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# Towards precision medicine in lymphoid malignancies

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Careful histopathologic examination remains the cornerstone in the diagnosis of the clinically and biologically heterogeneous group of lymphoid malignancies. However, recent advances in genomic and epigenomic characterization using high-throughput technologies have significantly improved our understanding of these tumors. Although no single genomic alteration is completely specific for a lymphoma entity, some alterations are highly recurrent in certain entities and thus can provide complementary diagnostic information when integrated in the hematopathological diagnostic workup. Moreover, other alterations may provide important information regarding the clinical course, that is, prognostic or risk-stratifying markers, or response to treatment, that is, predictive markers, which may allow tailoring of the patient's treatment based on (epi)genetic characteristics. In this review, we will focus on clinically relevant diagnostic, prognostic, and predictive biomarkers identified in more common types of B-cell malignancies, and discuss how diagnostic assays designed for comprehensive molecular profiling may pave the way for the implementation of precision diagnostics/medicine approaches. We will also discuss future directions in this rapidly evolving field, including the application of single-cell sequencing and other omics technologies, to decipher clonal dynamics and evolution in lymphoid malignancies.

**Keywords:** biomarkers, comprehensive molecular profiling, (epi)genetics, lymphoid malignancies, precision medicine, single-cell sequencing

## Introduction

The classification of lymphoid neoplasms has continuously undergone evolution with an increasing number of distinct diagnostic entities, and in the latest WHO classification from 2016, more than 80 entities of lymphoid malignancies were recognized [1]. Whilst the majority of lymphoid tumors derive from different stages of normal B-cell development, they can also originate from T cells or NK cells. There is an extensive heterogeneity of morphologic, immunophenotypic, and genetic features between and within different entities of lymphoid neoplasms. Similarly, there is a corresponding great clinical heterogeneity, ranging from patients with indolent forms to very aggressive clinical courses.

In the last 10 years, with the introduction of next-generation sequencing (NGS) technologies, we have witnessed a major leap in our understanding of the genomic landscapes of major entities of lymphoid malignancies [2, 3]. While a few entities showed a highly recurrent gene mutation [4, 5], most subtypes of lymphoid tumors demonstrated a very heterogeneous mutation landscape including hundreds of recurrently mutated genes [2, 3], probably mirroring the clinical heterogeneity observed between and among lymphoma entities. Based on a high number of publications in this field, novel

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biomarkers have been identified that may aid disease classification and risk stratification and guide therapy selection.

In parallel, efforts have focused on unraveling the epigenomic landscape of lymphoid malignancies [6]. From these studies, it has become apparent that many lymphoid malignancies carry a "footprint" from the cell of origin (COO), while they also gain tumor-specific epigenetic aberrations [7]. Using DNA methylation profiling, it is possible to classify patients into clinically relevant subgroups and also to assess the proliferative history of lymphoid tumors [8, 9]. In more recent years, we have also started to understand the complex landscape of histone modifications and their involvement in gene regulation and disease pathology [10].

In this review, we will discuss the recently uncovered genomic landscapes of the most frequent mature B-cell malignancies, and highlight important diagnostic, prognostic, and predictive biomarkers. We will also discuss how comprehensive genomic profiling may provide new tools for the implementation of precision diagnostics in everyday patient care. Furthermore, we will outline recent developments in deciphering the impact of epigenomics in the onset and evolution of lymphoid malignancies, but also discuss how single-cell sequencing and other omics technologies may further our understanding of the disease pathobiology of these tumors.

## Clinically relevant genomic markers in lymphoid malignancies

Since the REAL classification in 1994, detection of genetic aberrations is an integral part of diagnostics of lymphoid malignancies [11]. This has, until recently, mainly included detection of recurrent chromosomal aberrations using fluorescence in situ hybridization (FISH) technology that is more prevalent in certain lymphoid tumors. The most important include t(14;18) involving *BCL2* and IGH in follicular lymphoma (FL), t(8;14) involving *MYC* and IGH in Burkitt lymphoma (BL) and high-grade B-cell lymphomas (HGBCL) with *MYC* and *BCL2* and/or *BCL6* translocation (so-called double-hit lymphoma), and t(11;14) involving *CCND1* and IGH in mantle cell lymphoma (MCL) [1]. These different translocations can be captured using FISH probes designed for each translocation partner or using break-apart probes for individual genes and are applicable both for smears/imprints and formalin-fixed paraffin-embedded (FFPE) tissue.

