taille d'un noyau de lymphocyte normal. Histologiquement, la morphologie des cellules peut correspondre à des centrocytes de grande taille, de grands

centrocytes fusiformes et/ou des centroblastes. Il existe en effet une variante spécifique à la peau (absente dans le ganglion) qui correspond à un LBCF-GC d'architecture diffuse et constitué de grands centrocytes clivés prenant un aspect de cellules fusiformes d'aspect pseudo-sarcomateux.¹⁶⁻¹⁸

1.4.4. Pronostic et traitement

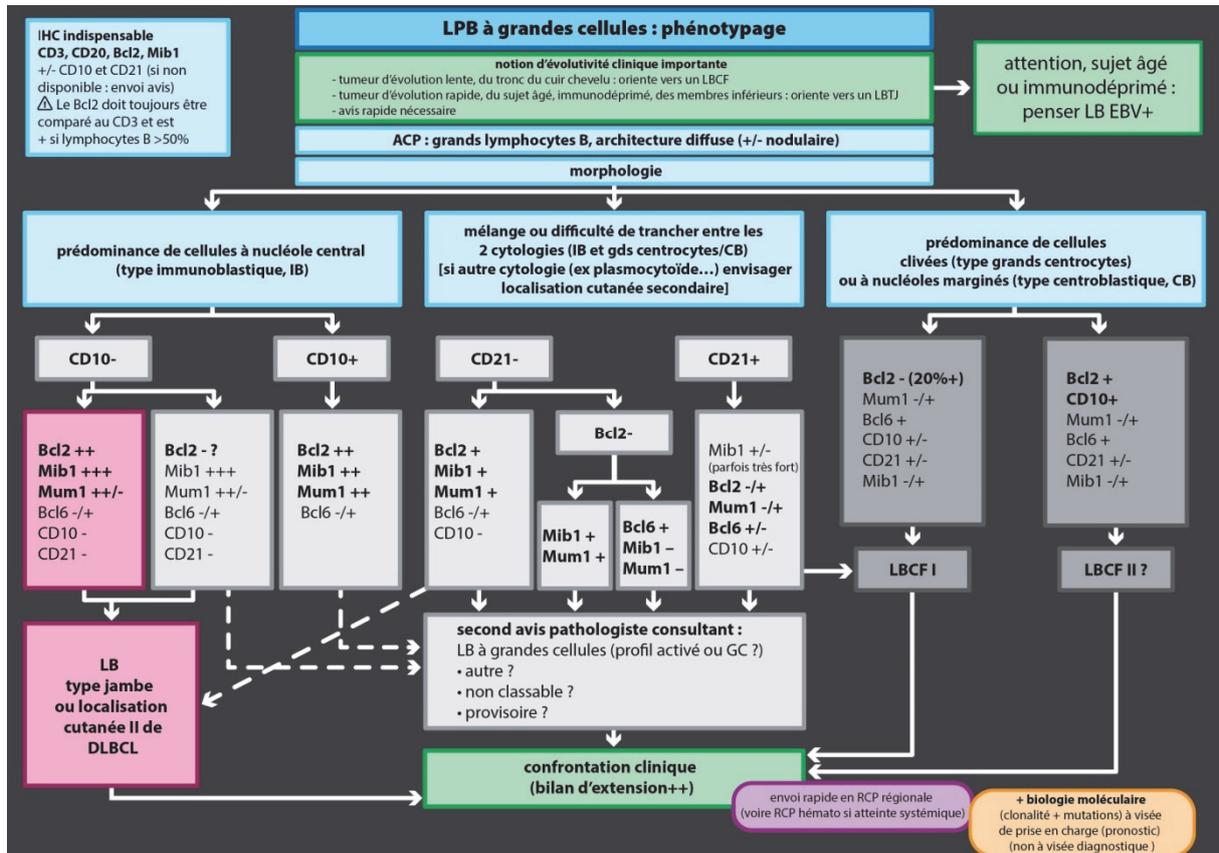
Les LBCF ont une évolution indolente avec une survie à 5 ans >95%.^{5,6} Aucune différence en terme de pronostic entre les LBCF à petites cellules et les LBCF-GC composés de grands centrocytes clivés n'est rapporté.¹⁰ Les LBCF avec des lésions cutanées aux membres inférieurs seraient de moins bon pronostic (survie globale à 5 ans d'environ 20 %).^{9,10} Les récurrences cutanées, observées dans environ 30% des cas, ne sont pas un signe de maladie évolutive.¹⁹ Une progression extra-cutanée de la maladie est très rare, approximativement 10%.^{10,13} Les patients avec des lésions localisées (la très grande majorité) sont traités en général par radiothérapie locale.^{20,21}

1.5. Le diagnostic différentiel des lymphomes B primitifs cutanés à grandes cellules.

Face à un lymphome B primitif cutané à grandes cellules, le pathologiste ne peut pas utiliser, comme pour leurs équivalents ganglionnaires le diagnostic de lymphome B diffus à grandes cellules (LBDGC). Avec la classification WHO 2017, il doit classer cette entité selon 2 diagnostics de pronostic et prise en charge différents: LBTJ ou LBCF-GC.⁴

Afin de parvenir au diagnostic, il tient compte de critères morphologiques et phénotypiques, comme décrit précédemment. Un algorithme établi par Laban et al a été validé pour le Groupe Français d'Etude des Lymphomes Cutanés (GFELC) afin d'aider les pathologistes dans la distinction de ces 2 entités.²² Il a également été montré dans ce travail, réalisé à partir des lymphomes revus en 2^o lecture du réseau Lymphopath, que ce diagnostic différentiel était difficile avec des erreurs en fréquence non négligeable. En effet 21,4% des discordances de type « discordance majeure » de classification (entraînant un changement de prise en charge du patient) correspondaient à cette problématique de diagnostic différentiel entre LBTJ et LBCF-GC.²² Le pronostic et le traitement de ces 2 entités étant très différents, il est important que les pathologistes puissent les différencier avec des outils morphologiques, phénotypiques et moléculaires adaptés.

Figure 3 : Algorithme diagnostique des LBPC-GC (Laban E et al. *Annales de Pathologie*. 2015)



2. OBJECTIFS DU TRAVAIL DE THESE

Ainsi nos différents travaux, réalisés dans le cadre de cette thèse, ont pour objectifs d'améliorer le diagnostic et donc la prise en charge des LBPC-GC en répondant aux questions suivantes :

2.1. Intérêt et applicabilité de la classification WHO 2017 pour les LBPC-GC ?

La classification WHO 2017 venant d'être publiée nous avons classé de façon rétrospective 64 cas de LBPC-GC (série multicentrique à partir de la base de données du GFELC), selon les définitions de cette nouvelle classification. L'objectif de ce travail (ARTICLE 1) était de voir si les critères diagnostiques de la WHO 2017 permettaient de catégoriser l'ensemble des LBPC-GC de cette série ?

2.2. Différence pronostique entre les LBTJ et les LBCF-GC ?

Une fois ces LBPC-GC classés selon la WHO 2017, nous avons étudié si la différence pronostique rapportée dans la WHO 2017 entre les LBTJ et les LBCF était réelle, en considérant que les LBCF de notre série étaient tous constitués de > 80% de grandes cellules (ARTICLE 1). Ce résultat justifiant l'importance pour le pathologiste du diagnostic différentiel entre un LBTJ et un LBCF-GC.

2.3. Comment améliorer le diagnostic différentiel des LBPC-GC ?

Nous avons alors cherché des outils moléculaires et immunohistochimiques qui permettraient d'améliorer ce diagnostic différentiel.

Premièrement, nous avons évalué l'apport des différents anticorps décrits dans la littérature pour les LBTJ et les LBCF-GC (ARTICLE 1).

Deuxièmement, nous avons testé différents algorithmes immunohistochimiques utilisés dans les LBDGC ganglionnaires pour évaluer leur impact diagnostique et pronostique dans les LBPC-GC (ARTICLE 1).

Troisièmement, nous avons voulu savoir si la mutation *MYD88* pouvait être un critère diagnostique dans les LBPC-GC (ARTICLE 2).

2.4. Existe-t-il des outils pronostiques pour les LBPC-GC ?

Enfin, nous avons appliqué aux LBPC-GC des outils pronostiques des LBDGC ganglionnaires : le statut double/triple hit et le profil double expresseur (ARTICLE 3).

3. ARTICLE 1

3.1. Introduction à l'ARTICLE 1

3.1.1. Cohorte de l'étude et principaux objectifs

Notre travail de thèse a porté sur une série multicentrique et rétrospective de 64 patients souffrant d'un LBPC composé de plus de 80% de grandes cellules lymphomateuses, issus du réseau du Groupe Français d'Etude des Lymphomes Cutanés (GFELC).

Les 2 principaux objectifs étaient :

- analyser l'applicabilité de la nouvelle classification WHO 2017 et confirmer son intérêt d'un point de vue pronostique.
- améliorer le diagnostic différentiel LBTJ versus LBCF-GC en testant d'une part des outils phénotypiques rapportés dans la littérature et d'autre part en se servant des algorithmes définissant le statut Germinal Center (GC) versus non-GC utilisés dans les équivalents ganglionnaires des LBDGC.

Ce travail a fait l'objet d'un article qui a été soumis à American Journal of Surgical Pathology le 13 juillet 2018.

3.1.2. Applicabilité et intérêt de la nouvelle classification WHO 2017 des LBPC

Dans la classification WHO 2008 des LBPC, reprise dans la classification WHO 2017, l'entité « lymphome primitif cutané B diffus à grandes cellules, autres » a disparu. La classification WHO2017 est donc composée, comme rappelé dans les généralités, de 3 entités :

- Lymphome de la zone marginale extra-nodal
- Lymphome primitif cutané B centro-folliculaire
- Lymphome primitif cutané B diffus à grandes cellules, de type jambe

« Les lymphomes primitifs cutanés B diffus à grandes cellules, autres » correspondaient à de rares cas n'entrant ni dans la catégorie des LBTJ ni dans la catégorie des LBCF avec infiltrat diffus de grand centrocytes.¹ Cette entité était controversée et hétérogène, incluant par exemple pour certains auteurs les LBTJ n'exprimant pas BCL2^{9,23}, alors que d'autres auteurs les considéraient comme des LBTJ.

Ainsi dans ce travail, notre objectif principal était d'évaluer si, en appliquant de façon stricte les critères morphologiques et phénotypiques définis dans la WHO 2017, les 64 cas de LBPC-GC pouvaient être classés selon les 2 entités décrites (LBTJ et LBCF) et quel était le pourcentage de cas non classables anciennement inclus dans l'entité « autres ».

3.1.3. Particularité des lymphomes B diffus à grandes cellules ganglionnaires : catégorisation GC versus non-GC

Les LBDGC ganglionnaires sont des lymphomes de phénotype B composés de cellules lymphomateuses de grande taille avec une architecture diffuse. Ils constituent 25-35% des lymphomes non Hodgkiens de l'adulte. C'est une pathologie vaste et hétérogène que l'on peut subdiviser notamment d'un point de vue moléculaire selon la cellule d'origine. Les LBDGC ganglionnaires se distinguent donc selon 2 sous-types de pronostic différent basés sur la cellule d'origine définie d'après le profil d'expression génique (GEP)²⁴ :

Sous-type GC ou Germinal center dont le profil d'expression est proche de celui d'une cellule B du centre germinatif

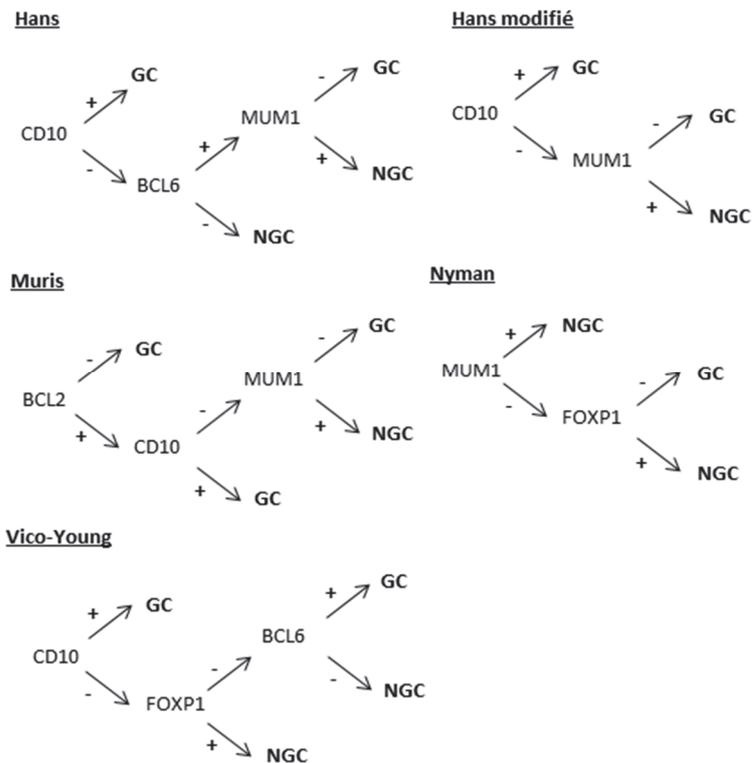
Sous-type non-GC ou ABC (Activated-B Cell) de profil d'expression d'un type cellulaire plus tardif, proche du plasmocyte.

Les LBDGC ganglionnaires de sous-type non-GC sont de moins bon pronostic que les LBDGC ganglionnaires de sous-type GC.

3.1.4. Algorithmes immunohistochimiques pour établir le statut GC/non-GC dans les LBDGC ganglionnaires

Différents algorithmes immunohistochimiques ont été développés dans les LBDGC ganglionnaires afin d'établir le statut GC et non-GC en conservant bien la différence pronostique entre les 2 sous-types : Hans²⁵, Muris²⁶, Nyman²⁷, Hans modifié²⁸, Visco-Young²⁹. L'algorithme aujourd'hui utilisé en routine est l'algorithme de Hans reposant sur 3 anticorps : CD10, BCL6 et MUM1.

Figure 4 : Cinq différents algorithmes pour établir le statut GC et non-GC



3.1.5. Equivalence du statut GC/non-GC dans les LBPC-GC ?

Des études de profiling ont montré que les LBCF avaient le même profil d'expression génique que les LBDGC ganglionnaires de sous-type GC et les LBTJ, le même profil d'expression génique que les LBDGC ganglionnaires de sous type non-GC.³⁰ Ainsi tester ces algorithmes prédictifs de la cellule d'origine dans les LBPC-GC dans un but diagnostique et pronostique semblait pertinent et n'avait pas été réalisé jusqu'à présent.

Nous avons donc testé 5 algorithmes différents établissant le statut GC/non-GC des 64 cas de LBPC-GC pour montrer la valeur diagnostique et pronostique de ce statut.

3.2. ARTICLE 1

ORIGINAL ARTICLE

**Evaluation of the 2017 WHO Classification Criteria to Diagnose Primary Cutaneous
Large B-Cell Lymphomas:**

Clinicopathologic and Molecular Analyses of 64 Cases

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ABSTRACT

We applied the 2017 WHO classification criteria to categorize a series of 64 primary cutaneous large B-cell lymphomas (PCLBCLs), with $\geq 80\%$ large cells to determine their reproducibility and clinical relevance. Morphology and phenotype identified 32 PCLBCLs, leg type (PCLBCLs-LT) and 25 primary cutaneous follicle center lymphomas with large cell morphology (PCFCLs-LC); 7 lesions (11%) remained unclassified. Morphology was less reproducible than immunophenotype. MUM1, BCL2, FOXP1, MYC and IgM expressions were significantly ($P < 0.03$) associated with PCLBCL-LT diagnosis, but BCL2 was also expressed by PCFCLs-LC (28%). CD10 and BCL6 expressions and CD21⁺ follicular dendritic cell meshwork were diagnostic for PCFCLs-LC, but 78% of PCLBCLs-LT expressed BCL6. Neither high Ki67 proliferative index nor P63 expression was discriminant. *MYD88* was mutated in 22 (69%) PCLBCLs-LT. Using Hans or Hans-modified algorithms (to classify nodal lymphomas), the 25 PCFCLs-LC had germinal center (GC) status and the 32 PCLBCLs-LT, non GC (NGC) status. Overall survival was poorer for PCLBCLs-LT than PCFCLs-LC, intermediate for unclassified cases ($P=0.0002$) and worst for NGC ($P=0.0002$). Our results support the 2017 WHO classification criteria for PCLBCL diagnosis. The Hans modified algorithm using CD10 and MUM1 distinguished PCFCLs-LC from PCLBCLs-LT. Rare unclassified cases may be a provisionally heterogeneous subgroup (previously called “other”). For these unclassified PCLBCLs, GC/NGC status (relevant for prognosis) may guide therapeutic decisions.

Key Words:

Cutaneous lymphoma

B-cell lymphoma

Primary cutaneous diffuse large B-cell lymphoma, leg type

Primary cutaneous follicle center lymphoma

Hans algorithm

INTRODUCTION

The 2017 WHO classification of primary cutaneous B-cell lymphomas (PCBCLs) divides them into the following 3 groups: primary cutaneous marginal zone lymphoma, primary cutaneous follicle center lymphoma (PCFCL), and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL-LT).^{1,2} The 2005 WHO–EORTC classification individualized a primary cutaneous large B-cell lymphoma (PCLBCL) “other” subgroup for rare cases not belonging to the group of PCLBCL-LT or PCFCL with a diffuse infiltration of large centrocytes.³ The term PCLBCL “other” should only be used for exceptional PCBCLs that do not satisfy the criteria of PCFCLs or PCLBCLs-LT.⁴ However, in practice, the “other” entity is poorly defined. For example, for some authors, the “other” category included BCL2[−] PCLBCLs-LT,^{5,6} considered as PCLBCLs-LT in other series.⁴ To facilitate the pathologist’s decision and make it more clinically pertinent, the PCLBCL “other” subgroup disappeared in the most recent 2017 WHO classification,^{2,7} leaving the pathologist with a binary choice for large B-cell cutaneous lymphoma diagnosis between 2 entities (PCLBCL-LT or PCFCL), with distinct prognoses and required therapies. PCFCLs have an indolent course, with a 5-year overall survival (OS) rate of >95%. Local therapeutic approaches are appropriate for solitary or localized lesions, which are the most frequently observed.⁸ Alternatively, PCLBCLs-LT have an aggressive course, requiring a combination of rituximab and polychemotherapy.⁹

Information contributing to make the diagnosis includes morphology, extensive phenotype and, when available, some recurrent molecular features. According to the WHO classification, the tumor cytology (immunoblasts, centroblasts or large centrocytes) and growth pattern may discriminate PCFCLs with large cell morphology (PCFCLs-LC) and PCLBCLs-LT. Immunolabelings of BCL2, MUM1, FOXP1, CD10, BCL6, CD21 (follicular dendritic cell meshwork) and Ki67 may help to differentiate PCFCLs from PCLBCLs-LT.¹⁰ IgM

expression also favors PCLBCL-LT.^{11,12} Robson et al suggested that P63 expression is specific to PCLBCL-LT.¹³ Our group showed that the *MYD88*^{L265P} mutation is a valuable criterion for PCLBCL-LT diagnosis, because it is not detected in PCFCLs-LC.^{14–16} Dual expression of MYC and BCL2 was also observed more frequently in PCLBCLs-LT than in PCFCLs-LC and characterizes patients with poorer prognoses.¹⁷

Notably, nodal diffuse large B-cell lymphomas (DLBCLs) are divided into 2 prognostic subgroups based on the cell-of-origin determined by transcriptome profiling: activated B cells or non-germinal center (NGC) DLBCLs and germinal center (GC) DLBCLs.^{18–21} Several immunohistochemical algorithms—Hans,²² Muris,²³ Nyman,²⁴ Hans modified²⁵ and Visco-Young²⁶—have been developed for nodal DLBCLs to predict the cell-of-origin.