With the advent and now widespread use of NGS technologies, a more comprehensive and unbiased molecular characterization of lymphoid malignancies was possible, particularly thanks to whole-exome (WES) or genome sequencing (WGS). In the next sections, the recently uncovered genomic landscapes of the most frequent mature B-cell malignancies will be reviewed and clinically relevant genomic markers highlighted.

### *Diagnostic markers*

In small B-cell lymphomas, where morphologic or immunophenotypic overlaps sometimes pose diagnostic difficulties, several alterations have been described to be recurrent and can help in the differential diagnosis. In 2011, a point mutation in the *BRAF* gene (*BRAF* V600E), which is present in almost all hairy cell leukemia (HCL) cases, could be first identified thanks to WES of one single patient [4], highlighting the utility of using a comprehensive rather than targeted approach, since *BRAF* was previously not known to be involved in lymphoid malignancies. Later studies have confirmed this finding and refined the mutational landscape, identifying additional recurrent mutations in *CDKN1B* [12] (16% of HCL) and *KMT2C* [13] (15% of HCL). The HCL variant does not carry *BRAF* mutations but was found to have mutations in *MAP2K1* (around 50% of cases), which pertains to the same signaling pathway [14]. *MAP2K1* mutations can also be found in classic HCL cases, which lack the *BRAF* V600E mutation and use the IGHV4-34 gene, although it remains unclear whether these cases are better classified as classic or variant HCL [14]. Similarly, the *MYD88* L265P mutation was first found to be present in a very high proportion of lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia (LPL/WM) by performing WGS in 30 patients [5]. Later studies confirmed this finding and found additional recurrent mutations, including *CXCR4* (30%–40%), *ARID1A* (17%), and *CD79B* (8%–15%), as well as the previously known frequent deletion of chromosome 6q [15]. *MYD88* L265P is not exclusive to LPL/WM and can be detected in a lower proportion of cases of chronic lymphocytic leukemia (CLL), marginal zone lymphomas (MZL) [16], and diffuse large B-cell lymphoma (DLBCL).

Several studies have investigated the genomic landscape of CLL [17–20], which shows a remarkable heterogeneity [21] when compared to the aforementioned LPL/WM or HCL, with highly

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recurrent alterations of single genes. The most frequent recurrent alterations in CLL are cytogenetic aberrations, which can be identified in more than 80% of cases and include deletion 13q (55%), deletion 11q (18%), trisomy 12 (16%), deletion 17p (7%), and deletion 6q (6%) [22]. Thus far, more than 60 "driver" mutations have been identified, although none of the mutations is highly prevalent nor specific to CLL. The most frequent mutations occur in *NOTCH1*, *SF3B1*, *TP53*, and *ATM*.

MCL includes two disease subtypes—conventional MCL (cMCL) and leukemic non-nodal MCL (nnMCL)—which originate from different B-cell maturation stages, but share the translocation t(11;14) [23]. The most frequently mutated genes include *ATM*, *KMT2D*, *CCND1*, and *KMT2D* [24]. A recent comprehensive genomic profiling of MCL [25] highlighted the differences in genetic alterations of the two subtypes—cMCL with frequent alterations of *ATM*, *KMT2D*, *BIRC3*, and *NSD2* and nnMCL with frequent *CCND1* somatic hypermutation (SHM) induced mutations and *TERT* alterations, whereas *TP53* alterations are relatively frequent in both subtypes, although slightly enriched in the non-nodal subtype. WES has also been performed in a series of primary and relapsed MCL samples [26] and novel mutation targets were identified, including *CARD11* (5.5%) and *S1PR1* (7.8%), with the latter significantly enriched in the relapsed samples [27].

FL is characterized by frequent alterations in epigenetic regulators (e.g., *KMT2D*, *CREBBP*, *EZH2*, *MEF2B*, *EP300*, and *HIST1* genes) [28, 29], which together with the *BCL2* translocation are involved in early FL pathogenesis [30]. Other involved pathways include B-cell receptor (BcR) signaling (e.g., *CARD11*), mTOR signaling (e.g., *RRAGC*) [31], JAK-STAT signaling (*SOCS1*, *STAT6*, and *STAT3*), as well as the NOTCH pathway [32]. Pediatric-type FL, which does not carry the IGH-*BCL2* translocation (by definition), shows a different landscape of alterations with frequent mutations in *TNFRSF11* (54%) and *MAP2K1* (49%), as well as *IRF8* (15%) [33].

The different types of MZL share common alterations and have few mutations that are prevalent in certain types [34]. Nodal marginal zone lymphoma (NMZL) has recurrent mutations in genes involved in chromatin remodeling/transcriptional regulation (71% of cases), NF-$\kappa$B (51% of cases), and NOTCH pathway (40% of cases), with *KMT2D*, *NOTCH2*, *PTPRD*, and *KLF2* mutations as the most frequent [35], of which only *PTPRD* seems to be enriched in NMZL. WES studies of splenic marginal zone lymphoma (SMZL) [36–41] have found frequent mutations in *TP53*, *KLF2*, *KMT2D*, *MYD88*, *NOTCH2*, and *TNFAIP3*, among others, in addition to the known cytogenetic aberrations of chromosomes 7 (deletion 7q) and chromosome 3 (trisomy 3 or gain of 3q). The recent large genetic characterization study of SMZL by the International Extranodal Lymphoma Study Group (IELSG; 303 spleen samples), confirmed previous results and separated the cases into four genetic subgroups based on the involved gene modules/pathways, termed NNK (involving NF-$\kappa$B, NOTCH, and KLF2), DMT (involving DNA damage response, MAPK, and TLR), CBS (involving cytokine, BcR signaling, and spliceosome), and PA (involving PI3K-AKT), of which NNK and DMT include 90% of all studied cases [42].

HGBCL or aggressive lymphomas have been extensively studied and several mutations can have diagnostic value. In addition to the translocation involving *MYC* and the IGH/IGK/IGL loci, BL was found to carry mutations in transcription factor *TCF3* or its negative regulator *ID3* in 70% of cases [43–47]—mutations that are not typical of DLBCL, although they can be encountered (especially in double-hit lymphomas). While not routinely used in diagnostics, transcriptomic analysis can be used for the diagnosis of BL, with a clear separation from DLBCL [48].

Efforts in the characterization of DLBCL through WES/WGS [49–51] have led to a refined molecular classification over the previous COO classification, which was mainly based on gene expression. Although there are slight differences in the proposed classifications between studies, there is a broad overlap among the new molecular subgroups; the major subgroups are BN2/C1 (*BCL6* translocation and *NOTCH2* mutation), A53/C2 (ABC subtype, *TP53* and *CDKN2A* inactivation), EZB/C3 (GCB subtype, *EZH2* mutations, and *BCL2* translocation/mutations), ST2/C4 (GCB subtype, *SGK1*, *NFKBIA*, and *SOCS1* mutations), MCD/C5 (ABC subtype, *MYD88* and *CD79B* mutations), and N1 (ABC subtype and *NOTCH1* mutations). Later studies [52–54] have developed classifiers that allow inferring of the DLBCL molecular subgroups when using targeted NGS gene panels. Although the prototypic cases in these subgroups carry the mentioned mutations, around 20%–35% of DLBCL cannot be assigned to a specific group. Furthermore, DLBCLs associated with infections

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such as hepatitis B were characterized with distinct genetic profiles and may represent either a different entity or subtype [55]. Hence, additional molecular subtypes of DLBCL are expected to be discovered in the coming years. In plasmablastic lymphomas, *MYC* translocations are the most frequent cytogenetic alteration [56], occurring in 50%–90% of cases, and frequently mutated pathways include MYC, MAPK, and JAK-STAT, with recurrent mutations of *STAT3*, *NRAS*, *TP53*, *MYC*, and *EP300* [57, 58].

Due to the low number of tumor cells, classic Hodgkin lymphomas (cHL) are not as easily accessible for WES or WGS as other lymphomas. Nevertheless, thanks to flow sorting [59, 60], circulating free tumor DNA [61, 62], as well as labor-intensive laser capture microdissection [63], the genomic landscape of cHL has been revealed, with finding of recurrent mutations in JAK-STAT, NF-$\kappa$B, and PI3K pathways. Primary mediastinal B-cell lymphoma (PMBCL) is thought to be related to cHL, and indeed shares mutational profiles in the same pathways, such as JAK-STAT, NF-$\kappa$B, and immune escape (e.g., *SOCS1*, *STAT6*, *NFKBIE*, and *CIITA*) [64, 65]. In addition, cytogenetic alterations involving 9p24 (which includes the genes encoding PD-L1 and PD-L2) are a characteristic and shared feature of cHL and PMBCL [66, 67].