Among primary cutaneous lymphomas, PCFCLs have a GC gene expression profile (GEP) while PCLBCLs-LT have the activated B-cell or NGC GEP subtype.²⁷ The evaluation of the abilities of those algorithms to predict cell-of-origin for the differential diagnoses between PCLBCLs-LT and PCFCLs-LC has not been specifically addressed.

This study was undertaken to classify a series of 64 PCLBCLs as PCLBCLs-LT or PCFCLs-LC, according to the 2017 WHO classification criteria: first, using morphological criteria and, then, extensive phenotyping analyses. The first objective was to assess the relevance of the 2017 WHO criteria. The second goal was to evaluate the abilities for PCLBCLs of the different algorithms developed to predict the cell-of-origin proposed for nodal DLBCLs. Finally, the prognostic impacts of the WHO PCLBCL classification criteria and the algorithms were investigated.

MATERIALS AND METHODS

Patient Selection

PCLBCLs containing $\geq 80\%$ large lymphomatous cells were retrieved from the French Study Group Cutaneous Lymphoma data base. Each case was selected based on the complete clinical file, including negative extracutaneous staging at diagnosis, and the availability of formalin-fixed paraffin-embedded (FFPE) skin biopsies for immunohistochemical and molecular analyses. Registration of the absence of opposition to the use of the information and tissue samples have been obtained according to the guidelines of French Bioethics Laws for retrospective, noninterventional, research studies.

Histologic Evaluation

For all specimens, 3- μm -thick FFPE tissue sections were stained with hematoxylin–eosin–saffron. Two pathologists (B.V., dermatopathologist, and M.P., hematopathologist) independently evaluated the architecture of the lymphoid infiltrates and tumor-cells cytomorphology. Architecture was defined as nodular, nodular and diffuse or diffuse. Cytological characteristics were based on nuclear morphology with 4 types of tumor cells: small-to-large centrocytes (cleaved cells with scant cytoplasm and inconspicuous nucleoli), centroblasts (large noncleaved cells with round nuclei and multiple peripheral nucleoli), immunoblasts (large noncleaved cells with round nuclei and prominent single central nucleoli), and spindle-cell aspect (large cleaved centrocytes).

Immunohistochemistry

FFPE slides were subjected to automated immunolabeling (Bond Max, Leica, Nanterre, France). We tested BCL2 (clone 124, Dako, Les Ulis, France), BCL2 (clone E17, Gene Tex, Irvine, CA), MUM1 (clone MUM1p, Dako), CD10 (clone 56C6, Leica), BCL6 (clone PGB6P, Dako), CD21 (clone 2G9, Leica), Ki67 (clone MM1, Leica), IgM (polyclonal,

Leica), p63 (clone 7JUL, Leica), FOXP1 (clone JC12, Abcam, Cambridge, UK) and MYC (clone Y69, Epitomics, Burlingame, CA). According to consensus criteria in literature, the positivity cut-offs for BCL2, MUM1, P63 and MYC expression, were respectively, by \geq 50%, \geq 30%, \geq 25% and \geq 40% of cells.^{13,22,28,29} Immunohistochemical labelings of P63, IgM, MYC and FOXP1 were reviewed double-blind by 2 pathologists (S.M. and B.V.); discrepancy led to slides being reviewed together to reach consensus. To define GC or NGC subgroups, the 5 algorithms were used with different marker combinations (Fig. 1). Immunohistochemical scoring was done without knowledge of the patient's outcomes.

2017 WHO Criteria (Morphology and Phenotype) for PCFCLs and PCLBCLs-LT

In the 2017 WHO textbook,² PCFCL is defined as “a tumour of neoplastic follicle centre cells, including centrocytes and variable numbers of centroblasts with a follicular, follicular and diffuse, or diffuse growth pattern.” The PCFCL neoplastic cells “consistently express BCL6. CD10 may be positive in cases with follicular growth pattern, but is generally negative with a diffuse growth pattern. Most cases either do not express BCL2 or only show faint BCL2 staining in a minority of neoplastic B cells.” “Staining for MUM1 and FOXP1 is negative in most cases.” There is a persistent meshwork of CD21+ follicular dendritic cells. PCFCLs “show a low proliferation rate” except for diffuse PCFCLs with large spindle-shaped centrocytes in which “the proliferation fraction is generally high.”

PCLBCL-LT is “a PCLBCL composed exclusively of diffuse infiltrate of centroblasts and immunoblasts.”^{30,31} The PCLBCL-LT neoplastic cells “usually strongly express BCL2, MUM1, FOXP1, MYC and IgM.^{4,5}” “BCL6 is expressed by

most cases, but may be dim, whereas CD10 staining is usually negative.³² “The proliferation rate is high.” “CD21⁺ follicular dendritic cell meshwork is absent.”

Molecular Biology

For all specimens, DNA was extracted from FFPE material and subjected to real-time polymerase chain-reaction analysis with *TaqMan* allele-specific probes to determine *MYD88* status, as reported elsewhere.¹⁴ Menguy et al previously investigated the *MYD88* status of 46 tumors.¹⁵ Epstein–Barr virus (EBV) status was tested by in situ hybridization using EBV-encoded RNAs (EBERs) (Bond™ Ready-to-Use ISH EBER Probe, Leica Biosystems Newcastle Ltd, UK).

Statistical Analyses

Quantitative parameters are expressed as median [range], and qualitative variables as number (%). Qualitative characteristics were compared between patient subgroups with Fisher’s exact test. The kappa agreement between 2 expert pathologists (B.V. and M.P.) for morphology analysis was assessed according to Landis et al.³³ Kappa agreements of the inter-observer reproducibility for immunohistochemical labeling (P63, MYC, IgM and FOXP1) between 2 pathologists (B.V. and S.M.) were also determined. Pathologists interpreted the slides independently. We tested the diagnostic value of algorithms predicting cell-of-origin by computation of sensitivity, specificity, positive- and negative-predictive values. Prognosis assessment considered OS, progression-free survival (PFS) (time between the date of diagnosis and the date of relapse or death) and specific survival (SS). Survival curves were plotted using the Kaplan–Meier method and compared with the log-rank test for each prognostic factor (univariate analysis). A 2-sided $P < 0.05$ defined significance. Statistical

analyses used MedCalc software, version 12.4.0 (Ostend, Belgium).

RESULTS

Clinical Characteristics of the Series

Sixty-four patients (35 (55%) women and 29 (45%) men, median age at diagnosis 78.5 [range: 42-97] years) with PCLBCLs defined by ≥ 80 % of large tumor cells were identified in the database of the French Study Cutaneous Lymphoma Group. Their clinical findings according to the 2017 WHO classification are reported in Table 1. All patients had primary cutaneous lymphoma, with their initial staging including computed-tomography scan confirming other-site negativity. Their clinical stages were T1 (39%) and T2 (55%) predominantly. The leg was the most frequent site (45%), followed by head and neck (25%), trunk (25%) and arm (5%). Fourteen tumors were ulcerated. Lactate dehydrogenase level were elevated in 14/58 (24%) patients (6 data missing). Mean and median times between first symptoms and diagnosis were 16.3 and 3.5 months, respectively. The most frequent initial treatment was rituximab-polychemotherapy (41%), followed by radiotherapy (30%), rituximab alone (11%) or surgery (9%); 6 (9%) received no specific therapy.

Microscopy

Histological classification based on morphology (growth pattern and cytology) criteria

Applying the WHO morphological definition of PCFCL to our 64 PCLBCLs, 26 (41%) were composed of centroblasts and/or centrocytes: 6 with a majority of centroblasts (Fig. 2A), 11 with a mixture of centroblasts and centrocytes, and 9 with

large spindle-shaped centrocytes (Fig. 2C). Among the 26, architecture was diffuse ($n=13$), follicular ($n=7$) or follicular and diffuse ($n=6$).

Applying the WHO morphological definition of PCLBCL-LT to our 64 PCLBCLs, 36 (56%) were composed of immunoblasts ($n=14$) (Fig. 2B) or a mixture of immunoblasts and centroblasts ($n=22$) (Fig. 2D). Among them, architecture was diffuse for 20 (55%), nodular and diffuse for 15 or nodular for 1. The latter unique case could not be classified as PCLBCL-LT according to the 2017 WHO criteria.

Two other PCLBCLs remained unclassified (PCLBCLs-U), 1 with nodular and diffuse architecture and a mixture of immunoblasts and large spindle-shaped centrocytes (Fig. 2E), and the other with nodular architecture and a mixture of immunoblasts, centroblasts and centrocytes (Fig. 2F).

Finally, based on morphology, 26 specimens could be considered as PCFCLs-LC and 35 as PCLBCLs-LT; and 3 could not be classified (PCLBCLs-U).

Morphology-interpretation reproducibility

The interobserver reliability kappa agreements for the 64 PCLBCLs were moderate: 0.425 [95% confidence interval (CI), 0.262–0.588] for cytology (30 (47%)); 0.413 [95% CI, 0.204–0.622] for architecture interpretation (43 (67%)); and 0.446 [95% CI, 0.235–0.657] for morphological classification (46 (72%)).

Immunophenotype

Profile

Among the 26 PCLBCLs classified morphologically as PCFCLs-LC, BCL6 and CD10 were expressed by 25 and 14 respectively, but some were also positive for

BCL2 ($n=8$), MUM1 ($n=2$), FOXP1 ($n=3$) (Fig. 3A), IgM ($n=2$) (Fig. 3C), P63 ($n=11$) (Fig. 3E), MYC ($n=12$). A meshwork of CD21⁺ follicular dendritic cells was seen in 15. Mean and median of Ki67 proliferation index were, respectively, 77.1% and 80% (Fig. 3G). Among these 26 cases, 1 had a phenotype profile close to PCLBCL-LT (BCL2⁺MUM1⁺MYC⁺FOXP1⁺BCL6⁻CD10⁻ and no CD21⁺ follicular dendritic cell meshwork), so it could not be categorized PCFCL-LC.

Among the 35 PCLBCLs categorized morphologically as PCLBCLs-LT, 32 had a standard PCLBCL-LT profile: BCL2⁺ ($n=34$), MUM1⁺ ($n=33$), FOXP1⁺ ($n=26$) (Fig. 3B) and CD10⁻ ($n=34$), with MYC ($n=27$), IgM ($n=15$) (Fig. 3D), BCL6 ($n=28$) and/or P63 ($n=15$) (Fig. 3F) also being positive more or less frequently. None had a CD21⁺ follicular dendritic cell meshwork. Mean and median of Ki67 proliferation indexes were, respectively, 85.3% and 90% (Fig. 3H). Three of these tumors did not have phenotypes consistent with PCLBCL-LT: 1 expressed CD10 (confirmed by CD10 and PAX5 double-labeling), another was MUM1⁻ FOXP1⁻, and the last was BCL2⁻MUM1⁻FOXP1⁻.

The 3 lesions morphologically unclassified had PCLBCL-LT phenotypes.

Phenotype-interpretation reproducibility

For the 64 PCLBCLs, kappa agreements were excellent for immunodetection of FOXP1 (61 (95%)) 0.906 [95% CI, 0.802–1.000] or IgM (60 (94%)) 0.850 [95% CI, 0.709–0.992]; good for P63 detection (54 (84%)) 0.660 [95% CI, 0.473–0.846]; and moderate for MYC detection (50 (78%)) 0.576 [95% CI, 0.398–0.754].

Final Classification According to 2017 WHO PCLBCL Classification Criteria

Combining microscopy and phenotype 2017 WHO criteria (Table 2), we identified 25 (39%) typical PCFCLs-LC and 32 (50%) typical PCLBCLs-LT, with 7 (11%) tumors remained PCLBCLs-U, because of morphology and phenotype mismatches (Table 3): 3 had unclassified morphological features, 1 had PCFCL-LC morphology and PCLBCL-LT phenotype, and 3 PCLBCL-LT morphology and PCFCL phenotype.

Diagnostic Relevance of Different Immunolabeling profiles and Algorithms

Diagnostic value of the 2017 WHO phenotype criteria

As shown in Table 4, MUM1, BCL2, FOXP1 and IgM expression contributed significantly to diagnosing PCLBCLs-LT, but 28% were discrepant for BCL2, which was frequently expressed in PCFCLs-LC. MYC positivity was also associated with PCLBCL-LT diagnosis ($P=0.028$). P63 had no discriminative impact ($P=1.00$).

CD10, BCL6 and meshwork of CD21⁺ follicular dendritic cell immunolabelings contributed significantly to diagnosing PCFCL-LC. However, BCL6 was also expressed by 78% of PCLBCL-LT.

The proliferative Ki67 indexes (Table 2) were high and comparable for both of those PCLBCL-LT and PCFCL-LC preceding entities.

Diagnostic Value of Algorithms for GC/NGC Status

Hans, Hans modified and Muris algorithms provided similar results. Twenty-eight (44%) PCLBCLs had GC status and 36 (56%) had NGC status. All 25 typical PCFCLs-LC (with final 2017 WHO classification) had GC status. All 32 typical PCLBCLs-LT had NGC status. Among the 7 PCLBCLs-U, 3 had GC status and 4 had NGC status. By comparison with the Hans algorithm, the Nyman algorithm provided different results for 3 tumors and the Visco-

Young algorithm for 7. GC/NGC status was associated with the final WHO diagnosis categorization ($P<0.001$). The diagnostic value of GC/NGC status according to sensitivity, specificity, positive-predictive value and negative-predictive value was optimal for Hans, Hans-modified and Muris algorithms (Table 5).

Molecular Biology

The *MYD88*^{L265P} mutation was detected in 25 (39%) PCLBCLs. Among the 32 typical PCLBCLs-LT, 22 (69%) harbored the *MYD88* mutation. Among the 25 typical PCFCLs-LC, none had a detected *MYD88* mutation.

Among the 7 PCLBCLs-U, 3 harbored a *MYD88* mutation: 2 with the PCLBCL-LT phenotype and the last with PCFCL phenotype (CD10⁺).

EBV was sought in 56 PCLBCLs by EBER in situ detection and were negative.

Survival Analyses

After median follow-up of 29.5 months (range: 1–287), 18 of the 64 PCLBCL (28%) patients had died. Death was attributed to the lymphoma for 11 of them (9 PCLBCLs-LT and 2 PCLBCLs-U). Regardless of the criteria (morphology, phenotype) applied for classification, the PCLBCL-LT subgroup had worse OS and SS. NGC status, classified with the Hans, Hans modified or Muris algorithms, had significantly poorer OS and SS than those with GC status ($P=0.0002$ and $P=0.007$ respectively) (Table 5 and Fig. 4A). The 7 patients with PCLBCLs-U, had intermediate OS (Fig. 4B): 2 died of their lymphoma and the 5 others were alive in complete remission. Median PFS was better for PCFCLs-LC (131 months) compared to PCLBCLs, leg type or PCLBCLs-U (32 and 30 months respectively).

Twenty-five (39%) of the 64 patients developed cutaneous relapses with similar percentages for the 3 subgroups (Table 1): 10 PCFCLs-LC; 12 PCLBCLs-LT and 3 PCLBCLs-U. Conversely, among the 11 (17%) patients with extracutaneous spreading, 8 of them had PCLBCLs-LT, and the 3 PCFCLs-LC with extracutaneous spreading had a mixture of centroblasts and centrocytes and no particular phenotype profile. Tumors with dual expression of BCL2 and MYC had significantly worse OS than the others ($P=0.005$). *MYD88* mutation was associated with poorer OS ($P=0.03$) but was not associated with relapse, extracutaneous progression, OS or PFS.

In the PCLBCL-LT subgroup, leg location did not influence relapses or extracutaneous progression ($P=0.379$ and $P=0.327$ respectively) and OS, SS and PFS did not differ between immunoblasts or mixture of centroblast-and-immunoblast cytology patterns. For this group, only MYC expression was significantly associated with cutaneous relapses ($P=0.014$).

The 2 PCLBCL-U patients with disease-attributed deaths did not have a *MYD88* mutation. The other 5 patients, including 3 harboring the *MYD88* mutation, were in complete remission at end point. Finally, no phenotype or molecular criteria were able to predict survival or relapse for this small PCLBCL-U subgroup and GC/NGC status did not impact OS ($P=0.592$).

DISCUSSION

We evaluated the applicability of the 2017 WHO classification criteria for PCLBCLs and found them accurate for classifying for 89% out our 64 tumors categorized as either PCFCLs-LC (n=25) or PCLBCLs-LT (n=32), but 7 (11%) remained unclassified. These PCLBCLs-U had intermediate prognoses between PCFCLs-LC and PCLBCLs-LT, which could suggest

individualizing such cases, but this subgroup was too heterogeneous to be considered an entity.

We analyzed the reproducibility of those 2017 WHO classification criteria to distinguish between PCFCLs-LC and PCLBCLs-LT. The only moderate kappa agreements led us to conclude that microscopic criteria (architecture and cytology) were not very reproducible, in accordance with Grange et al's³⁰ previously reported considerable interobserver variation. Immunohistochemical analyses provided more objective results even with unusual antibodies, eg FOXP1 and IgM (strong kappa agreement). Phenotype categorization as PCFCL-LC or PCLBCL-LT seemed to be more accurate with real impact on outcomes. The Ki67 proliferative index was not discriminant. Alternatively, *MYD88* mutation seemed to be a specific criterion for PCLBCL-LT, as our group previously reported.¹⁴⁻¹⁶ MYC expression was associated with PCLBCL-LT cutaneous relapses which might also correspond to the poorer prognoses of these patients with dual expression of MYC and BCL2.¹⁷

As observed for nodal DLBCLs, we also demonstrated the prognostic impact of GC/NGC status of PCLBCLs, since patients with NGC status had worse prognoses than the GC subgroup. Using immunohistochemical labeling to determine GC/NGC status, the Hans, Hans modified and Muris algorithms distinguished PCLBCLs-LT and PCFCLs-LC with 100% sensitivity and specificity. Moreover, the Hans modified algorithm, which uses only CD10 and MUM1, performed similarly and eliminates the need for BCL6 immunolabeling, which had poor reproducibility among laboratories.³⁴ In skin, interpretation of CD10 immunolabeling requires finesse because of the positivity of dermis fibers, which can lead to false-positives. MUM1 is a reliable nuclear marker that is easy to interpret.

Our PCLBCL-U lesions could correspond to the subgroup previously referred to as "other" by some authors. First, their 11% rate herein is close to that of "other" tumors with round cell morphology but BCL2⁻ and corresponding to 9 (9.7%) of 93 PCLBCLs.⁵ Twenty-six (32.9%)

of 79 PCLBCLs were classified as “other” by Plaza et al.³⁵ In other series (including small and large cell PCBCLs), “other” or “not otherwise specified” cases represented 3.4% – 25%.^{36–38} Discordance of the percentages of “others” highlights the heterogeneity of this subgroup.

Our results also suggest that morphology and phenotype are associated in the majority of PCLBCLs with the GC or NGC status based on the simplified Hans algorithm, with excellent agreement. When excluding morphological criteria for the 7 PCLBCLs-U, it should also be emphasized that the 4 with PCLBCL-LT phenotype had NGC status and the 3 with PCFCL phenotype had GC status, including 1 harboring a *MYD88* mutation, located on the leg. That latter case was indeed considered PCFCL-LC because its tumor cells expressed CD10. Indeed, that case raises the question of whether CD10 positivity is sufficient to diagnose PCFCL-LC and whether such case could not be considered PCLBCL-LT because its *MYD88* mutation.^{14,15} That patient achieved complete remission.