As illustrated in this section and Table 1 (small cell and indolent B-cell lymphomas and leukemias) and Table 2 (classic Hodgkin, large-cell, and aggressive B-cell lymphoma), there is a significant overlap between different entities, highlighting how more comprehensive genetic characterization could be helpful in the diagnostic procedure, for example by using targeted NGS panels that include frequent genetic alterations, as discussed below.

### *Prognostic markers*

In several lymphoma entities, a substantial number of genetic alterations have been demonstrated to be prognostically significant and could ideally provide information to guide treatment decisions. However, most of the described prognostic alterations have not yet entered clinical routine. CLL is the best-studied example, where several molecular prognostic biomarkers are now included in the standard workup for all patients with CLL. The IGHV gene mutation status [68] allows to separate CLL patients with mutated IGHV genes (M-CLL) and a favorable prognosis from patients with unmutated IGHV genes (U-CLL) and inferior outcome, and in more recent years, studies have shown that the IGHV mutation status also has a predictive impact [69]. Based on IG gene sequencing, it is also possible to identify subsets of patients expressing quasi-identical or stereotyped BcRs [70], which can be identified in up to 41% of cases. In particular, patients belonging to subset 2 (IGHV3-21/IGLV3-21) or carrying the IGLV3-21 R110 mutation have clinically aggressive disease and do not benefit from chemoimmunotherapy [71, 72], while subset 8 patients have the highest risk of Richter transformation [73, 74].

The previously mentioned frequent cytogenetic aberrations in CLL [22] are routinely investigated with FISH and have prognostic significance, from worst to best: del(17p), del(11q), trisomy 12, no alteration, and del(13q). In addition to del(17p) (includes *TP53*), *TP53* sequence analysis [75] has also entered routine practice, having an adverse impact even without del(17p) [76–78]. Interestingly, TP53 mutations detected at low (subclonal) frequency also has a negative impact on the outcome, at least in patients treated with chemoimmunotherapy [79, 80]. Considering the independent prognostic strength of *TP53* alterations and IGHV subtype, they were included in the clinical prognostic index CLL-IPI [33] and the iwCLL guidelines [81]. There are several other genes that are promising prognostic biomarkers, for example, *NOTCH1, SF3B1* [20, 82–86], *ATM* [87], *EGR2* [88], and *BIRC3* [89] among others, although it will be important to study which of them will remain as independent prognostic biomarkers in well-characterized larger patient series [90–92].

In MCL, proliferation signatures have long been described [93] to have an important prognostic impact and can now be investigated in FFPE material [94–97]. *TP53* is also an adverse prognostic factor in MCL [98, 99], as well as in DLBCL [100–103] and LPL/WM [104]. In FL, similar to CLL, genetic alterations have been included in a risk model termed m7-FLIPI [105], which combines clinical parameters with mutations in seven genes (*EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP*, and *CARD11*) and improves the prognostication of FL patients. *MYC* and *BCL2* and/or *BCL6* translocation confer an adverse prognosis in comparison to other DLBCL [106, 107], but these lymphomas are now classified as a separate entity (HGBCL with *MYC* and *BCL2* and/or *BCL6* translocation).

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**Table 1.** *Overview of mutations in selected genes in small cell and indolent B-cell lymphomas and leukemias*

| Pathway | Gene | CLL (%) | MCL (%) | HCL (%) | LPL (%) | NMZL (%) | SMZL (%) | FL (%) |
|---|---|---|---|---|---|---|---|---|
| JAK-STAT | *SOCS1* | | | | | | | 5–15 |
| | *STAT3* | | | | | | | <5 |
| | *STAT6* | | | | | | | 5–15 |
| MAPK | *BRAF* | <5 | | >90 | | | <5[DMT] | |
| | *MAP2K1* | <5 | | <5[a] | | | | [b] |
| | *NRAS* | <5 | | | | | | |
| | *KRAS* | <5 | | | | | | |
| BcR | *CD79B* | | | | 5–15 | | | <5 |
| | *CARD11* | | 5–10 | | | 5–10 | 5–10 | 5–15 |
| Epigenetic | *KMT2D* | | 10–20% | | | 20–30 | 5–15 | 40–50 |
| | *KMT2C* | | 5–15 | 10–20% | | | | |
| | *CREBBP* | | | 5–10 | | | <5 | 40–50 |
| | *EP300* | | | <5 | | 5–10 | 5–10 | 10–20 |
| | *EZH2* | | | | | | | 10–20 |
| | *HIST1*[c] | <5 | | | | 5–10 | 5–10 | 30–40 |
| | *MEF2B* | | 5–10 | | | | | 5–15 |
| | *ARID1A* | | | | 10–20% | 10–20% | 5–10 | 5–15 |
| | *TERT* | | 10–20% | | | | | |
| | *NSD2* | | 5–15 | | | | | |
| Cell cycle | *CCND1* | | 20–30 | | | | | |
| | *CDKN1B* | | 5–15 | 10–20% | | | | |
| | *CDKN2A* | | 10–20% | | | | | |
| DNA damage | *TP53* | 5–10 | 20–30 | | 5–10 | <5 | 10–20% DMT | 5–10 |
| | *ATM* | 5–10 | 40–50 | | | <5 | <5 DMT | |
| NF-κB | *NFKBIE* | | <5 | | | | <5 | |
| NOTCH | *NOTCH1* | 5–15 | 5–10 | <5 | | | 5–10 NNK | <5 |
| | *NOTCH2* | | <5 | <5 | <5 | 10–20% | 20–30% NNK | <5 |
| | *SPEN* | | | | | 5–15 | 5–10 NNK | |
| TLR | *MYD88* | <5 | | | >90 | 5–10 | 5–10 DMT | |
| Apoptosis | *BCL2* | | | | | | | 20–30 |
| | *BIRC3* | <5 | 10–20% | | | 5–10 | 5–10 | |
| Immune | *TNFRSF14* | | | | | 10–20% | | 20–30 |
| | *CXCR4* | | | | 30–40 | | <5 | |
| | *TNFAIP3* | | | | | 10–20% | 5–15 NNK | |
| RNA editing | *SF3B1* | 10–20% | | | | | | |
| | *RPS15* | <5 | | | | | | |
| Transcription | *ID3* | | | | | | 5–15 | |
| | *EGR2* | <5 | | | | | | |
| | *KLF2* | | | 10–20% | | 10–20% | 20–30[NNK] | |

(*Continued*)

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**Table 1.** *(Continued)*

| Pathway | Gene | CLL (%) | MCL (%) | HCL (%) | LPL (%) | NMZL (%) | SMZL (%) | FL (%) |
|---|---|---|---|---|---|---|---|---|
| mTOR | *RRAGC* | | | | | | | 10–20% |
| Others | *PTPRD* | | | | | 10–20% | | |

| Mutation frequency ranges (%) | |
|---|---|
| <5 | 20–30 |
| 5–10 | 30–40 |
| 5–15 | 40–50 |
| 10–20 | >90 |

*Note*: Frequency ranges are approximated, in a majority of cases stemming from two or more studies.
Abbreviations: BcR, B-cell receptor; CLL, chronic lymphocytic leukemia; DMT, DNA damage response, MAPK, and TLR; FL, follicular lymphoma; HCL, hairy cell leukemia; LPL, lymphoplasmacytic lymphoma; MCL, mantle-cell lymphoma; NMZL, nodal marginal zone lymphoma; NNK, NF-$\kappa$B, NOTCH, and KLF2; SMZL, splenic marginal zone lymphoma; TLR, toll-like receptor.
[a]Frequent in *BRAF* unmutated HCL and in HCL variant.
[b]40%–50% in pediatric-type FL.
[c]HIST1 gene family.
[DMT]Mutations identifying DMT SMZL subtype.
[NNK]Mutations identifying NNK SMZL subtype.

### *Predictive markers*

Identification of predictive markers is the least developed biomarker field in B-cell malignancies. However, there is promising mounting evidence, especially when considering signaling pathways, rather than single genes. The best example for this is BcR pathway inhibitors (BTK, STK, and PI3K inhibitors), which show high activity in lymphomas with an activated BcR pathway [108]. In CLL, the presence of *TP53* alterations or unmutated IGHV genes identifies patients that benefit from treatment with BcR [109, 110] or BCL2 inhibitors [111]. *NOTCH1* mutations in CLL seem to not benefit from the addition of an anti-CD20 antibody [91]. In MCL, the presence of *TP53* mutations in MCL identifies a group of young patients who do not benefit from traditional intensified chemoimmunotherapy regimens [112], highlighting the need for alternative treatment regimens for these patients. In LPL/WM, *CXCR4* warts, hypogammaglobulinemia, infections and myelokathexis (WHIM)-like mutations identify patients with higher disease activity [113] and lower response to the BTK inhibitor ibrutinib [114–116].