Finally, considering that NGC status was associated with shorter OS and SS for the entire cohort, determining the GC/NGC status could be a valuable diagnostic tool for therapeutic decision-making in this particular PCLBCL-U subgroup. New molecular tools, such as multiplex polymerase chain reaction-based prognostic models developed for DLBCLs could be tested.^{39,40} Indeed, other authors suggested that gene signatures are relevant for the prognosis of intermediate-risk patients with DLBCLs.^{41, 42}

In conclusion, the 2017 WHO classification criteria for PCLBCL diagnosis accurate classification of 89% of our PCFCLs-LC or PCLBCLs-LT tumors. A third provisional PCLBCL-U subgroup corresponded to tumors with discordant morphologies and phenotypes in 11% of the patients. Because morphology had weak reproducibility, our results suggest that the Hans modified algorithm be used to diagnose PCLBCLs.

GC/NGC status also proved relevant for prognosis and may help therapeutic decision, making for these rare discordant PCLBCLs.

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FIGURE LEGEND

FIGURE 1. Diagrams illustrating the 5 individual algorithms used to classify nodal diffuse large B-cell lymphoma into germinal center (GC) or non germinal center (NGC) status subgroups.

FIGURE 2. Histological characteristics of primary cutaneous large B-cell lymphomas (hematoxylin–eosin–safranin, original magnification $\times 400$). **A.** Diffuse infiltration of the dermis by large lymphomatous cells with centroblastic morphology in a primary cutaneous follicle-center lymphoma-large cell (PCFCLs-LC). **B.** Diffuse infiltration of dermis by large lymphomatous cells with immunoblastic cytology in a primary cutaneous large B-cell lymphoma, leg-type (PCLBCL-LT). **C.** Diffuse infiltration of the dermis by large lymphomatous cells with morphology of large spindle-shaped centrocytes in a PCFCL-LC. **D.** Diffuse infiltration of the dermis by large lymphomatous cells with immunoblastic and centroblastic cytology in a PCLBCL-LT. **E.** Diffuse infiltration of the dermis by large lymphomatous cells composed of a mixture of immunoblasts and large spindle-shaped centrocytes in primary cutaneous large B-cell lymphoma-unclassified (PLBCL-U). **F.** Diffuse infiltration of the dermis by large lymphomatous cells composed of a mixture of immunoblasts, centroblasts and centrocytes in a PLBCL-U.

FIGURE 3. Phenotypic characteristics of primary cutaneous large B-cell lymphoma (original magnification $\times 400$). **A.** FOXP1 negative expression in a primary cutaneous follicle center lymphoma, large cell (PCFCL-LC). **B.** FOXP1 positive expression in a primary cutaneous large B-cell lymphoma, leg-type (PCLBCL-LT). **C.** IgM negative expression in a PCFCL-LC. **D.** IgM positive expression in a PCLBCL-LT. **E.** P63 negative expression in a PCFCL-LC. **F.** P63 positive expression in a PCLBCL-LT. **G.** Ki67 high expression in a PCFCL-LC. **H.** Ki67 high expression in a PCLBCL-LT.

FIGURE 4. Survival curves of patients with primary cutaneous large B-cell lymphomas (PCLBCLs) ($n=64$). **A.** Overall survival (OS) according to germinal center (GC)/non-germinal center (NGC) status, established with the Hans algorithm: GC status ($n=28$) and NGC status ($n=36$) differed significantly ($P=0.0002$). **B.** OS according to subgroup: primary cutaneous follicle-center lymphomas, large cell (PCFCLs-LC; $n=25$), PCLBCL-LT (PCLBCLs-LT; $n=32$) or PCLBCL, unclassified (PCLBCLs-U) ($n=7$) differed significantly.

TABLES

TABLE 1. Clinical Characteristics of Primary Cutaneous Large B-Cell Lymphomas according to the Final 2017 WHO-Defined Classification

	PCFCLs-LC	PCLBCLs-LT	PCLBCLs-U	Total
Characteristic	n=25	n=2	n=7	n=64
Age, median [range]	63 [42–86]	82 [61–97]	82 [42–90]	78.5 [42–97]
Male sex, n (%)	18 (72)	9 (28)	2 (29)	29 (45)
Location of skin lesions, n (%)				
Leg	1 (4)	25 (78)	3 (43)	29 (45)
Arm	1 (4)	2 (6)	0	3 (5)
Head and neck	12 (48)	2 (6)	2 (29)	16 (25)
Trunk	11 (44)	3 (9)	2 (29)	16 (25)
Stage, n (%)				
T1	11 (44)	10 (31)	4 (57)	25 (39)
T2	12 (48)	20 (63)	3 (43)	35 (55)
T3	2 (8)	2 (6)	0	4 (6)
Ulceration*, n (%)	5 (20)	9* (30)	0	14* (23)
Months before diagnosis, mean [range]	33.8 [0–240]	5.5 [0–37]	3.1 [0–16]	16.3 [0–240]
Treatment, n (%)				
None	3 (12)	3 (9)	0	6 (9)
Surgery	3 (12)	2 (6)	1 (14)	6 (9)
Radiotherapy	11 (44)	6 (19)	2 (29)	19 (30)
Rituximab	5 (20)	1 (3)	1 (14)	7 (11)
Rituximab–PCT	3 (12)	20 (63)	3 (43)	26 (41)
Response to initial treatment, n (%)				
Complete response	21 (84)	24 (75)	6 (86)	51 (80)
Partial response	1 (4)	2 (6)	0	3 (5)
Stable disease	1 (4)	3 (9)	0	4 (6)
Progression	2 (8)	3 (9)	1 (14)	6 (9)
Skin relapse, n (%)	10 (40)	12 (38)	3 (43)	25 (39)

Median months to relapse [range],	39.6 [3–158]	19.5 [0–102]	25.3 [21–30]	28.24 [0–158]
Extracutaneous spread, n (%)	3 (12)	8 (25)	0	11 (17)
Survival, months				
Median OS	not reached	41	not reached	not reached
Median [range] PFS	131 [3–287]	32 [0–106]	30 [14–65]	102 [0–287]
Status at end point, n (%)				
DOD	0	9 (28)	2 (29)	11 (17)
DOC	0	7 (22)	0	7 (11)
ACR	23 (92)	15 (47)	5 (71)	43 (67)
AWD	2 (8)	1 (3)	0	3 (5)

PCFCLs-LC indicates primary cutaneous follicle center lymphomas, large cell; PCLBCLs-LT: primary cutaneous large B-cell lymphomas, leg type; PCLBCL-U: primary cutaneous large B-cell lymphomas, unclassified; PCLBCL: primary cutaneous large B-cell lymphoma; PCT: polychemotherapy; OS: overall survival; PFS: progression free survival; DOD: died of disease; DOC: died of another cause; ACR: alive in complete remission.

*Data missing for 2 patients.

TABLE 2. Baseline Morphological Features, Immunohistochemical Phenotypes and Molecular Data of Primary Cutaneous Large B-Cell Lymphomas According to 2017 WHO Classification Criteria-Attributed Final Diagnoses

	PCFCLs-LC	PCLBCLs-LT	PCLBCLs-U	PCLBCLs
Parameter	n=25	n=32	n=7	n=64
Architecture, n (%)				
Nodular	7 (28)	0	2 (29)	9 (14)
Nodular and diffuse	6 (24)	14 (44)	2 (29)	22 (34)
Diffuse	12 (50)	18 (56)	3 (43)	33 (52)
Cytology, n (%)				
Majority of centroblasts	6 (24)	0	0	6 (9)
Mixture of centroblasts and centrocytes	11 (44)	0	0	11 (17)
Large spindle-shaped centrocytes	8 (32)	0	1 (14)	9 (14)
Majority of immunoblasts	0	13 (41)	1 (14)	14 (22)
Mixture of immunoblasts and centroblasts	0	19 (59)	3 (43)	22 (34)
Others	0	0	2 (29)	2 (3)
Phenotype, n (%)				
BCL2	7 (28)	32 (100)	6 (86)	45 (70)
MUM1	1 (4)	32 (100)	5 (71)	38 (59)
CD10	14 (56)	0	1 (14)	15 (23)
BCL6	25 (100)	25 (78)	5 (71)	55 (86)
Meshwork of CD21 ⁺ follicular dendritic cells	15 (60)	0	1 (14)	17 (27)
MYC	11 (44)	24 (75)	7 (100)	43 (67)
P63	11 (44)	13 (41)	2 (29)	26 (41)
IgM	2 (8)	14 (44)	2 (29)	18 (28)
FOXP1	2 (8)	25 (78)	4 (57)	31 (48)
Ki67, mean (%) [range]	78 [40–100]	85 [60–100]	83 [60–100]	82 [40–100]
Dual expression (BCL2 ⁺ MYC ⁺)	5 (20)	24 (75)	6 (86)	35 (55)
MYD88 mutation, n (%)	0	22 (69)	3 (43)	25 (39)

PCFCLs-LC indicates primary cutaneous follicle-center lymphomas, large cell; PCLBCLs-LT: primary cutaneous large B-cell lymphomas, leg type; PCLBCLs-U: primary cutaneous large B-cell lymphomas, unclassified; PCLBCLs: primary cutaneous

large B-cell lymphomas; GC: germinal center.

TABLE 3. Morphologies, Phenotypes, Molecular and Clinical Findings and Outcomes of the 7 Unclassified Primary Cutaneous Large B-Cell Lymphomas

Classification		Hans	MYD88	Clinical Features				
Morphology	Phenotype	Phenotyping Results	Status	Mutation	T; site	Treatment	Skin Relapse	Status at End Point
FC	LT	BCL2 ⁺ MUM1 ⁺ CD10 ⁻ BCL6 ⁻ FOXP1 ⁺ IgM ⁻ MYC ⁺ p63 ⁻	NGC	+	T1; leg	RTX-PCT	-	ACR
LT	FC	BCL2 ⁻ MUM1 ⁻ CD10 ⁻ BCL6 ⁺ FOXP1 ⁻ IgM ⁻ MYC ⁺ p63 ⁺	GC	-	T2; neck	RTX-PCT	-	ACR
LT	FC	BCL2 ⁺ MUM1 ⁻ CD10 ⁻ BCL6 ⁺ FOXP1 ⁻ IgM ⁻ MYC ⁺ p63 ⁻	GC	-	T1; trunk	RRX-PCT	+	DOD
LT	FC	BCL2 ⁺ MUM1 ⁺ CD10 ⁺ BCL6 ⁺ FOXP1 ⁺ IgM ⁺ MYC ⁺ p63 ⁺	GC	+	T1; leg	Surgery	+	ACR
U	LT	BCL2 ⁺ MUM1 ⁺ CD10 ⁻ BCL6 ⁻ FOXP1 ⁺ IgM ⁻ MYC ⁺ p63 ⁻	NGC	-	T2; leg	RTX	+	DOD
U	LT	BCL2 ⁺ MUM1 ⁺ CD10 ⁻ BCL6 ⁺ FOXP1 ⁻ IgM ⁻ MYC ⁺ p63 ⁻	NGC	-	T3; trunk	Radiotherapy	-	ACR
U	LT	BCL2 ⁺ MUM1 ⁺ CD10 ⁻ BCL6 ⁺ FOXP1 ⁺ IgM ⁺ MYC ⁺ p63 ⁻	NGC	+	T1; head	Radiotherapy	-	ACR

FC indicates morphological aspect of primary cutaneous follicle-center lymphoma; LT: morphological aspect of primary cutaneous large B-cell lymphoma, leg type; GC: germinal center; NGC: non germinal center; T; pathological T stage; RTX-PCT: rituximab-polychemotherapy; ACR: alive in complete remission; DOD: died of disease.

TABLE 4. Diagnostic Value of Each Marker Used for the 2017 WHO PCLBCL-Classification Criteria, According to Subtype-Matching and -Discrepancy Percentages of the Final Diagnoses

Diagnosis Supported			
Marker	Matching (%)	Discrepancy (%)	P
PCLBCL-leg type			
MUM1	100	4	<0.001
BCL2	100	28	<0.001
FOXP1	78	8	<0.001
IgM	44	8	0.003
MYC	75	44	0.028
P63	41	44	1.000
PCFCL-LC			
CD10	56	0	<0.001
CD21	60	0	<0.001
BCL6	100	78	0.015

PCLBCLs-LT indicates primary cutaneous large B-cell lymphomas, leg type; PCFCLs-LC, primary cutaneous follicle-center lymphomas, large cell.

TABLE 5. Algorithms Defining Germinal Center/Non germinal Center Status, Their Diagnostic Relevance and Their Prognostic Value

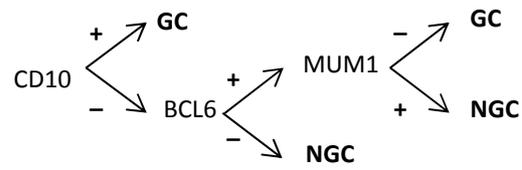
Algorithm	GC Status, n (%)				Diagnostic Value of GC/NGC Status (%) [95% CI%]						P
	PCFCLs-LC, n=25	PCLBCLs-LC, n=32	PCLBCLs-U, n=7	PCLBCL, n=64	Sensitivity	Specificity	PPV	NPV	OS	SS	
Hans	25 (100)	0	3 (43)	28 (44)	100 [100–100]	100 [100–100]	100 [100–100]	100 [100–100]	0.0002	0.007	
Hans modified	25 (100)	0	3 (43)	28 (44)	100 [100–100]	100 [100–100]	100 [100–100]	100 [100–100]	0.0002	0.007	
Muris	25 (100)	0	3 (43)	28 (44)	100 [100–100]	100 [100–100]	100 [100–100]	100 [100–100]	0.0002	0.007	
Nyman	23 (92)	0	2 (29)	25 (39)	100 [100–100]	92 [85–99]	94 [88–100]	100 [100–100]	0.0013	0.022	
Visco-Young	25 (100)	6 (19)	4 (57)	35 (55)	81 [72–91]	100 [100–100]	100 [100–100]	81 [71–90]	0.0001	0.024	

PCFCLs-LC indicates primary cutaneous follicle center lymphomas, large cell; PCLBCLs-LT: primary cutaneous large B-cell lymphomas, leg-type; PCLBCLs-U: primary cutaneous

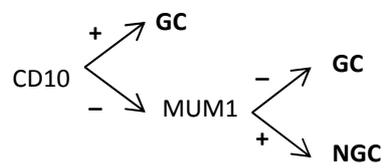
large B-cell lymphomas, unclassified; PCLBCLs: primary cutaneous large B-cell lymphoma; GC: germinal center; NGC: non germinal center; PPV: positive-predictive value; NPV:

negative-predictive value; OS: overall survival; SS: specific survival.

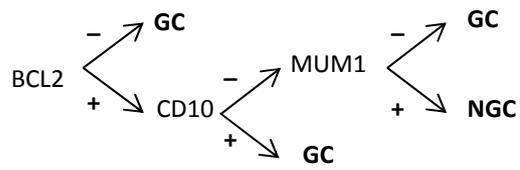
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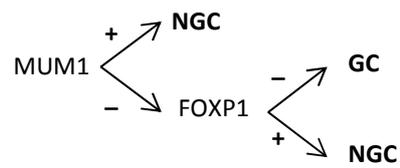
Hans modified



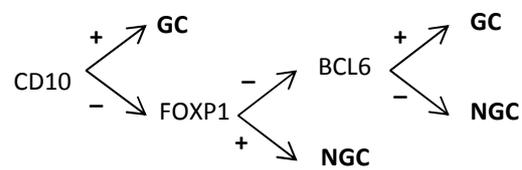
Muris



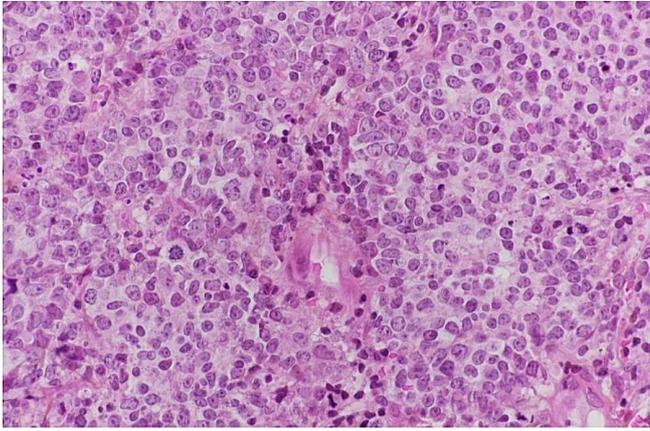
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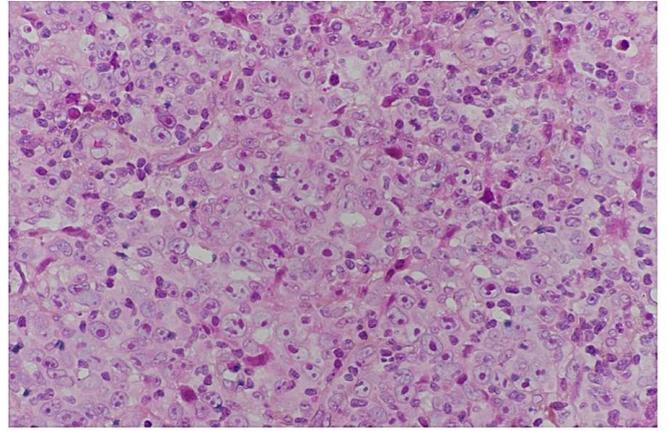
Vico-Young