Another example of a potential predictive marker is the aforementioned new molecular subgroups of DLBCL, where the hope is that targeted treatments exploiting dependencies of malignant cells towards certain pathways will improve the outcome compared to the classic R-CHOP treatment regimen [53], although this still has to be proven.

Despite the promising efficacy of these novel agents targeting signaling pathways, resistance to treatment can be observed over time, as was seen with targeted treatments in other malignancies (e.g., EGFR-targeting tyrosine kinase inhibitors in lung cancer or BCR-ABL1 inhibitors in chronic myeloid leukemia). In this context, predictive biomarkers of treatment resistance have been described, for example, *BTK* and *PLCG2* mutations, which confer resistance to ibrutinib treatment [117–119]. Similarly, acquisition of the *BCL2* G101V mutation has been shown to confer resistance to the BCL2 inhibitor venetoclax [120].

## Comprehensive genomic profiling

Considering the increasing number of clinically relevant aberrations in lymphoid malignancies, custom NGS-based targeted sequencing has been developed, which allows cost-effective screening of many genes simultaneously and with a higher sensitivity than Sanger sequencing [121, 122]. For this purpose, commercial or laboratory-developed amplicon-based NGS assays have been designed to cover the most recurrent genes in major entities of lymphoid malignancies [3]. While these gene panels can detect single nucleotide variants (SNVs)

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**Table 2.** *Overview of mutations in selected genes in classic Hodgkin, large cell, and aggressive B-cell lymphomas*

| Pathway | Gene | BL (%) | DLBCL (%) | PMBCL (%) | cHL (%) | PBL (%) |
|---|---|---|---|---|---|---|
| JAK-STAT | *SOCS1* | | ST2 | 40–50 | 30–40 | 5–10 |
| | *STAT3* | | ST2 | | 5–15 | 30–40 |
| | *STAT6* | | <5 | 30–40 | 30–40 | |
| | *JAK1* | | | 5–15 | | 10–20% |
| MAPK | *NRAS* | | | | | 10–20% |
| | *KRAS* | | <5 | | | 5–10 |
| BcR | *CD79B* | | MCD | | | |
| | *CARD11* | | | | | 5–15 |
| Epigenetic | *KMT2D* | 5–10 | EZB | | 5–15 | |
| | *CREBBP* | 5–10 | EZB | | 5–10 | |
| | *EP300* | | EZB | | | 10–20% |
| | *EZH2* | | EZB | 10–20% | 30–40 | |
| | *HIST1*[a] | 30–40 | 5–15 | 20–30 | 10–20% | |
| | *ARID1A* | 5–10 | | | 10–20 | |
| | *TET2* | | ST2 | | <5 | 5–15 |
| | *ZNF217* | | | 20–30 | 10–20% | |
| Cell cycle | *MYC* | 40–50 | | 5–15 | | 10–20% |
| DNA damage | *TP53* | 10–20% | 10–20% | 10–20% | 5–10 | 20–30 |
| | *ATM* | <5 | <5 | | 5–15 | |
| NF-κB | *NFKBIE* | | <5 | 10–20% | 10–20% | |
| NOTCH | *NOTCH1* | 5–10 | N1 | | <5 | |
| | *NOTCH2* | | BN2 | | <5 | |
| | *SPEN* | | 5–10 | | 5–15 | |
| TLR | *MYD88* | | MCD | | | |
| Apoptosis | *BCL2* | | EZB[b] | | | |
| Immune | *TNFRSF14* | | EZB | | <5 | |
| | *CXCR4* | | <5 | | <5 | |
| | *B2M* | | 5–10 | 20–30 | 20–30 | |
| | *TNFAIP3* | | BN2 | 20–30 | 20–30 | |
| | *IL4R* | | | 20–30 | | |
| Transcription | *ID3* | 30–40 | | | <5 | |
| | *TCF3* | 20–30 | | | | |
| | *KLF2* | | 5–15 | | <5 | |
| Others | *PTPN1* | | | 20–30 | 20–30 | |