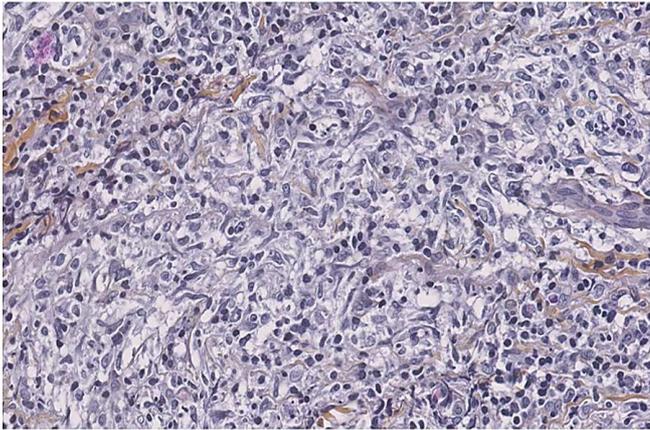
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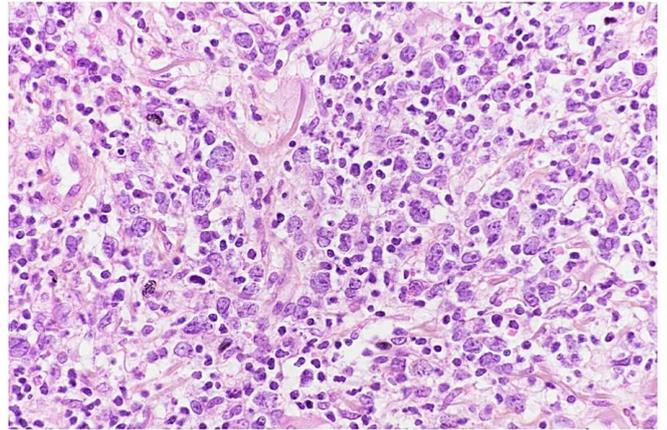
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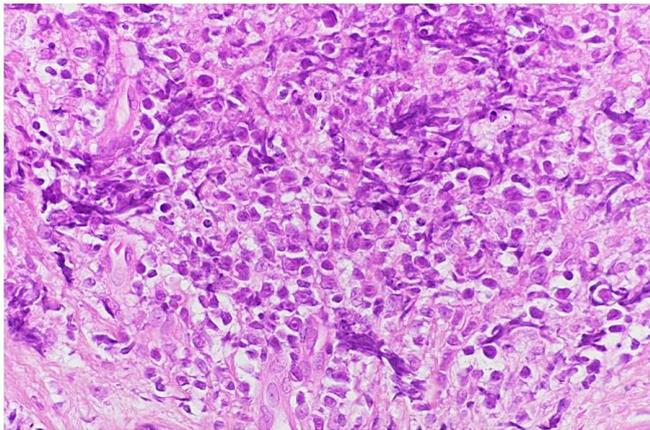
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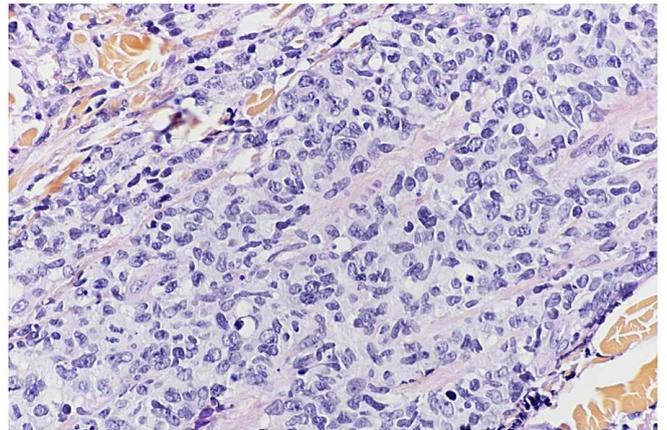
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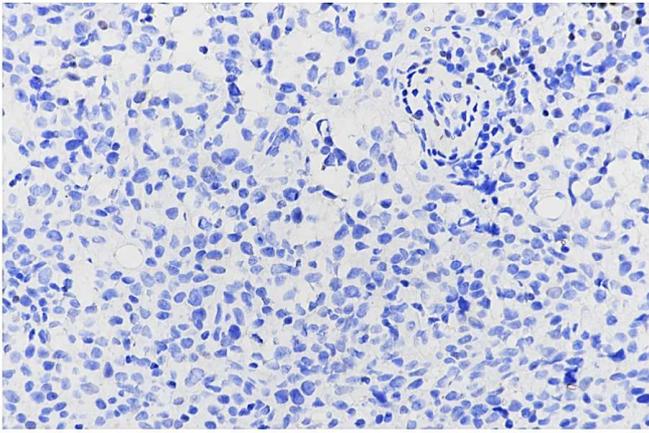
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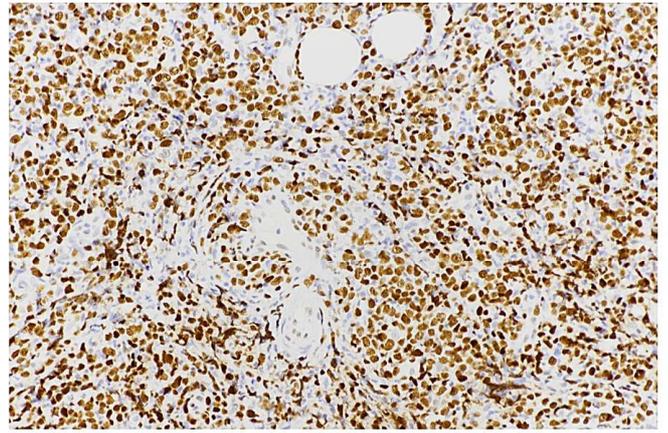
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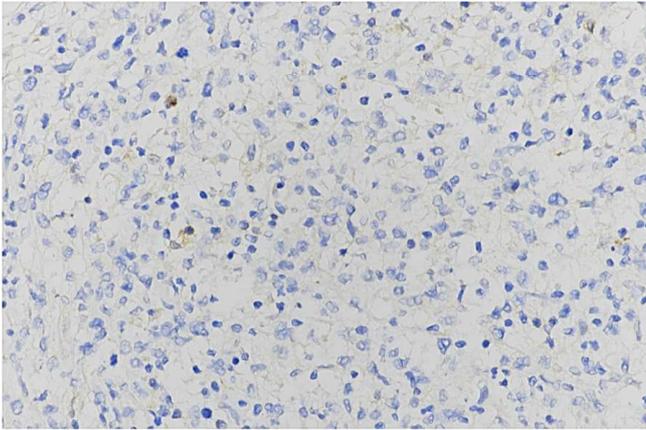
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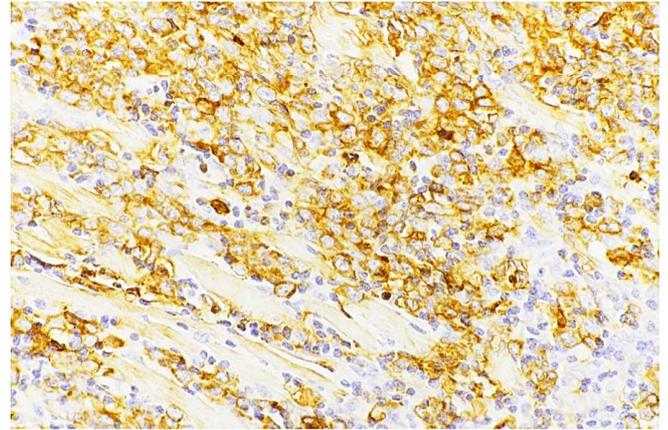
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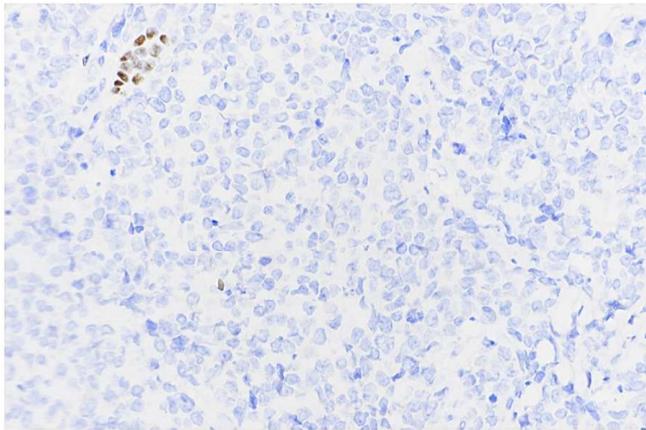
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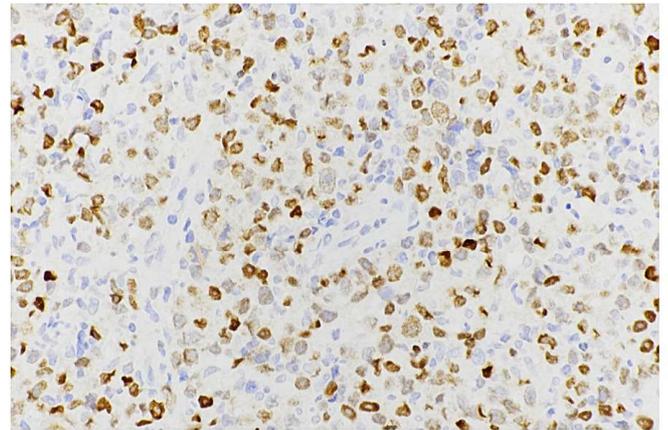
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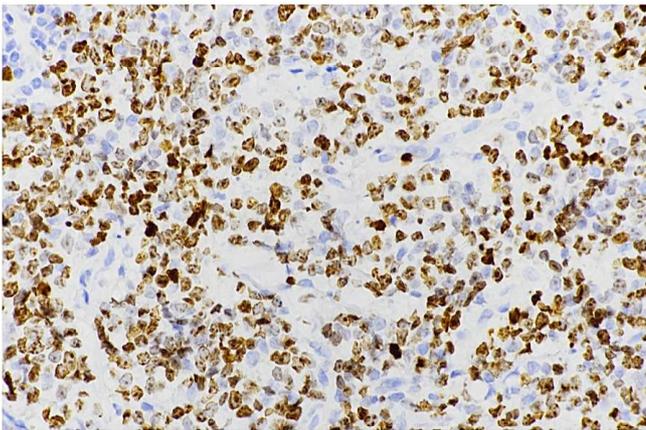
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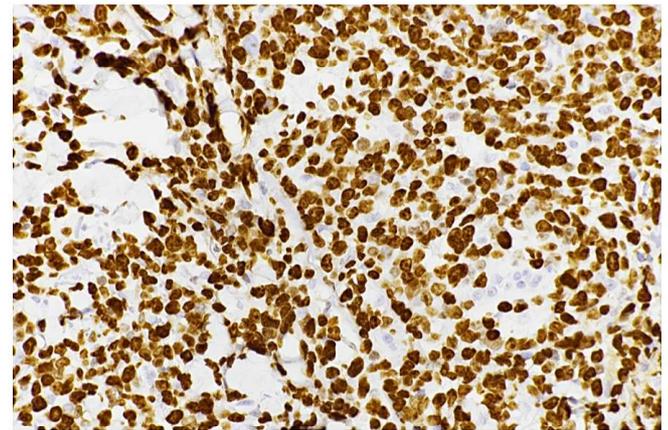
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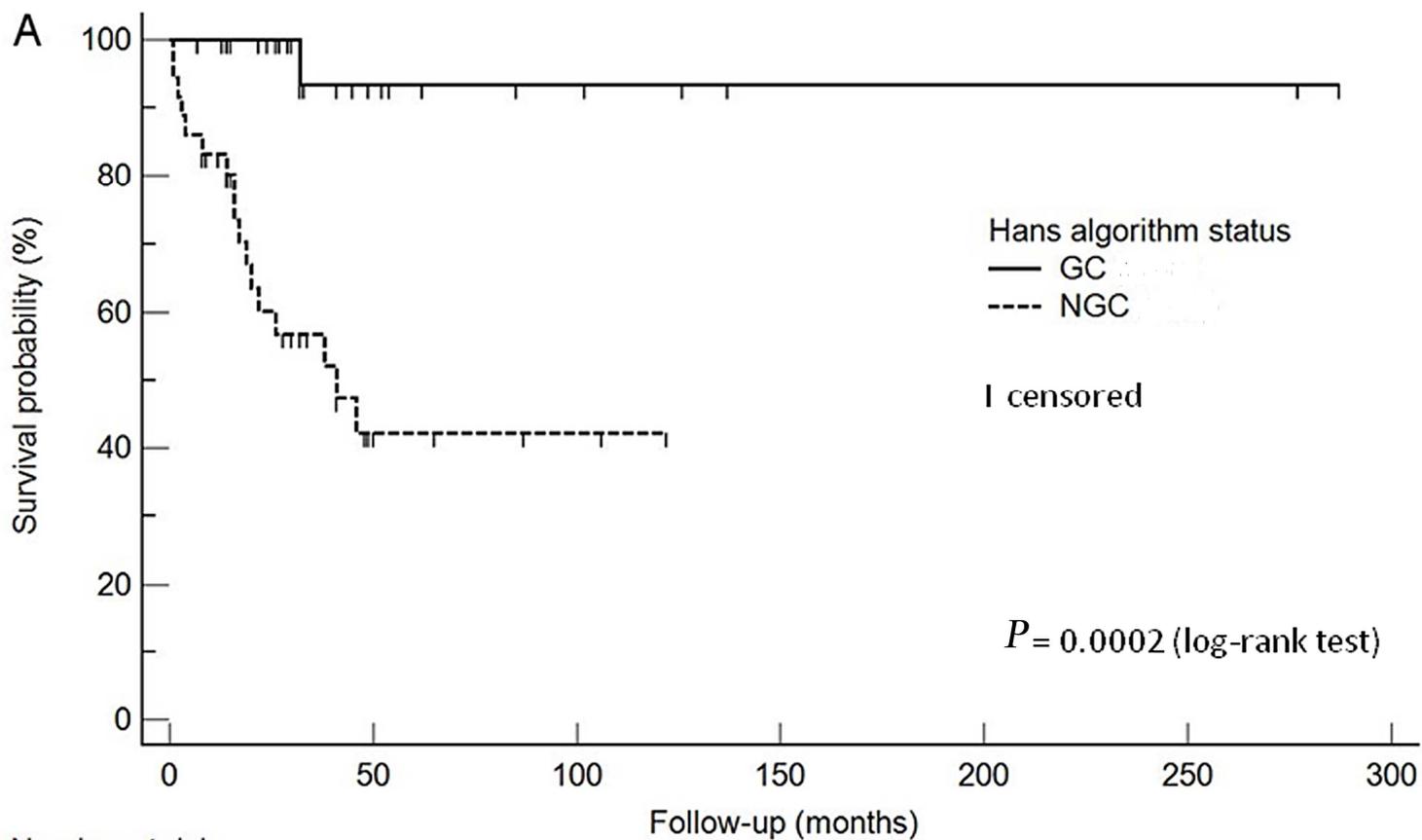


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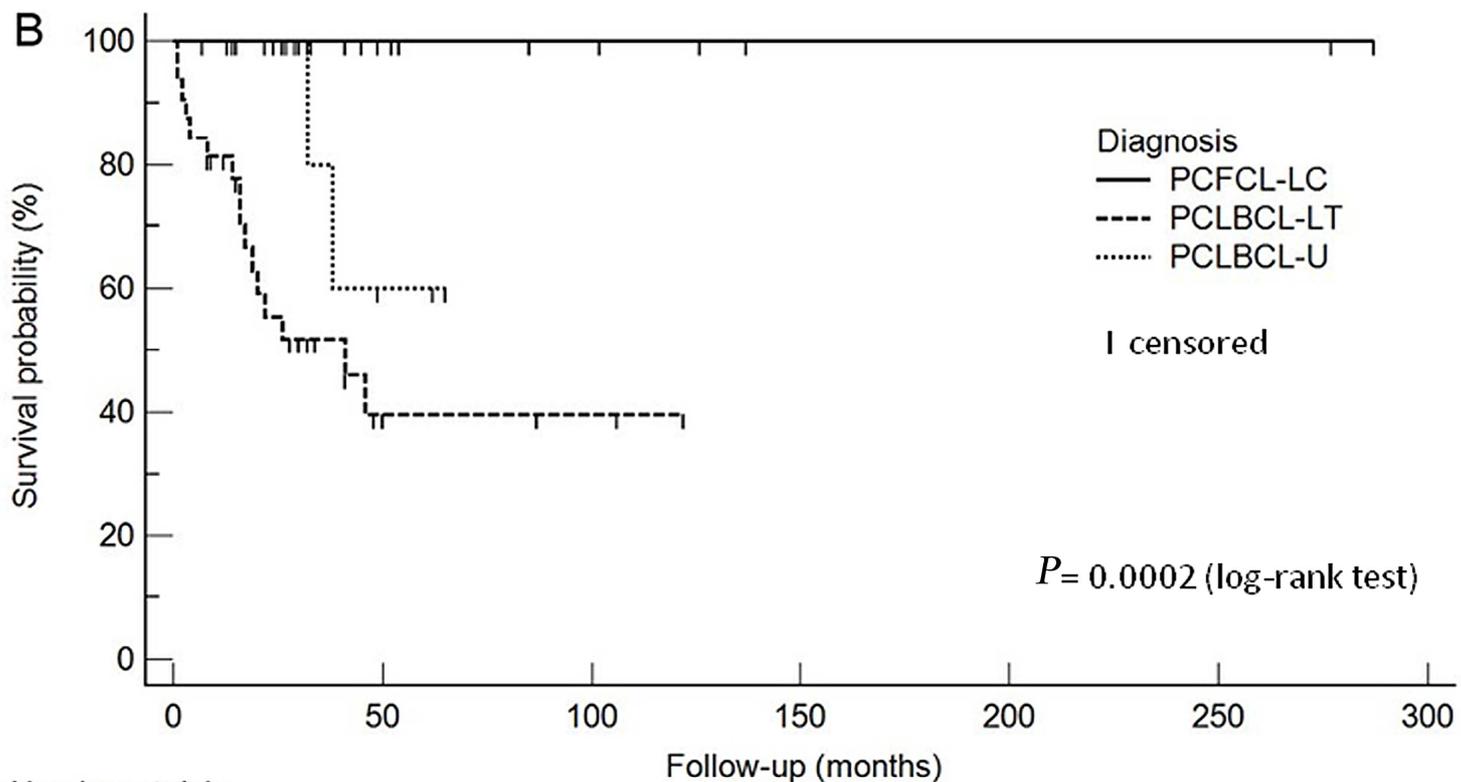
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Number at risk

Group	0	50	100	150	200	250	300
Group: GC	28	9	5	2	2	2	0
Group: NGC	36	4	2	0	0	0	0



Number at risk

Group	0	50	100	150	200	250	300
Group: PCFCL-LC	25	8	5	2	2	2	0
Group: PCLBCL-LT	32	3	2	0	0	0	0
Group: PCLBCL-U	7	2	0	0	0	0	0

4. ARTICLE 2

4.1. Introduction à l'ARTICLE 2

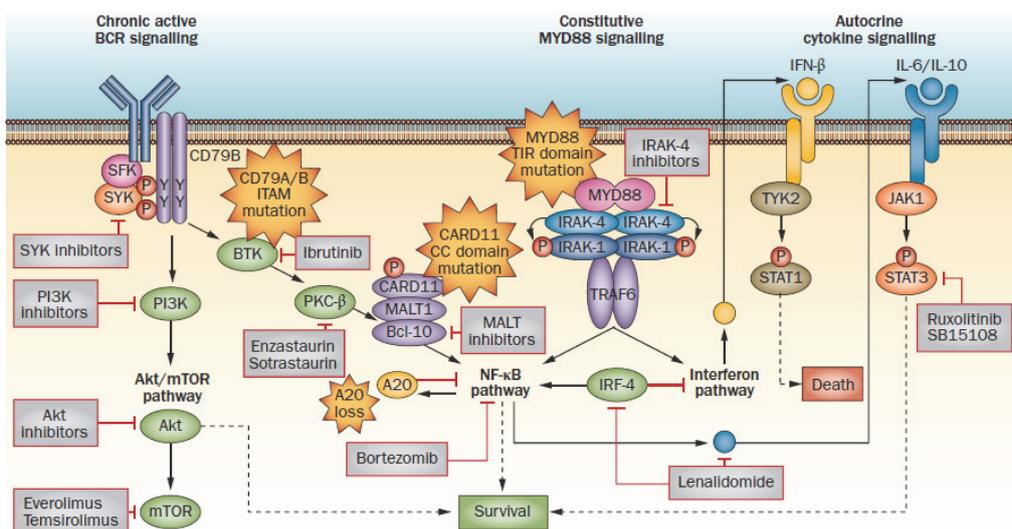
4.1.1. Anomalie moléculaire principale des LBTJ : la mutation *MYD88*

D'un point de vu moléculaire, il a été montré qu'une proportion importante de LBTJ présentait la mutation *MYD88*^{L265P} (69%).³¹ Il a été démontré que la mutation *MYD88*^{L265P} était associée à un moins bon pronostic, indépendamment du traitement reçu.³² Il existe également une association avec des facteurs cliniques liés à cette maladie (localisation à la jambe et âge avancé).

4.1.2. Généralités sur la mutation *MYD88*

Ngo et al ont mis en évidence la présence de mutation activatrice de l'oncogène impliquant le gène *MYD88*, qui encode pour une protéine adaptatrice des Toll-like récepteur dans 29% des cas de lymphomes B diffus à grandes cellules ganglionnaires.³³ Cette mutation activatrice impute la liaison du Toll-like récepteur à un complexe de signalisation composé de sous unités appartenant à la famille de sérine/thréonine kinase IRAK (Interleukine-receptor-associated kinase), IRAK1 et IRAK4. Alors le complexe IRF4 est surexprimé, ce qui entraîne une stimulation de la voie NF- κ B (survie cellulaire), l'inhibition de la voie interféron B (mort cellulaire) et la sécrétion d'IL-6/IL-10 (survie cellulaire).