Mutation frequency ranges (%)

| | |
|---|---|
| <5 | 20–30 |
| 5–10 | 30–40 |
| 5–15 | 40–50 |
| 10–20 | >90 |

*Note*: Frequency ranges are approximated, in a majority of cases stemming from two or more studies. In the DLBCL column, EZB, BN2, MCD, N1, and ST2 denote mutations typically present in the said molecular subtype.
Abbreviations: BcR, B-cell receptor; BL, Burkitt lymphoma; BN2, *BCL6* fusion and *NOTCH2* mutation; cHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; EZB, *EZH2* mutation and *BCL2* translocation; MCD, *MYD88* and *CD79B* mutations; N1, *NOTCH1* mutation; PBL, plasmablastic lymphoma; PMBCL, primary mediastinal B-cell lymphoma; ST2, *SGK1* and *TET2* mutations; TLR, toll-like receptor.
[a]HIST1 gene family.
[b]*BCL2* translocation.

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and indels accurately, polymerase chain rection (PCR) amplification can introduce biases in amplification and sequence artifacts (in particular with FFPE tissue as input), and sequencing of GC-rich regions can be problematic. To study the robustness of amplicon-based assays, a multicenter study, testing three different amplicon-based gene panels (including 11 recurrently mutated CLL-related genes) and 48 CLL samples, was carried out at six European centers [123]. While a very high concordance was demonstrated between assays and centers above a variant allele frequency (VAF) of 5%, more diverse results were seen for variants with low VAF (<5%). A conclusion from the study was that the introduction of unique molecular identifiers is necessary to reliably detect variants with lower VAFs.

In more recent years, the focus has shifted to hybridization-based targeted enrichment panels. Using baits that capture the regions of interest, these assays give a more uniform sequencing quality, and hence more difficult regions and difficult samples can be sequenced [124, 125]. While a larger number of genes can be included in capture-based gene panels, different types of genomic aberrations can also be assessed, that is, SNVs/indels, copy-number aberrations (CNAs), and structural variants (e.g., translocations). In addition, they can provide information on more complex markers, such as IG gene and T-cell receptor rearrangements. Two such capture-based gene panels were recently published focusing on lymphoid malignancies, that is, the LYNX panel [126] and the EuroClonality-NGS capture panel [127]. Another advantage with broad capture-based panels is that they may allow detection of the genomic aberrations needed to identify disease subgroups, for instance, the recently proposed new subtypes of DLBCL [53]. Moreover, capture-based panels are not limited to the detection of genomic aberrations but can also include targeted RNA sequencing and even epigenomic alterations [124]. With this type of panel, it will be possible to combine different types of molecular tests currently performed in routine diagnostics into a single test. This will hopefully pave the way for the implementation of precision diagnostics for patients with lymphoid malignancies.

Another option for comprehensive genomic profiling would be to perform WGS or WES to capture all the relevant genomic markers needed for diagnostic purposes. Clinical WGS is tested for acute leukemias within different national/local initiatives with the goal to replace the many different genetic analyses currently performed for these patients [128, 129]. A pilot study in CLL has also compared WGS versus targeted NGS and FISH with high concordance for SNVs/indels, while the concordance was lower for CNAs. However, considering the cost for WGS is still high and that WGS is challenging for FFPE samples, this is currently not a viable option.

### Epigenomics of lymphoid malignancies

The altered phenotype and function of neoplastic lymphoid cells are not only achieved through the acquisition of genetic alterations, but also a variety of epigenetic changes [6]. Generally speaking, epigenetics is defined as the discipline that studies changes in gene expression in the absence of any genetic alteration [6, 7]. This gene expression regulation is achieved through different layers that act in concert, including DNA methylation, histone modifications, chromatin accessibility, and 3D folding of the genome. DNA methylation thus far is the best-characterized layer, for the most part consisting of addition of a methyl group to cytosines mostly in a cytosine-guanine dinucleotide (CpG) context. Although DNA methylation is generally perceived as a repressive epigenetic mark, with gene silencing through promoter hypermethylation as a widely reported phenomenon [130], its role depends on the genomic and chromatin context where methylation takes place [131, 132]. In addition to DNA methylation, histone marks comprise a variety of chemical modifications of histones that strongly associate with particular genomic functions [133]. Looking at a combination of histone marks, different chromatin states can be defined (e.g., active, weak or poised promoters, active or weak enhancers, transcriptional elongation, as well as various modes of silent chromatin). Chromatin accessibility gives information on the open regions of the genome where transcription factors bind. Finally, the 3D structure of the genome provides epigenetic information regarding distant regulation between enhancers and promoters through DNA looping [134–137]. Therefore, to gain a deep insight into epigenetic deregulation in lymphoid neoplasms, an integrative analysis of several epigenetic layers is essential. Epigenetics in precision medicine is currently an understudied aspect in comparison to genetics, but has the potential to give a plethora of information, ranging from a better understanding of

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pathophysiological processes, diagnostic and prognostic information, as well as potentially actionable targets for treatment.