Figure 5 : Voies de signalisation impliquées dans le LBDGC avec notamment l'implication de la mutation *MYD88* (Roschewski M. *et al. Nat. Rev. Clin. Oncol.* 2014)



La mutation *MYD88* est peu fréquente dans les LBDGC ganglionnaires mais elle est souvent retrouvée dans les LBDGC extra-ganglionnaires du système nerveux central (75%) et du testicule (71%).³⁴ On la retrouve également dans la maladie de Waldenström (lymphome lymphoplasmocytaire) dans plus de 90% des cas.^{35,36}

4.1.3. La mutation *MYD88* : critère diagnostique des LBPC-GC ?

Nous avons recherché la mutation *MYD88* dans une série de 46 LBPC-GC, composée de 21 LBCF-GC et 25 LBTJ. Les cas inclus dans cette étude étaient identiques à ceux inclus dans l'ARTICLE 1 mais ce travail ayant été publié avant, le nombre de cas était moins important (46 versus 64 patients). La question posée par ce travail était de savoir si la mutation *MYD88* décrite en proportion élevée dans les LBTJ était retrouvée dans les LBCF-GC et dans quelle mesure cette analyse permettait d'améliorer la classification des LBPC-GC ?

Ce travail a fait l'objet d'un article publié dans *Journal of Investigative Dermatology* en 2016 :

MYD88 somatic mutation is a diagnostic criterion in primary cutaneous large B-cell lymphoma. Menguy S, Gros A, Pham-Ledard A, Battistella M, Ortonne N, Comoz F, Balme B, Szablewski V, Lamant L, Carlotti A, Lorton MH, de Muret A, Le Gall F, Franck F, Croue A, Cappellen D, Beylot-Barry M, Merlio JP, Vergier B. *J Invest Dermatol.* 2016 Aug;136(8):1741-4.

4.2. ARTICLE 2

conjunctival lesions. *J Invest Dermatol* 2015; 135:304–8.

Martincorena I, Roshan A, Gerstung M, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 2015;348:880–6.

South AP, Purdie KJ, Watt SA, et al. NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. *J Invest Dermatol* 2014;134:2630–8.

Su F, Viros A, Milagre C, et al. RAS mutations in cutaneous squamous-cell carcinomas in pa-

tients treated with BRAF inhibitors. *New Engl J Med* 2012;366:207–15.

Wang NJ, Sanborn Z, Arnett KL, et al. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. *Proc Natl Acad Sci USA* 2011;108:17761–6.



MYD88 Somatic Mutation Is a Diagnostic Criterion in Primary Cutaneous Large B-Cell Lymphoma

Journal of Investigative Dermatology (2016) 136, 1741–1744; doi:10.1016/j.jid.2016.04.018

TO THE EDITOR

Primary cutaneous B-cell lymphoma (PCBCL) have been divided into the following three 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). Although PCBCL in the last category display an aggressive behavior requiring a combination of rituximab and polychemotherapy (Grange et al., 2014), frontline therapy for PCBCLs in first two categories, which have an indolent course, consists of 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 systemic therapy (Senff et al., 2007). Moreover, differential diagnosis between PCDLBCL-LT and PCFCL may be challenging, especially when PCFCLs exhibit large-cell morphology. Hierarchical determination of CD10, Bcl-6 and MUM1 expression according to the Hans algorithm (Hans et al., 2004) is not a reliable tool for cutaneous B-cell lymphoma diagnosis, because CD10 expression may be lost in PCFCL, and Bcl-6 may be expressed as frequently in PCDLBCL-LT than in PCFCL (Dewar et al., 2015). Moreover, this algorithm does not include Bcl-2 immunostaining, which is a major diagnostic and

Table 1. Clinical findings at diagnosis, immunohistochemistry data concerning Bcl-2 and MUM1 and results of MYD88 mutation status in two groups: PCFCL with large-cell morphology and PCLBCL-LT

Characteristic	PCFCL-LC	PCLBCL-LT
Patients, n	21	25
Median age, years	62.3	83.3
Male, n (%)	15 (71.4)	7 (28.0)
Female, n (%)	6 (28.6)	18 (72.0)
Location, n (%)		
Leg, n (%)	2 (9.5)	20 (80.0)
Elsewhere, n (%)	19 (90.5)	5 (20.0)
Phenotype, n (%)		
Bcl-2 expression by > 50% cells	4 (19.0)	25 (100.0)
MUM1 expression by > 80% cells	0	20 (80.0)
MYD88 status, n (%)		
MYD88 L265P mutation	0 (0.0)	19 (76.0)
MYD88 wild type	21 (100.0)	6 (24.0)

Abbreviations: PCFCL-LC, primary cutaneous follicle center lymphoma with large-cell morphology; PCLBCL-LT, primary cutaneous large B-cell lymphoma-leg type.

prognostic factor for PCLBCL (Grange et al., 2004).

MYD88, a gene encoding for an adaptor protein of both Toll-like receptors and IL-1 receptors was recently shown to exhibit driver oncogenic mutations in nodal activated B-cell diffuse large-cell lymphoma, resulting in NF- κ B signaling pathway activation (Ngo et al., 2010). We previously reported the high prevalence of MYD88^{L265P} mutation in more than 60% of 58 PCDLBCL-LT patients studied using a robust and highly sensitive, allele-specific PCR

technique (Pham-Ledard et al., 2014), but others described a lower rate of 40% in 10 patients (Koens et al., 2014). Whether such assessment could discriminate PCFCLs with large-cell morphology from PCDLBCL-LTs has not been evaluated. Only three patients with PCFCLs with large-cell morphology were included in our initial study, and we found that these PCFCLs exhibited a wild-type MYD88 status by 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 through 2015. These cases were compared with 25 cases of PCDLBCL-LT recorded from 2004 through 2015, including four cases

Abbreviations: GC, germinal center; 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

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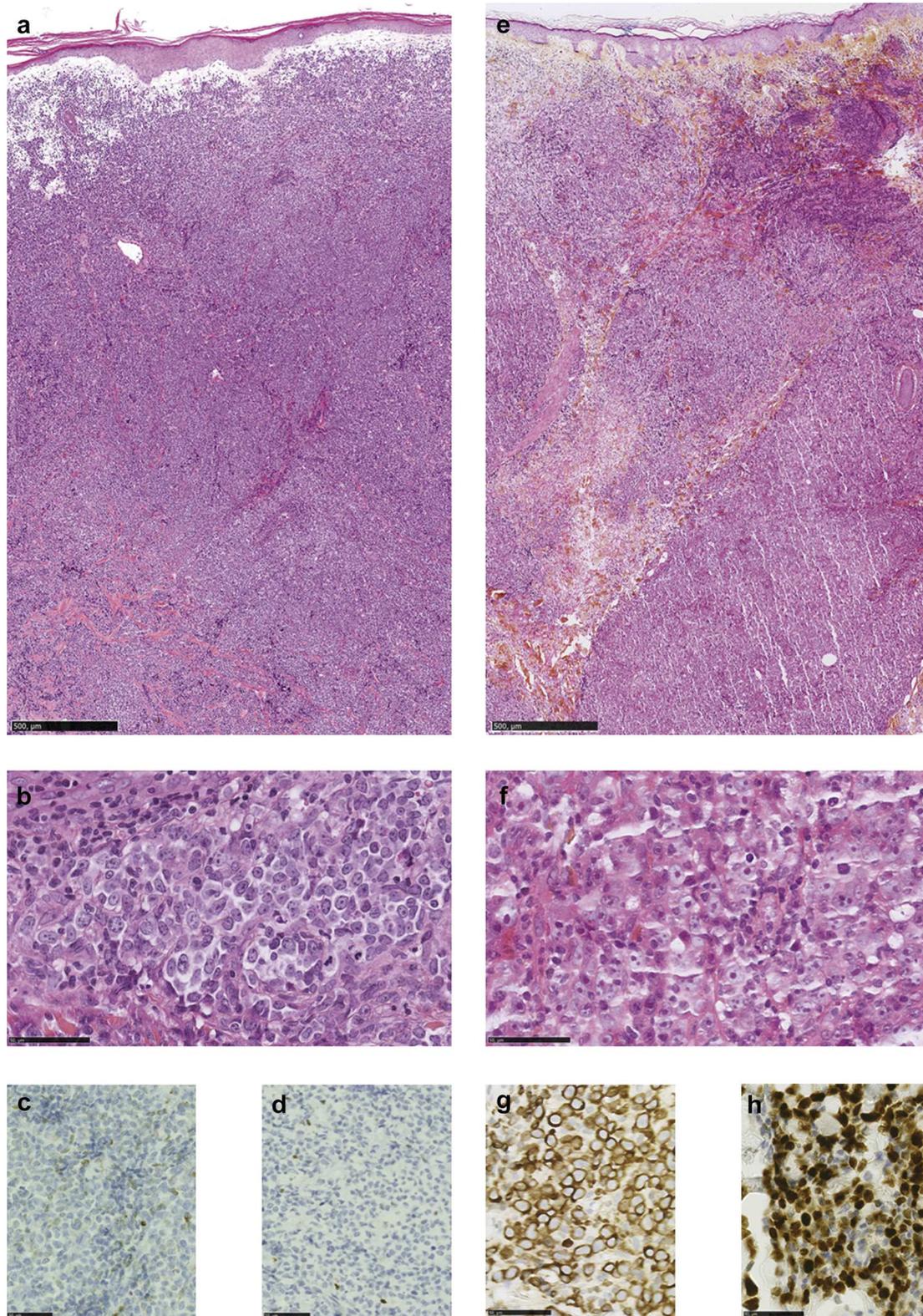


Figure 1. Two examples of primary cutaneous large B-cell lymphoma subtypes included in this study. Left panel, primary cutaneous follicle center lymphoma with large-cell morphology. (a) Diffuse infiltration of the dermis by lymphomatous cells (HE&S, original magnification $\times 100$). (b) Large tumor cells with centroblastic morphology and small nucleoli (HE&S, original magnification $\times 400$). (c) Negative Bcl-2 immunostaining result (original magnification $\times 400$). (d) Negative MUM1 immunostaining result (original magnification $\times 400$). Right panel, primary cutaneous large B-cell lymphoma-leg type. (e) Diffuse infiltration of the dermis by lymphomatous cells (HE&S, original magnification $\times 100$). (f) Large tumor cells with immunoblastic morphology and prominent nucleolus (HE&S, original magnification $\times 400$). (g) Strong Bcl-2 immunostaining result (original magnification $\times 400$). (h) Positive of MUM1 immunostaining result (original magnification $\times 400$). Scale bars for **a** and **e** = 0.5 mm. Scale bars for **b**, **c**, **d**, **f**, **g** and **h** = 0.05 mm. HE&S, hematoxylin, eosin and safranin staining.

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 (Sigma-Aldrich, Saint-Quentin Fallavier, France) as reported (Pham-Ledard et al., 2014).

All patients had primary cutaneous lymphoma and the initial staging results, including computed tomography scan, were negative. Both clinical and histopathological features are summarized in Table 1. The sex ratio (male to 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. Although Bcl-2 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 Bcl-2 (81%) and MUM1 (100%) expression when using a cut-off criteria of 50% for Bcl-2 and 80% for MUM1 (Figure 1). 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). MUM1 expression was observed in less than 80% of tumor cells but more than 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 Bcl-2 immunostaining result in more than 50% of tumor cells, only one of them occurring at the leg site. These four cases, however, all had negative results for MUM1 expression. A MYD88^{L265P} mutation was detected in 19 of 25 (76%) PCDLBCL-LT cases, with a slight difference between those developing at the leg site (16/20, 80%) and those developing at other sites (3/5, 60%) (Table 1). Interestingly, the MYD88^{L265P} mutation was never detected in the 21 PCFCL cases. Both specificity and positive predictive value for MYD88 status were determined to be 100%,

and negative predictive value was 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 differences 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 poor prognosis factor in patients with PCLBCL-LT, whatever treatment they have received (Pham-Ledard et al., 2014). This feature was also recently reported in patients with nodal or systemic diffuse large B-cell lymphoma 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 frontline therapy in association with polychemotherapy (Grange et al., 2014), a significant proportion of these patients will relapse and have lymphoma dissemination to extracutaneous sites. Therefore, determination of MYD88 status, along with other mutations, could also be relevant to identify patients who could respond to specific therapy targeting either the B-cell antigen receptor, JAK/signal transducer and activator of transcription, or NF- κ B signaling pathways (Wilson et al., 2015). Altogether, the present study supports that MYD88 status is a necessary biomarker in patients with PCBCLs for diagnosis, prognosis, and management.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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5. ARTICLE 3

5.1. Introduction à l'ARTICLE 3

5.1.1. Lymphome B de haut grade ganglionnaire double/triple hit

Les LBDGC ganglionnaires présentant un réarrangement de *MYC* et *BCL2* et/ou *BCL6* font à présent partie d'une entité à part dans la nouvelle classification WHO 2017 : lymphome B de haut grade. Il a en effet été montré que ces lymphomes double ou triple hit sont plus fréquemment de sous-type GC (entre 60 et 95% environ).^{37,38} Ils sont de plus mauvais pronostic que les LBDGC ganglionnaires sans réarrangement.³⁹⁻⁴¹

5.1.2. Lymphome B diffus à grandes cellules ganglionnaire double expresseur

Les LBDGC ganglionnaires ayant un profil double expresseur sont un sous-groupe de LBDGC qui co-exprime *BCL2* et *MYC*. Ils sont le plus souvent de sous-type non-GC selon l'algorithme de Hans (entre 70% et 80% environ).^{42,43} Ils ont été individualisés car ils présentent un pronostic péjoratif^{37,38,44,45} et notamment un risque augmenté d'extension au système nerveux central.⁴⁶

5.1.3. Le statut double hit et le profil double expresseur dans les LBPC-GC

Une troisième étude a consisté à évaluer le statut double hit et le profil double expresseur dans une série de 44 LBPC-GC constituée de 23 LBTJ et 21 LBCF-GC. Nous avons recherché une corrélation entre le statut double hit, le profil double expresseur et le sous-type histologique puis nous avons évalué si ces sous-groupes avaient un impact pronostique.

Ce travail a fait l'objet d'un article publié dans *Modern Pathology* en 2018 :

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5.2. ARTICLE 3



Double-hit or dual expression of MYC and BCL2 in primary cutaneous large B-cell lymphomas

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Abstract

In nodal diffuse large B-cell lymphoma, the search for double-hit with *MYC* and *BCL2* and/or *BCL6* rearrangements or for dual expression of *BCL2* and *MYC* defines subgroups of patients with altered prognosis that has not been evaluated in primary cutaneous large B-cell lymphoma. Our objectives were to assess the double-hit and dual expressor status in a cohort of 44 patients with primary cutaneous large B-cell lymphoma according to the histological subtype and to evaluate their prognosis relevance. The 44 cases defined by the presence of more than 80% of large B-cells in the dermis corresponded to 21 primary cutaneous follicle centre lymphoma with large cell morphology and 23 primary cutaneous diffuse large B-cell lymphoma, leg type. Thirty-one cases (70%) expressed *BCL2* and 29 (66%) expressed *MYC*. Dual expressor profile was observed in 25 cases (57%) of either subtypes ($n = 6$ or $n = 19$, respectively). Only one primary cutaneous follicle centre lymphoma, large-cell case had a double-hit status (2%). Specific survival was significantly worse in primary cutaneous diffuse large B-cell lymphoma, leg type than in primary cutaneous follicle centre lymphoma, large cell ($p = 0.021$) and for the dual expressor primary cutaneous large B-cell lymphoma group ($p = 0.030$). Both overall survival and specific survival were worse for patients belonging to the dual expressor primary cutaneous diffuse large B-cell lymphoma, leg type subgroup ($p = 0.001$ and $p = 0.046$, respectively). Expression of either *MYC* and/or *BCL2* negatively impacted overall survival ($p = 0.017$ and $p = 0.018$ respectively). As the differential diagnosis between primary cutaneous follicle centre lymphoma, large cell and primary cutaneous diffuse large B-cell lymphoma, leg type has a major impact on prognosis, dual-expression of *BCL2* and *MYC* may represent a new diagnostic criterion for primary cutaneous diffuse large B-cell lymphoma, leg type subtype and further identifies patients with impaired survival. Finally, the double-hit assessment does not appear clinically relevant in primary cutaneous large B-cell lymphoma.

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Introduction

Primary cutaneous B-cell lymphoma have been divided into the 3 following groups: primary cutaneous marginal zone lymphoma, primary cutaneous follicle centre lymphoma and primary cutaneous diffuse large B-cell lymphoma, leg type [1, 2]. Differential diagnosis between these entities is crucial to adapt the therapy. Primary cutaneous marginal zone lymphoma and primary cutaneous follicle centre lymphoma are mostly small B-cell lymphomas forming unique or multiple skin lesions. A conservative treatment with excision and/or radiotherapy for localized disease, or Rituximab for diffuse skin involvement permit to achieve complete response with a 95% 5-year survival despite frequent relapses [3]. On the other hand, primary cutaneous diffuse large B-cell lymphoma, leg type displays an aggressive behavior despite therapeutic improvement by the combination of rituximab and age-adjusted chemotherapy reaching a 74% 5-year overall survival [4]. In these patients, refractory disease or relapse are associated with a high risk of secondary extracutaneous spreading as opposed to primary cutaneous follicle centre lymphoma where relapse may occur without impairing prognosis [4, 5]. Question still remains whether cutaneous large B-cell lymphomas with borderline features between primary cutaneous follicle centre lymphoma and primary cutaneous diffuse large B-cell lymphoma, leg-type could define a distinct category [6–8]. Indeed, the 2005 WHO/EORTC classification individualized a primary cutaneous large B-cell lymphoma “other” subgroup for rare cases not belonging to the two categories [1]. Conversely, the most recent WHO classification considered these cases as a large cell and diffuse variant of primary cutaneous follicle centre lymphoma on the basis of their clinical features and favorable outcome [2, 9]. For the pathologist, the accurate classification of primary cutaneous B-cell lymphoma displaying diffuse pattern and predominant large cell morphology is therefore an important challenge and there is an obvious need for evaluating new diagnostic criteria. Moreover, a study of 161 cases of primary cutaneous B-cell lymphoma other than primary cutaneous marginal zone lymphoma individualized 40 cases (30%) corresponding to primary cutaneous large B-cell lymphoma not otherwise specified with an intermediate behavior between primary cutaneous follicle centre lymphoma and primary cutaneous diffuse large B-cell lymphoma, leg type according to their cell of origin profile determined by the immunohistochemical Hans’ algorithm [6, 10].

Indeed, the hierarchical determination of CD10, BCL6 and MUM1 expression [10] is compulsory in nodal diffuse large B cell lymphoma as the non-germinal centre subtype also called activated B-cell displays more

aggressive clinical and evolutive features than the germinal centre subtype [11, 12]. In primary cutaneous B-cell lymphoma, gene expression profiling has shown that primary cutaneous follicle centre lymphoma derives from germinal centre cells while most primary cutaneous diffuse large B-cell lymphoma, leg type cases are of non-germinal centre subtype [13]. However, the Hans’ algorithm may not permit the differential diagnosis as non-germinal centre type of primary cutaneous follicle centre lymphoma with large cell morphology or germinal centre type of primary cutaneous diffuse large B-cell lymphoma, leg type cases have been reported [6, 14, 15]. Indeed, the WHO classification textbook points to some limitations in using the immunohistochemical algorithm for primary cutaneous B-cell lymphoma classification as CD10 expression may be lost in primary cutaneous follicle centre lymphoma, large cell while BCL6 may be expressed as frequently in primary cutaneous diffuse large B-cell lymphoma, leg-type as in primary cutaneous follicle centre lymphoma [2, 16]. Among primary cutaneous B-cell lymphoma with large cell morphology, the combined BCL2 and MUM1 expression together with a high proliferative index as assessed by Ki-67 immunostaining have been employed as diagnostic features of primary cutaneous diffuse large B-cell lymphoma, leg type [16, 17]. TP63 expression and the *MYD88*^{L265P} mutation have also been proposed as criteria for primary cutaneous diffuse large B-cell lymphoma, leg-type diagnosis as they are not present in primary cutaneous follicle centre lymphoma, large-cell [18, 19].

Interestingly, diffuse large B-cell lymphoma with double-hits involving *MYC* and *BCL2* and/or *BCL6* rearrangement are now classified in the high grade B-cell lymphomas category according to their inferior survival [2, 20–22]. The co-expression of BCL2 and MYC in DLBCL without double-hit is now referred to as dual expressor diffuse large B-cell lymphoma, a subgroup more frequently observed in the non-germinal centre-type diffuse large B-cell lymphoma [23–25]. Patients with dual expressor-diffuse large B-cell lymphoma have an altered prognosis and an increased risk of central nervous system relapse [25–27]. The parallel evaluation of double-hit and dual expression has not been so far evaluated in primary cutaneous B-cell lymphoma with large cell morphology.

Therefore, we retrieved in the French Study group on cutaneous lymphoma database a series of typical cases corresponding to either primary cutaneous follicle centre lymphoma, large cell or to primary cutaneous diffuse large B-cell lymphoma, leg type. Our goal was to determine if double-hit or dual expressor status correlates with the histological subtype and may identify a subgroup of patients with specific evolutive features.

Methods

Patient selection

Primary cutaneous B-cell lymphoma containing at least 80% large tumoral cells were prospectively reviewed and included between 1993 and 2016 in the database of the French Study group on cutaneous lymphomas between 1993 and 2016. We retrieved cases on the basis of complete clinical record and available formalin-fixed paraffin-embedded skin biopsy to perform fluorescent in situ hybridization (FISH) and immunochemical techniques. Diagnoses were reevaluated according to the 2016 WHO classification criteria which does not retain an “other” category to finalize the primary diagnosis into two separate categories [2]. Primary cutaneous diffuse large B-cell lymphoma, leg type exhibited a diffuse pattern with immunoblastic and centroblastic features with strong expression BCL2 and MUM1. The presence of the *MYD88*^{L265P} mutation was also used as a diagnostic criterion [18]. Primary cutaneous follicle centre lymphoma, large-cell were selected as diffuse and/or nodular infiltration by large cells with centroblastic and/or centrocytic features associated with low expression or negativity for BCL2 and MUM1. Six cases of primary cutaneous diffuse large B-cell lymphoma, leg-type have been already investigated by FISH by Pham-Ledard et al. [28] and 35 cases for *MYD88* status by Menguy et al. [18].

International staging criteria for B-cell cutaneous lymphomas were used and secondary cutaneous lymphoma excluded [29]. The study was performed after registration of absence of opposition according to the guidelines of the French Bioethical law for retrospective non interventional research studies.

Immunohistochemistry

Automated immunostaining was performed on formalin-fixed paraffin-embedded slides (Bond Max, Leica, Nanterre, France). We tested BCL2 (clone 124, DAKO, Les Ulis, France), BCL2 (clone E17, Gene Tex, Irvine, CA), MUM1 (clone MUM1p, DAKO), BCL6 (clone PGB6P, DAKO), Ki-67 (clone MM1, Leica Biosystems, Newcastle Ltd, UK), and MYC (clone Y69, Epitomics, Burlingame, CA). BCL2 (clone 124), BCL6, Ki-67, and MUM1 immunostainings were part of the original diagnosis criteria. MYC immunochemistry was performed during 2016 and BCL2 clone E17 during June 2017. BCL2 and MUM1 were considered positive if more than 50% of the cells were stained. A 40% positive cells threshold was used for MYC according to criteria used in diffuse large B-cell lymphoma [2, 23]. Each immunohistochemical staining was independently interpreted by 3 pathologists (SM, BV, and JPM). In case of

discrepancy, slides were reviewed together to reach agreement. The immunohistochemical scoring was performed without knowledge of FISH results or patient outcome.

Interphase fluorescence in situ hybridization (FISH)

Such analysis was performed in June and July 2017, on formalin-fixed paraffin-embedded slides as reported previously [28]. Hybridization was conducted with labeled probes for *BCL2*, *BCL6*, and *MYC* (FISH DNA split signal, DAKO). Slides were analyzed with a fluorescence microscope (Imager Z2, Zeiss, Jena, Germany) by two independent observers (SM and JPM). In case of discrepancy, slides were reviewed together to reach agreement. Images were collected using workstation equipped with Isis software (Metasystems, Altussheim, Germany). FISH patterns were recorded by analyzing 100 non-overlapped tumor cell nuclei and abnormal patterns were taken into account above a 10% threshold. We investigated gene breakpoint with split of red and green signal and copy number variation such as extra copy (more than 2 fusions pattern). Low copy-number gain was evaluated as more than 3 copies and less than 6 copies and amplification as more than or equal to 6 copies.

MYD88 status determination

The *MYD88* status was assessed by allele specific real time PCR as reported elsewhere [30]. The *MYD88* status was reported in 35 patients by Menguy et al. [18] and determined for 9 patients of the present series.

Statistical analysis

Quantitative characteristics were described by median and range, and qualitative characteristics were described by number and frequency. Comparisons of qualitative characteristics between subgroups of patients were performed using Fisher’s exact test. Progression free survival was calculated as the time between the date of diagnosis and the date of recurrence or death. Overall survival was determined from the date of diagnosis to the date of death of any cause. Specific survival duration was calculated from diagnosis to date of disease-related death, with patients whose death was unrelated to lymphoma considered censored at death time. Both overall survival and specific survival were evaluated considering the advanced age of the patients, especially those with primary cutaneous diffuse large B-cell lymphoma, leg type. The administrative date of end of follow-up was set to 1 August 2017. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test for each prognostic factor (diagnosis type, dual expressor status, double-hit status). A two-sided $p < 0.05$ was considered significant. Statistical analysis was

performed using MedCalc software, version 12.4.0 (Ostend, Belgium).

Results

Cohort

The multicentre study analyzed the features of a series of 44 patients with primary cutaneous large B-cell lymphoma defined by the presence of more than 80 per cent large cells. Patients with primary cutaneous follicle centre lymphoma, large cell ($n = 21$) were 5 women (24%) and 16 men (76%) with median age at diagnosis of 64 years (range: 42–86). Patients with primary cutaneous diffuse large B-cell lymphoma, leg type ($n = 23$) were 15 women (65%) and 8 men (35%) with a median age at diagnosis of 82 years (range: 61–95).

In the 21 patients with primary cutaneous follicle centre lymphoma, large cell, nine (43%) were at T1 stage, 10 (48%) at T2 stage and 2 (9%) at T3 stage. The most frequent localization was head and neck (52%, $n = 11$). The other localizations were trunk (38%, $n = 8$), leg (5%, $n = 1$) and arm (5%, $n = 1$).

Among the 23 patients with primary cutaneous diffuse large B-cell lymphoma, leg type, six (26%) had a solitary lesion (T1), 15 (65%) had a regional skin involvement (T2) and 2 (9%) had generalized skin involvement (T3). The most frequent site was the leg (78%, $n = 18$). The other sites were arms (9%, $n = 2$), trunk (9%, $n = 2$) and head and neck (4%, $n = 1$).

All patients had primary cutaneous lymphoma and the initial staging including computed tomography scan was negative. Clinical data are summarized in Table 1.

Histological features

Among the 21 primary cutaneous follicle centre lymphoma, large cell cases, 10 cases (48%) showed a diffuse pattern, 5 cases (24%) a nodular and diffuse pattern and 6 cases (28%) a nodular pattern (Table 2). Among the 23 primary cutaneous diffuse large B-cell lymphoma, leg type cases, 13 cases (57%) had diffuse pattern and 10 cases (43%) a nodular and diffuse pattern. Concerning cytomorphology, the majority of cells of 4 cases (19%) out of 21 primary cutaneous follicle centre lymphoma, large cell had centroblastic appearance, 8 cases (38%) a spindle cell aspect and 9 cases (43%) contained a mixture of centroblasts and large centrocytes (Fig. 1a). Concerning the 23 primary cutaneous diffuse large B-cell lymphoma, leg type cases, 10 cases (44%) had an immunoblastic morphology with a prominent nucleolus and 13 cases (56%) contained a mixture of immunoblasts and centroblasts (Fig. 1b).

Table 1 Clinical features at baseline, and follow-up of patients with primary cutaneous large B-cell lymphoma, diagnosed between 1993 and 2016—French Study group on cutaneous lymphoma database, France ($n = 44$)

	Primary cutaneous follicle centre cell lymphoma, large cell ($n = 21$)	Primary cutaneous diffuse large B-cell lymphoma, leg type ($n = 23$)
Baseline characteristics		
Age, median (range), years	64 (42–86)	82 (61–95)
Male, No (%)	16 (76)	8 (35)
Female, No (%)	5 (24)	15 (65)
Location, No (%)		
Leg	1 (5)	18 (78)
Arm	1 (5)	2 (9)
Head and neck	11 (52)	1 (4)
Trunk	8 (38)	2 (9)
Tumor node metastasis stage, No (%)		
T1	9 (43)	6 (26)
T2	10 (48)	15 (65)
T3	2 (9)	2 (9)
Cutaneous relapse, No (%)		
Extra cutaneous spreading, No (%)	2 (9)	6 (26)
Survival outcome		
Median overall survival, months		46
Median progression free survival, months	131	32

In the 21 primary cutaneous follicle centre lymphoma, large cell cases, all but one (5%) were negative for MUM1. BCL2 was found expressed in 6 cases (29%). In 12 BCL2-negative cases according to the anti-BCL2 clone 124 immunostaining, a second round of immunostaining for BCL2 was performed with the clone E17 and 2 cases were then considered positive. Altogether using both anti-BCL2 antibodies, 8 cases (38%) were considered BCL2 positive. Ten cases (48%) expressed MYC above the 40% threshold. All cases (100%) were positive for BCL6. The mean of proliferation index Ki-67 was 79%.

In the 23 primary cutaneous diffuse large B-cell lymphoma, leg type cases, BCL2 and MUM1 expression was a diagnostic feature used for inclusion (100%) (Fig. 1c). Nineteen (83%) of 23 primary cutaneous diffuse large B-cell lymphoma, leg type cases expressed MYC (Fig. 1d). Twenty cases (87%) were positive for BCL6. The mean of proliferation index Ki-67 was 86%.

There was a significant association between BCL2 and MYC expression ($p = 0.003$). Expression of either MYC

Table 2 Baseline morphological features, immunohistochemical profile and cytogenetic data of patients with primary cutaneous large B cell lymphoma, diagnosed between 1993 and 2016—French Group of Cutaneous Lymphoma database, France ($n = 44$)

	Primary cutaneous follicle centre lymphoma, large cell ($n = 21$)	Primary cutaneous diffuse large B-cell lymphoma, leg type ($n = 23$)
Architecture, No (%)		
Diffuse proliferation	10 (48)	13 (57)
Nodular and diffuse proliferation	5 (24)	10 (43)
Nodular proliferation	6 (28)	0 (0)
Cytology, No (%)		
Centroblasts	4 (19)	0 (0)
Spindle cells	8 (38)	0 (0)
Mix of centroblasts and centrocytes	9 (43)	0 (0)
Immunoblasts	0 (0)	10 (44)
Mix of immunoblasts and centroblasts	0 (0)	13 (56)
Phenotype		
BCL2 expression by >50% cells, No (%)	8 (38)	23 (100)
CMYC expression by >40% cells, No (%)	10 (48)	19 (83)
MUM1 expression by >50% cells, No (%)	1 (5)	23 (100)
BCL6 expression by >50% cells, No (%)	21 (100)	20 (87)
Mean proliferation index Ki-67 (%)	79	86
Dual expression of MYC/BCL2, No (%)	6 (29)	19 (83)
FISH analysis		
<i>BCL2</i> translocation, No (%)	0 (0)	0 (0)
<i>BCL6</i> translocation, No (%)	1 (5)	4 (18)
<i>CMYC</i> translocation, No (%)	1 (5)	1 (5)
<i>BCL2</i> extra copy, No (%)	0 (0)	4 (18)
<i>BCL6</i> extra copy, No (%)	0 (0)	3 (14)
<i>CMYC</i> extra copy, No (%)	1 (5)	0 (0)
Double-Hit, No (%)	1 (5)	0 (0)
Dual expression + double-hit, No (%)	1 (5)	0 (0)
<i>MYD88</i> mutation, No (%)	0 (0)	16 (70)

or BCL2 was significantly associated with the diagnosis of primary cutaneous diffuse large B-cell lymphoma, leg type ($p = 0.025$, $p < 0.001$ respectively). Out of 29 cases which expressed MYC, 10 cases (34%) corresponded to primary cutaneous follicle centre lymphoma, large cell and 19 cases (66%) to primary cutaneous diffuse large B-cell lymphoma, leg type. Among the 31 cases which expressed BCL2, 8 cases (26%) corresponded to primary cutaneous follicle centre lymphoma, large cell and 23 cases (74%) to primary cutaneous diffuse large B-cell lymphoma, leg type.

In the entire cohort ($n = 44$), the 25 dual-expressor cases (57%) corresponded to as 6 primary cutaneous follicle centre lymphoma, large cell cases (29%) or to 19 primary cutaneous diffuse large B-cell lymphoma, leg type cases (83%) (Fig. 1c, d) and such distribution was statistically different ($p = 0.006$)

Cytogenetic analysis

FISH analysis for *BCL2*, *BCL6*, and *MYC* was found interpretable in 41 cases because of hybridization failure in

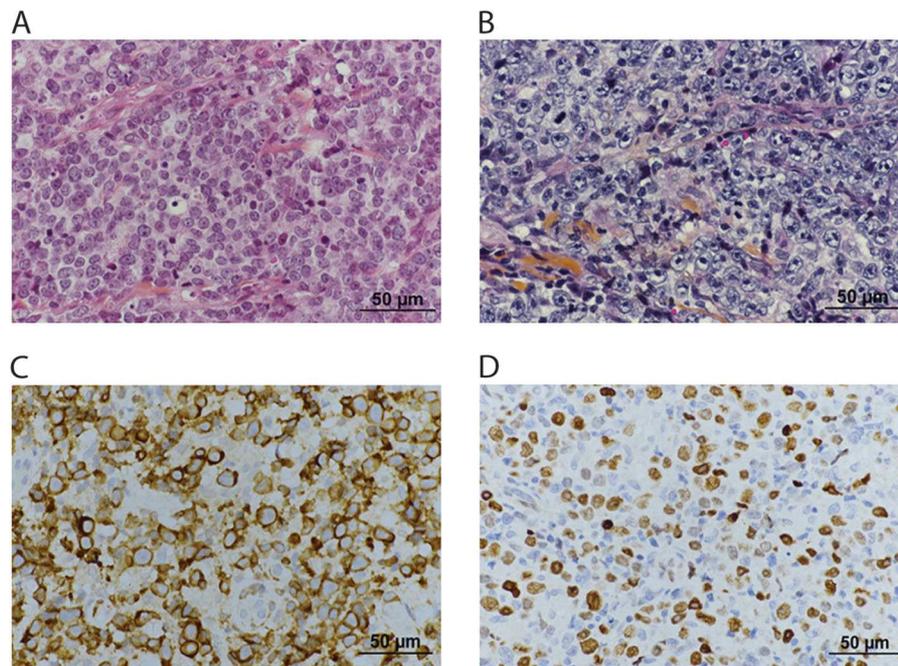
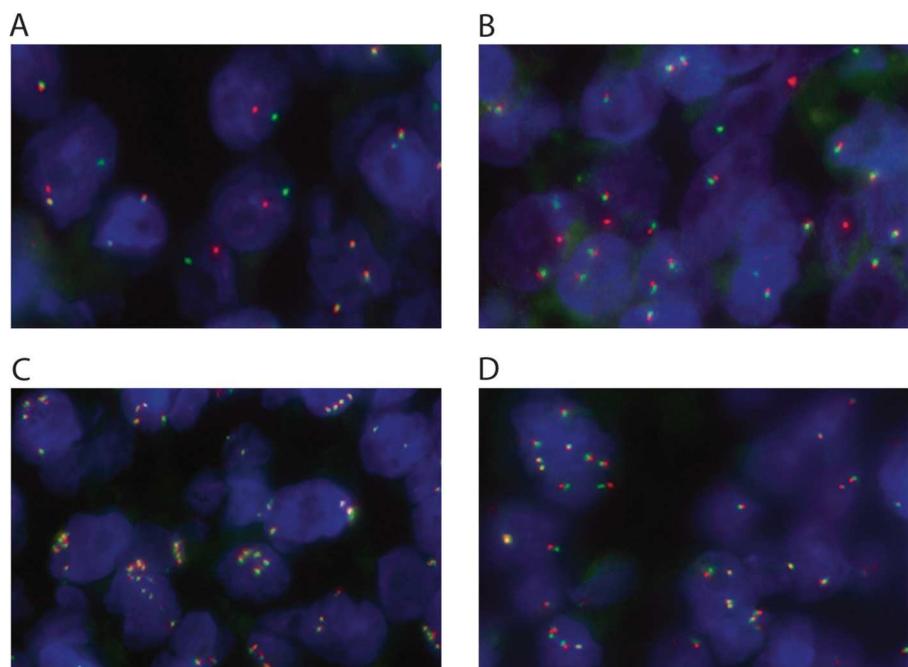


Fig. 1 Histological and phenotype features of primary cutaneous large B-cell lymphoma. **a** Diffuse infiltration of the dermis by large lymphomatous cells with centroblastic morphology in a case of primary cutaneous follicle centre lymphoma, large cell (Hematoxylin–eosin–saphranin, original magnification $\times 400$). **b** Diffuse infiltration of dermis by large lymphomatous cells with immunoblastic cytology in a

case of primary cutaneous diffuse large B cell lymphoma, leg type (Hematoxylin–eosin–saphranin, original magnification $\times 400$). **c** BCL2 expression in a case of primary cutaneous diffuse large B-cell lymphoma, leg type (original magnification $\times 400$). **d** MYC expression in a case of primary cutaneous diffuse large B-cell lymphoma, leg type (original magnification $\times 400$)

Fig. 2 Cytogenetic analysis of *BCL2*, *BCL6*, *MYC* by fluorescent in situ hybridization in primary cutaneous large B-cell lymphoma. **a** Split of *MYC* signals in a case of primary cutaneous diffuse large B-cell lymphoma, leg type. **b** Split of *BCL6* signals in a case of primary cutaneous follicle centre lymphoma large cell. **c** Amplification of *BCL2* locus in a case of primary cutaneous diffuse large B-cell lymphoma, leg type. **d** Gain of *BCL6* locus in a case of primary cutaneous diffuse large B-cell lymphoma, leg type



3 cases (Table 2). Concerning *MYC*, 2 cases (5%) displayed a rearrangement or hit, 1 primary cutaneous diffuse large B-cell lymphoma, leg type case (5%) and 1 primary cutaneous follicle centre lymphoma, large cell case (5%) (Fig. 2a). A

low copy-number gain of the *MYC* was only observed in 1 primary cutaneous follicle centre lymphoma, large cell case (2%). A rearrangement of the *BCL6* locus was observed in 5 cases (12%) with 1 primary cutaneous follicle centre

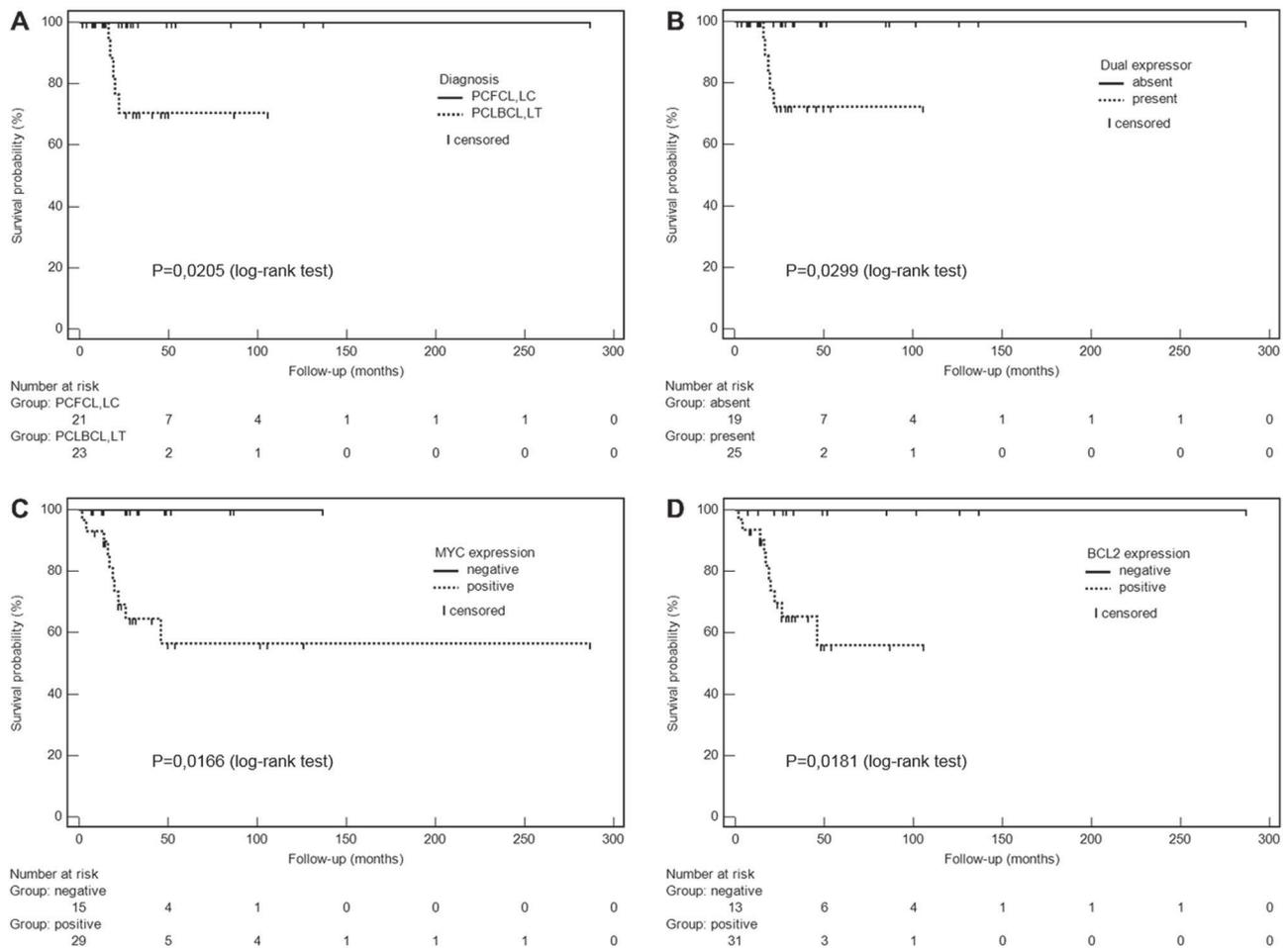


Fig. 3 Survival curves of patients with primary cutaneous large B-cell lymphoma, diagnosed between 1993 and 2016—French Study group on cutaneous lymphoma database, France ($n = 44$). **a** Specific survival according to histological diagnosis in 21 patients with primary cutaneous follicle centre lymphoma, large cells (PCFCL, LC) and in 23 patients with primary cutaneous large B-cell lymphoma, leg type (PCLBCL,LT). The global difference between curves was significant ($p = 0.021$). **b** Specific survival according to MYC and BCL2 dual

expression: no dual expression (19 patients), dual expressor status (25 patients). The global difference between curves was significant ($p = 0.030$). **c** Overall survival according to MYC expression: No MYC expression (15 patients) and MYC expression (29 patients). The global difference between curves was significant ($p = 0.017$). **d** Overall survival according to BCL2 expression: No BCL2 expression (13 patients) and BCL2 expression (31 patients). The global difference between curves was significant ($p = 0.018$)

lymphoma, large cell case (5%) and 4 primary cutaneous diffuse large B-cell lymphoma, leg type cases (18%) (Fig. 2b). A low copy-number gain of the *BCL6* locus was identified in 3 primary cutaneous diffuse large B-cell lymphoma, leg type cases (7%) (Fig. 2d). No *BCL2* rearrangement or hit was observed but a low copy-number gain or an amplification of *BCL2* were observed in 3 primary cutaneous follicle centre lymphoma cases (7%) and in 1 case (2%) of primary cutaneous diffuse large B-cell lymphoma, leg type, respectively (Fig. 2c). Only one primary cutaneous large B-cell lymphoma (2%) displayed a double-hit pattern with translocation of *MYC* and *BCL6*. This case was a primary cutaneous follicle centre lymphoma, large cell which also expressed *MYC*, *BCL2*, and *BCL6*. Two primary cutaneous diffuse large B-cell lymphoma, leg type

cases (5%), harbored a double low copy-number gain of *BCL2* and *BCL6* loci.

Cytogenetic alterations (gain or translocation) of *BCL6* were significantly more frequent in primary cutaneous diffuse large B-cell lymphoma, leg-type than in primary cutaneous follicle centre lymphoma, large cell (33 vs. 1.5%, $p = 0.049$).

MYD88 status determination

The *MYD88*^{L265P} mutation was detected in 16 out of 23 primary cutaneous diffuse large B-cell lymphoma, leg type cases (70%) but not in the 21 primary cutaneous follicle centre lymphoma, large cell cases (Table 2). There was no significant correlation between *MYD88* status and *MYC*

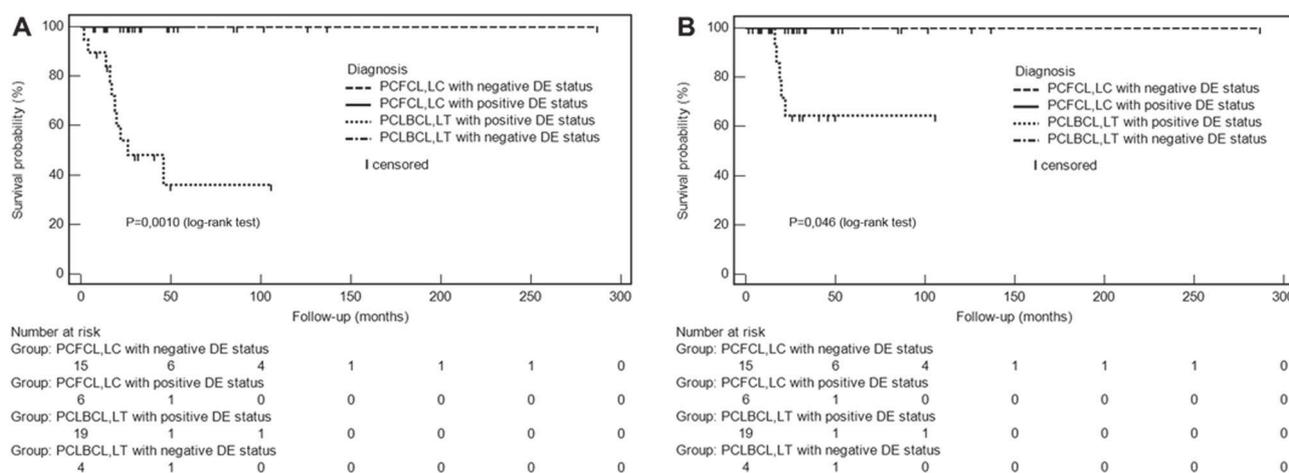


Fig. 4 Survival curves of patients with primary cutaneous large B-cell lymphoma according to the dual expressor (DE) status of MYC and BCL2 (positive or negative) evaluated against the histopathological diagnosis of primary cutaneous follicle centre lymphoma, large cells (PCFCL,LC) and primary cutaneous diffuse large B-cell lymphoma,

leg type (PCLBCL,LT). The dual expressor primary cutaneous diffuse large B-cell lymphoma, leg type subgroup had a significant worse overall survival (a) and specific survival (b) ($p = 0.001$ and $p = 0.046$, respectively)

expression ($p = 0.51$) and between *MYD88* status and dual expressor phenotype ($p = 0.113$).

Survival analysis

On the whole series of 44 primary cutaneous large B-cell lymphoma cases, the median follow-up was 29 months (range: 2–287). Ten patients (23%) died during follow-up, all in the subgroup of primary cutaneous diffuse large B-cell lymphoma, leg type. The death was related to the disease in 5 patients (11%). Eighteen patients out of 44 (40%) exhibited cutaneous relapses and 8 (18%) had extra-cutaneous spreading. The median overall survival was not reached and median progression free survival was 41 months.

Overall survival and specific survival were significantly worse in primary cutaneous diffuse large B-cell lymphoma, leg type than in primary cutaneous follicle centre lymphoma, large cell ($p = 0.001$ and $p = 0.021$, respectively) (Fig. 3a). Progression free survival was not different between the two histological subgroups ($p = 0.722$).

According to MYC and BCL2 expression profile, the dual expressor group showed a worse overall survival ($p = 0.002$) and a worse specific survival ($p = 0.030$) (Fig. 3b). Expression of MYC was associated with a worse overall survival ($p = 0.017$) (Fig. 3c). Similarly, expression of BCL2 was associated with a worse overall survival ($p = 0.018$) (Fig. 3d).

Concerning cytogenetic results, *BCL6* cytogenetic alterations (gain and translocation) significantly affected negatively specific survival ($p = 0.024$) but not overall survival ($p = 0.379$). There was a non-significant trend for a worse prognosis in patients with *BCL6* translocation ($p =$

0.086). *MYC* or *BCL2* cytogenetic alterations did not significantly impact overall survival ($p = 0.56$ and $p = 0.525$, respectively).

In the subgroup of primary cutaneous follicle centre lymphoma, large cell, overall survival or specific survival were not a workable factor because all patients were alive at end point. Nine patients (43%) harbored cutaneous relapses and extracutaneous progression concerned 2 patients (9%). Median progression free survival was 131 months.

In the subgroup of primary cutaneous diffuse large B-cell lymphoma, leg type, median overall survival was 46 months. Nine patients (40%) harbored cutaneous relapses. Extra-cutaneous progression concerned 6 patients (26%). Median progression free survival was 32 months. Overall survival seemed to be negatively influenced by dual expression profile in our limited cohort ($p = 0.099$). The single criterion affecting overall survival was MYC expression.

When analyzing survival according to both the histopathological diagnosis and the dual expressor status, the subgroup of dual expressor primary cutaneous diffuse large B-cell lymphoma, leg type displayed a significant worse overall survival ($p = 0.001$) and specific survival ($p = 0.046$) than each of the three other groups (Fig. 4). Indeed, all deaths were observed in this specific subgroup.

Discussion

Owing to the difficulty in the differential diagnosis between primary cutaneous follicle centre lymphoma, large-cell and primary cutaneous large B-cell lymphoma leg type which has major impact, we have evaluated on a retrospective

series of 44 typical primary cutaneous large B-cell lymphoma cases the new criteria used for nodal diffuse large B-cell lymphoma categorization to define double-hit of *MYC* and *BCL2* and/or *BCL6* genes rearrangement or the dual expression of *MYC* and *BCL2* proteins. The series corresponded to cases that were prospectively included in our national database with standardized criteria for diagnosis, staging and follow-up. Neither secondary skin involvement by systemic lymphoma nor primary cutaneous follicle centre lymphoma small cell was included as they do not raise the diagnosis and therapeutic issues of primary cutaneous large B-cell lymphoma. Beyond the identification of double-hit or dual expressor cases, we measured the diagnostic relevance and impact on prognosis of such categorization of primary cutaneous large B-cell lymphoma.

One main finding of our study is that double-hit primary cutaneous large B-cell lymphoma is extremely rare as it was observed in a single patient with primary cutaneous follicle centre lymphoma, large cell who is still alive with complete response after 29 months' follow-up. Our findings are in accordance with partial data of other groups evaluating the status of *MYC*, *BCL2*, or *BCL6* locus in a various set of primary cutaneous B-cell lymphoma mixing cases with either small-cell or large-cell morphology [6, 28, 31–34]. Therefore, our study would not support the evaluation of double-hit in primary cutaneous large B-cell lymphoma as opposed to the recommendation for nodal diffuse large B-cell lymphoma [2].

Moreover, we did not find any *BCL2* rearrangement in both primary cutaneous follicle centre lymphoma, large cell or primary cutaneous diffuse large B-cell lymphoma, leg type as generally observed [32, 35]. Other studies including one from our group observed a low rate of *BCL2* rearrangement in primary cutaneous follicle centre lymphoma [6, 36, 37]. *MYC* was found rearranged in rare cases of either primary cutaneous follicle centre lymphoma, large cell or primary cutaneous diffuse large B-cell lymphoma, leg type subtype. As reported previously [7, 29], *BCL6* cytogenetic alterations were slightly more frequent in primary cutaneous diffuse large B-cell lymphoma, leg type cases than in primary cutaneous follicle centre lymphoma, large cell but such event is too rare to be used for differential diagnosis between the two entities. As opposed to their impact in nodal diffuse large B-cell lymphoma [38–42], no impact on primary cutaneous large B-cell lymphoma prognosis was found for most of the cytogenetic alterations including double-hit, single hit or abnormality of *MYC* or *BCL2*. Only a non-significant trend for a worse prognosis of primary cutaneous large B-cell lymphoma was observed with an abnormal *BCL6* status (split or gain), as already reported in a series of nodal diffuse large B-cell lymphoma [43]. However, neither *BCL6* translocation alone nor double-hit involving *MYC* and *BCL6* were found to predict poorer

outcome in another large series of 898 diffuse large B-cell lymphoma cases [44]. In primary cutaneous large B-cell lymphoma, the relevance of single hit or double-hit evaluation will remain elusive as such event is too occasional in such rare disease.

Alternatively, our study brings up that determination of the dual expressor status (*MYC* and *BCL2*) has impact on both diagnosis and prognosis. Indeed, *MYC* expression has not been investigated routinely in primary cutaneous diffuse large B-cell lymphoma, leg type which usually express *BCL2*. Dual expressor-primary cutaneous large B-cell lymphoma represent nearly 60% of cases but was more frequently seen in primary cutaneous diffuse large B-cell lymphoma, leg type (83%) than in primary cutaneous follicle centre lymphoma, large cell (29%). The single expression of *MYC* or *BCL2* and the dual expressor status were associated with an inferior outcome in primary cutaneous large B-cell lymphoma, as recently reported for *MYC* in primary cutaneous diffuse large B-cell lymphoma, leg-type [45]. The expression of *BCL2* is already used as an immunohistochemical criterion for primary cutaneous diffuse large B-cell lymphoma, leg type diagnosis [3, 16, 18, 46]. The altered prognosis of patients with dual expression was found significant at the level of the entire primary cutaneous large B-cell lymphoma cohort but also in the primary cutaneous diffuse large B-cell lymphoma, leg type subgroup as patients with dual expression had the most aggressive disease.

A single study has previously reported that *MYC* and *BCL2* co-expression in primary cutaneous B-cell lymphoma may impact overall survival and progression free survival [6]. They individualized, a third separate category of primary cutaneous large B-cell lymphoma, previously named primary cutaneous diffuse large B-cell lymphoma, not otherwise specified [8], according to its intermediate prognosis between primary cutaneous follicle centre lymphoma and primary cutaneous diffuse large B-cell lymphoma, leg type [6]. Such study however differs from ours as it focused on the impact on prognosis of the immunohistochemical Hans' algorithm which had practical limitations as 20% of primary cutaneous diffuse large B-cell lymphoma, leg type displayed a paradoxical germinal centre profile [6]. A prognostic value of *BCL2* rearrangement was also found that contrasts with its absence in our series suggesting heterogeneity in inclusion criteria. Alternatively, our findings are in agreement with the EORTC and WHO classification considering primary cutaneous follicle centre lymphoma, large cell as a subtype of primary cutaneous follicle centre lymphoma [2, 9]. In our study, all patients with primary cutaneous follicle centre lymphoma, large cell subgroup displayed a very good prognosis which was statistically different from that of primary cutaneous diffuse large B-cell lymphoma, leg type.

For primary cutaneous large B-cell lymphoma, the histological categorization into two separate subgroups has a major impact for the management of patients making the pathologist's decision a crucial issue. Ancillary testing such as the detection of the *MYD88*^{L265P} mutation may help pathologists to confirm the diagnosis of primary cutaneous diffuse large B-cell lymphoma, leg type [18]. Our study would suggest that *MYC* immunostaining could be added to those of *BCL2* and *MUM1* for the diagnosis of primary cutaneous diffuse large B-cell lymphoma, leg type as dual expression of *MYC* and *BCL2* was significantly predominant in this lymphoma subtype. Investigating the mutational profile of 28 primary cutaneous diffuse large B-cell lymphoma, leg type, we recently reported *MYC* mutation in about 20% of cases which also supports a *MYC*-driven oncogenesis [47].

Our pilot study performed on typical primary cutaneous large B-cell lymphoma cases needs to be confirmed on larger validation sets of patients in order to determine if dual-expression or single *MYC* expression represent new prognostic factors in multivariate analysis. This will require standardization of diagnostic criteria between primary cutaneous follicle centre lymphoma, large cell and primary cutaneous diffuse large B-cell lymphoma, leg type using consensus immunohistochemical algorithms and/or ancillary molecular testing. Conversely to nodal diffuse large B-cell lymphoma, our study does not argue for the need of double-hit determination in primary cutaneous large B-cell lymphoma. Dual-expressor status was found as a differential diagnosis criterion for primary cutaneous diffuse large B-cell lymphoma, leg type vs. primary cutaneous follicle centre lymphoma, large cell but also identified among patients with primary cutaneous diffuse large B-cell lymphoma, leg type those with the most impaired prognosis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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6. RECAPITULATIF DES RESULTATS DES 3 ARTICLES : REPONSES AUX OBJECTIFS DE DEPART

6.1. La classification WHO 2017 des LBPC : applicabilité et impact pronostique

6.1.1. Applicabilité de la classification WHO 2017 des LBPC

Nous avons montré dans l'ARTICLE 1 que les critères histologiques et phénotypiques définis dans le WHO 2017 permettaient de classer la très grande majorité des 64 cas de LBPC-GC (89%) : 25 LBCF-GC et 32 LBTJ. Sept cas (11%) n'ont pas pu être classés. Cette catégorie était très hétérogène d'un point de vue clinique, histologique et phénotypique.

6.1.2. Différence pronostique entre les LBTJ et les LBCF-GC

Selon la catégorisation faite grâce aux critères de la WHO 2017, nous avons confirmé la très nette différence de pronostic entre les LBTJ et les LBCF-GC, en soulignant le fait que dans cette série les LBCF étaient constitués de >80 % de grandes cellules (ARTICLE 1). Les 7 cas de LBPC-GC inclassés avaient une survie intermédiaire entre le LBTJ et le LBCF-GC ($p=0.0002$).

6.2. Les outils pour le diagnostic différentiel des LBPC-GC

6.2.1. Valeur diagnostique des critères phénotypiques

A l'inverse de l'interprétation morphologique qui était peu reproductible entre 2 pathologistes ayant une expérience différente (hématopathologiste versus dermatopathologiste) (Kappa = 0,45), l'interprétation phénotypique (évaluée pour FOXP1, IgM, MYC et P63) était plus fiable (Kappa compris entre 0,58 et 0,91 selon l'anticorps). Les marqueurs MUM1, BCL2, FOXP1, MYC et IgM avaient une valeur diagnostique significative pour le LBTJ, mais BCL2 était également souvent exprimé dans les LBCF-GC (28%). Les expressions de CD10, BCL6 et du réseau folliculaire dendritique CD21+ avaient une valeur diagnostique significative pour le LBCF, mais BCL6 était également souvent positif dans les LBTJ (78%).

L'expression de P63 et l'index de prolifération Ki67, élevé dans les 2 entités, n'étaient pas des marqueurs discriminants contrairement à ce qui est décrit dans la littérature pour P63 et rapporté pour les lymphomes ganglionnaires pour Ki67.

6.2.2. Le statut GC/non-GC : aide au diagnostic des LBPC-GC

Nous avons testé 5 algorithmes sur les 64 cas LBPC-GC (Hans, Muris, Nyman, Hans modifié et Visco-Young) dans l'ARTICLE 1. Les algorithmes de Hans, Hans modifié et Muris avaient les mêmes résultats : les 25 LBCF-GC étaient bien de statut GC et les 32 LBTJ étaient bien de statut non-GC. La valeur diagnostique du statut GC/non-GC était optimale avec ces 3 algorithmes : sensibilité =100%, spécificité =100%, valeur prédictive positive = 100% et valeur prédictive négative = 100%. Sachant que l'algorithme de Hans modifié ne repose que sur 2 anticorps (le CD10 et le MUM1), ces 2 anticorps suffiraient à distinguer les LBTJ et les LBCF, ce qui est intéressant d'un point de vue économique (matériel et financier).

6.2.3. La mutation *MYD88* : critère diagnostique des LBPC-GC

Nous n'avons pas mis en évidence de mutation *MYD88* dans les LBCF sur l'ensemble de la série des 64 cas (ARTICLE 1 et 2). Dans l'ARTICLE 2, la mutation *MYD88* était retrouvée dans 19 des 25 cas de LBTJ (76%). Dans l'ARTICLE 1 (18 cas supplémentaires par rapport à l'ARTICLE 2), la mutation *MYD88* était présente dans 25 sur 64 cas (39%) de l'ensemble de la série : 22 sur 32 cas de LBTJ (69%) et 3 sur 7 cas de LBPC-GC inclassés (41%), aucun des cas classés en LBCF-GC n'était muté.

6.3. De nouveaux critères pronostiques dans les LBPC-GC

6.3.1. Valeur pronostique de l'étude phénotypique

De façon intéressante, nous avons mis en évidence que dans le sous-groupe LBTJ, l'expression de MYC était significativement associée à un risque de récurrence cutanée ($p=0.014$) (ARTICLE 1).

6.3.2. Le statut GC/non-GC : valeur pronostique dans les LBPC-GC

Dans la série de 64 cas de LBPC-GC (ARTICLE 1), les cas non-GC, classés selon les algorithmes Hans, Hans modifié et Muris avaient une moins bonne survie globale et spécifique que les cas GC ($p=0.0002$ et $p=0.007$ respectivement).

6.3.3. Double hit dans les LBPC-GC

Dans la série de 44 de LBPC-GC (ARTICLE 3), nous avons montré que le statut double hit était un événement très rare dans les LBPC-GC : un seul cas (5%) avec un réarrangement de *MYC* et *BCL6*. Ce cas correspondait à un LBCF-GC et le patient au bout de 29 mois de suivi était en vie en rémission complète. Au contraire des LBDGC ganglionnaires, la recherche du statut double hit ne semble donc pas pertinente dans les LBPC-GC.

6.3.4. Double expresseur dans les LBPC-GC

Dans la série de 44 de LBPC-GC (ARTICLE 3), nous avons montré que le profil double expresseur était en revanche fréquent : 25 (57%). Il était significativement associé au diagnostic de LBTJ : 19 sur 23 cas (89%). L'expression de *BCL2* seul, *MYC* seul et la double expression *MYC* et *BCL2* étaient associées à une moins bonne survie dans les LBPC-GC (respectivement $p=0.018$, $p=0.016$ et $p=0.03$). Et nous avons montré qu'également dans le sous-groupe des LBTJ, les patients avec un profil double expresseur avaient une maladie plus agressive ($p=0.001$). La recherche phénotypique de double expression *MYC* et *BCL2* est donc intéressante dans les LBPC-GC tant d'un point de vue diagnostique que pronostique.

7. DISCUSSION ET PERSPECTIVES

7.1. Classification WHO 2017 de LBPC : intérêt de l'entité « autres »

Nous avons montré que la classification WHO 2017 de LBPC était applicable dans la très grande majorité des cas avec seulement 7 cas non classables entre LBTJ et LBCF-GC. Ces rares cas de LBPC-GC inclassés pourraient correspondre à l'ancien sous-groupe «Lymphome primitif cutané B diffus à grandes cellules, autres » de la précédente classification WHO/EORTC 2005. Ce sous-groupe « autre » a été rapporté dans plusieurs publications concernant les LBPC avec des pourcentages variables et sans définition précise des cas classés dans cette catégorie.^{9,47-50} Il est donc difficile de comparer ces études entre elles.

Ces 7 cas avaient dans notre étude un pronostic intermédiaire reflétant probablement leur hétérogénéité. En analysant les différentes caractéristiques (cliniques, morphologiques, phénotypiques et moléculaire avec la mutation *MYD88*) de ces 7 cas inclassés avec leur évolution clinique, nous n'avons pas pu mettre en évidence de critères significatifs pour prédire le pronostic et donc guider la décision thérapeutique. Il s'agit d'un groupe très hétérogène, difficilement individualisable et définissable en tant que tel. Il semble préférable comme le préconise la WHO 2017 de ne pas en faire une entité à part, car les pathologistes risqueraient de l'utiliser de façon peu reproductible. A ce jour, il paraît plus juste d'admettre qu'un petit nombre de cas de LBPC-GC ne peuvent être définitivement classés en LBTJ ou LBCF-GC.

Cependant, nous avons montré que les LBPC-GC de type non-GC avaient un moins bon pronostic, ainsi que les cas avec un profil double expresseur. Ces 2 critères, associés au tableau clinique, pourraient être des propositions d'orientation pronostique et d'aide à la prise en charge thérapeutique de ces rares cas inclassés. De nouveaux outils moléculaires seraient nécessaires pour catégoriser et prédire l'évolution de ces cas discordants.

7.2. Mutation *MYD88* : valeur pronostique et enjeux thérapeutiques

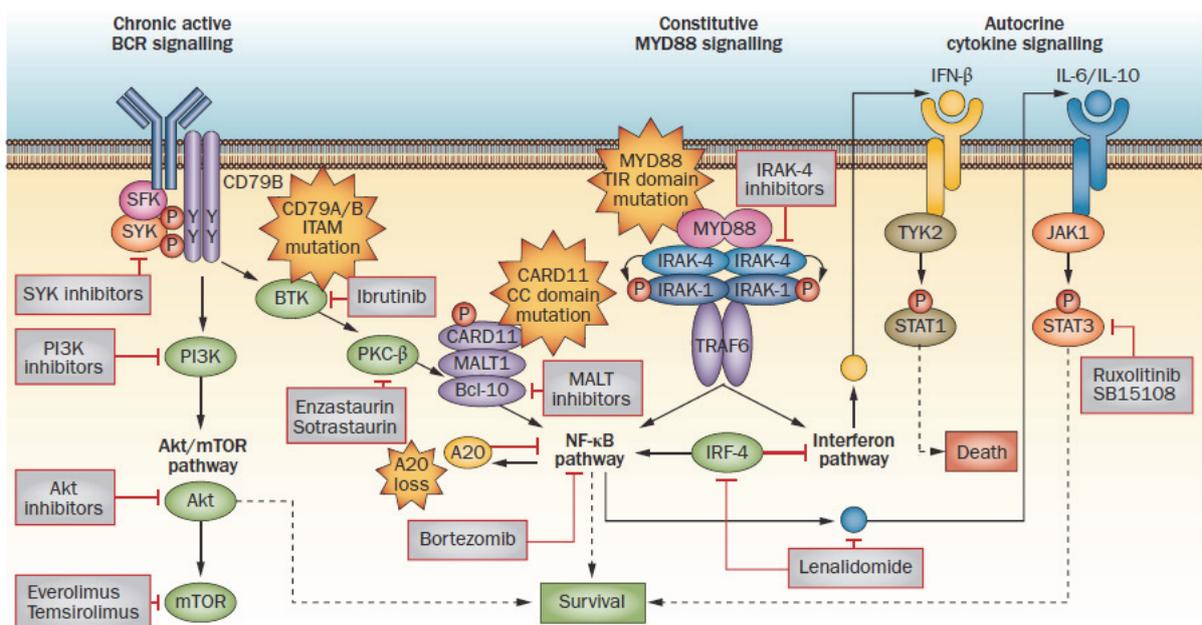
Pham-Ledard et al ont montré que la présence de mutation de *MYD88* était associée à un plus mauvais pronostic pour les patients atteints de LBTJ.³² Nous n'avons pas retrouvé ce résultat probablement du fait d'une cohorte trop petite.

En plus de sa valeur pronostique, la détermination du statut de *MYD88* peut être intéressante pour identifier des patients qui pourraient répondre à des thérapies spécifiques ciblant par exemple le BCR, la voie JAK ou la voie de signalisation NF-KB.⁵¹

Le lenalidomide (Revlimid®; Celgene Corporation) est un agent anticancéreux immunomodulateur, ayant un effet anti angiogénique et antiprolifératif qui induit une inhibition des voies de signalisation NF-KB et INF Béta.^{52,53} Un essai de phase II par lenalidomide a été conduit chez des patients atteints de LBTJ en rechute ou réfractaire au traitement standard et a montré des résultats encourageants.⁵⁴ L'absence de mutation *MYD88* était associée à un taux de réponse globale meilleure au traitement.

Des inhibiteurs de *MYD88* ciblant son homodimérisation ont également été développés. Ces molécules ont pour but d'inhiber la prolifération tumorale liée à l'activation aberrante du récepteur TLR, par la mutation *MYD88*.⁵⁵ Il n'existe pas d'étude in vivo ou in vitro sur les LBDGC ganglionnaires non-GC mutés *MYD88*. Une étude in vitro a montré l'inhibition de l'immunomarquage P65 (composant du complexe NF-KB), après culture en présence d'inhibiteur de *MYD88* homodimérisé, sur des cellules issues d'une maladie de Waldenström.⁵⁶

Figure 6 : Voies de signalisation impliquées dans les LBDGC et thérapies ciblées en lien (Roschewski M. *et al. Nat. Rev. Clin. Oncol.* 2014)



7.3. Vers de nouveaux outils moléculaires

7.3.1. Anomalies moléculaires des LBTJ

Récemment, une étude du génome par séquençage nouvelle génération (NGS) a été réalisée sur 28 cas de LBTJ. Ces analyses, soit par technique de whole exome ou en séquençage ciblé (Lymphopanel), ont permis de mettre en évidence la grande fréquence (>40%) des mutations *MYD88*, *CD79B* et *PIM1* dans ce type de lymphome. Les gènes *TBLIXR1* (33%), *MYC* (26%), *CREBBP* (26%), *IRF4* (21%) et *HIST1H1E* (41%) étaient aussi fréquemment mutés.⁵⁷ La connaissance de ces gènes et des combinaisons de mutations pourraient permettre de préjuger de l'efficacité de certaines thérapies ciblant le BCR, comme par exemple l'ibrutinib, inhibiteur de Bruton Tyrosine Kinase (BTK).

7.3.2. Anomalies moléculaires des LBCF

La présence de réarrangement de *BCL2* dans les LBCF est controversée. La plupart des études montre une absence ou une fréquence très faible de réarrangement de *BCL2* dans les LBCF.^{15,58,59} Quelques études montrent un taux plus élevé de réarrangement de *BCL2*.^{14,60,61} La présence d'un réarrangement de *BCL2* doit soulever la question d'un lymphome folliculaire ganglionnaire avec localisation secondaire à la peau. Des réarrangements de *BCL6* sont également retrouvés en faible proportion dans les LBCF.⁵⁹ Les LBCF présentent fréquemment une amplification de *REL*.⁶² La délétion de 1p36, anomalie cytogénétique décrite dans les lymphomes folliculaires ganglionnaires, est rapportée dans la littérature dans 17% des LBCF.⁵⁹ Contrairement au LBTJ, l'inactivation du locus des gènes *CDKN2A* et *CDKN2B* est rarement retrouvée.⁶² D'un point de vue thérapeutique, la recherche de ces anomalies n'apporte rien pour le moment. D'un point de vue pronostique, Szablewski et al ont montré qu'il n'y avait pas de différence de pronostic entre les LBCF avec ou sans réarrangement de *BCL2*.¹⁴

7.3.3. Développement de nouveaux outils moléculaires dans les LBDGC ganglionnaires

Gentles & Alizadeh suggèrent que la signature moléculaire permettrait d'avoir des informations supplémentaires chez les patients de risque intermédiaire, comme nos 7 cas de LBPC-GC inclassés.^{63,64} Des techniques d'amplification ligature-dépendante multiplexe de sonde (MLPA) et de transcriptase inverse de la MLPA (RT-MLPA) ont été développées dans les LBDGC ganglionnaires et pourraient être testées sur ces cas inclassés.^{65,66}

7.4. Perspectives

Nous souhaiterions réaliser une étude comparative moléculaire des LBPC-GC, en comparant des LBTJ typiques et des LBCF-GC typiques, selon 2 différentes techniques et combiner les résultats :

- par NGS ciblé en utilisant le Lymphopanel établi par le Dr A. Gros (Service de Biologie des Tumeurs, Hôpital Haut-Lévêque, CHU Bordeaux)
- par RT-MLPA, technique à mettre au point avec le Dr A. Gros.

Après avoir défini les profils moléculaires de ces 2 entités, nous pourrions alors tester les 7 cas de LBPC-GC inclassés et tenter de les classer afin de pouvoir prédire leur évolution et guider la stratégie thérapeutique.

Aucune étude exhaustive du génome des LBCF n'a été publiée jusqu'à présent. Même si la survie est excellente dans ce type de lymphome, il serait peut-être intéressant d'un point de vue scientifique de réaliser une étude du génome de LBCF à morphologie à petites cellules et à morphologie à grandes cellules car il est étonnant que des lymphomes avec une morphologie proche d'un lymphome agressif se comportent comme un lymphome de bas grade avec une bonne évolution clinique. Ces résultats pourraient être comparés avec les données existantes sur les lymphomes folliculaires ganglionnaires de différents grades et les LBDGC ganglionnaires de type GC pour établir vers quel profil génomique ils se rapprochent le plus.^{67,68}

8. CONCLUSION

Nous avons démontré à travers ces 3 articles que la classification WHO 2017 des LBPC-GC (>80% de cellules tumorales) permettait de classer la majorité des cas et que cette classification LBTJ (de profil non-GC) versus LBCF-GC (de profil GC) restait importante, car malgré les progrès thérapeutiques, le pronostic des LBTJ était moins bon que celui des LBCF. Pour aboutir à ce diagnostic différentiel nous proposons de modifier l'algorithme diagnostique de départ en utilisant des outils immunohistochimiques et moléculaires d'intérêt diagnostique (expression de CD10 et MUM1, et mutation *MYD88*) et pronostiques (expression de *MYC* et *BCL2*) (Figure 7). Restent au final 11% de LBPC-GC non classés (qui nécessiteront probablement de techniques moléculaires plus sophistiquées comme le NGS ciblé ou RT-MLPA) et dont la prise en charge doit être discutée en réunion de concertation pluridisciplinaire au cas par cas (clinique, phénotype GC/non-GC, profil double expresseur, mutation *MYD88*).

Figure 7 : Algorithme diagnostique des lymphomes B primitifs cutanés à grandes cellules (LBPC-GC)

LBTJ : lymphome B diffus à grandes cellules, de type jambe

LBCF-GC : lymphome B cutané centro-folliculaire à grandes cellules

GC : centre germinatif

LBPC-GC

Prolifération lymphomateuse de grands lymphocytes B sans atteinte extra-cutanée lors du bilan d'extension au diagnostic

1. Morphologie

LBCF-GC

- Architecture : nodulaire ou nodulaire et diffuse ou diffuse
- Cytologie : centrocytique de taille petite ou grande +/- centroblastique

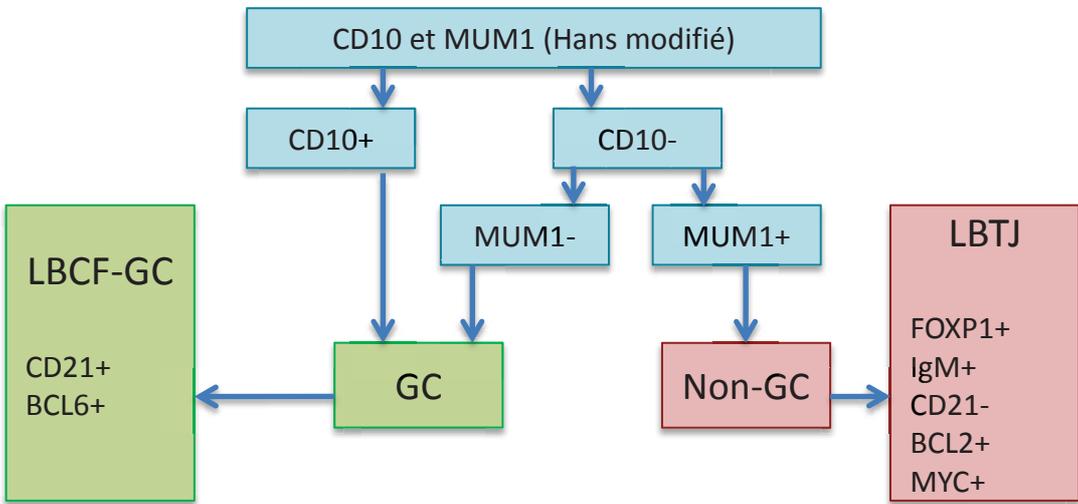
LBTJ

- Architecture : diffuse ou nodulaire et diffuse
- Cytologie : immunoblastique +/- centroblastique

Inclassable

- Cytologie douteuse
- Discordance entre architecture et cytologie

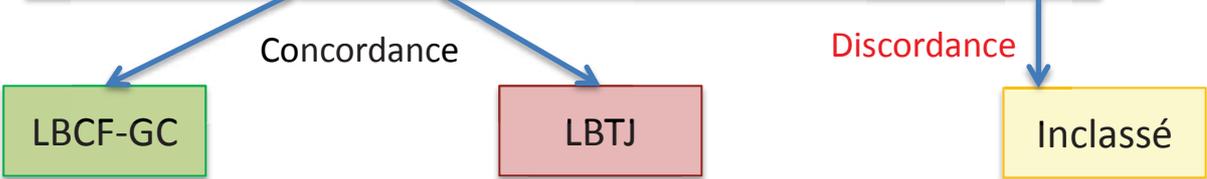
2. Phénotype



3. Mutation *MYD88*^{L265P}



4. Confrontation des résultats



5. Facteurs pronostiques péjoratifs

Profil non-GC
Double expresseur (MYC+BCL2+)
Mutation *MYD88*

6. RCP : confrontation anatomo-clinique → Décision thérapeutique

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SERMENT D'HIPPOCRATE

Au moment d'être admis à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences. Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me les demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçu à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonoré et méprisé si j'y manque.