*DNA methylation changes during normal B-cell differentiation*

To understand how the DNA methylome of B-cell neoplasms changes, it is essential to define what happens during the physiological maturation of B cells. In this context, two seminal works have described the dynamics of DNA methylation changes during the entire B-cell differentiation program at a single base-pair resolution [138, 139]. The first observation is that during B-cell maturation, gradual and global demethylation of the genome can be observed, particularly at mature stages (GC B cells, memory B cells, and plasma cells). A more detailed look reveals the presence of local demethylation in B-cell-related enhancers and promoters, and more abundantly, changes that do not imply a direct impact on gene expressions, such as gains of methylation in CpG-rich regions marked by the polycomb group, and methylation loss in heterochromatic regions. Different steps in the maturation of B cells are accompanied by specific changes in DNA methylation, most prominently upon entering the GC and when differentiating to memory B cells or plasma cells, and can accordingly be used to infer the differentiation stage of B cells, which is also possible with lower coverage methods, such as methylation microarrays or even just by analyzing a reduced set of CpGs [139]. Importantly, the characterization of normal-B-cell differentiation allows interpretation of methylation changes of B-cell lymphomas from a COO standpoint.

*Methylation-based proliferative clock*

As mentioned above, normal B-cell maturation involves DNA methylation changes without an impact on gene expression. Based on recent studies, these changes (hypermethylation in polycomb regions and hypomethylation in heterochromatin) have been linked to cell division [140–142]. Therefore, DNA methylation in these repressed regions becomes accumulated during sequential cell divisions and can be used as a mitotic clock. In normal and neoplastic B cells, this concept has led to the development of an epigenetic mitotic clock, termed epiCMIT (epigenetically-determined cumulative mitoses), which captures both DNA hypo- and hypermethylation associated with cell proliferation [8]. The epiCMIT of normal B cells at different differentiation stages reflects the past proliferative history, rather than their current proliferation state, highlighting how DNA methylation can provide information on the past processes a cell has gone through. In malignant B cells, the epiCMIT reflects the sum of proliferation during normal B-cell development and during malignant transformation. In comparison to other epigenetic clocks like the Horvath clock [143] (which predicts the chronological age), the epiCMIT is independent of the age of the individual. Analysis of the epiCMIT not only traces the past proliferative history but has an important role in predicting the future clinical behavior of the patients. Changes in the epiCMIT have been shown to independently predict the clinical outcome in ALL, CLL, and MCL, as well as being associated with a greater number of genetic driver lesions, reflecting the accumulation of alterations with time [8].

*Methylation landscape and subtypes of B-cell lymphomas*

The DNA methylation landscape of a majority of B-cell lymphomas has already been characterized, in most cases by means of high density, single base-pair resolution methylation microarrays (i.e., 450k or EPIC methylation arrays). A general observation is that across mature B-cell lymphomas, there is a global DNA methylation loss when compared to normal B cells, most likely due to loss of methylation in heterochromatic regions occurring with proliferation [6].

Early work on CLL [144] showed that approximately half of the differentially methylated CpGs between CLL subtypes (U-CLL and M-CLL) are related to their respective resemblance to the COO. In addition, a new epigenetic subtype termed intermediate CLL (i-CLL) could be identified, besides naïve-like CLL (n-CLL, corresponding to U-CLL) and memory-like CLL (m-CLL, corresponding to M-CLL). This finding was confirmed in a later study [140] using an alternative terminology (low-programmed CLL for n-CLL, intermediate-programmed CLL for i-CLL, and high-programmed CLL for m-CLL) and in several clinical series [145–148], showing intermediate clinical behavior of i-CLL, between the more aggressive n-CLL and m-CLL. Of interest, recent work has shed further light on the i-CLL subtype, finding that a majority of cases with adverse clinical behavior harbor a point mutation in the lambda light chain variable gene (IGLV3-21$^{R110}$), which leads to constitutively active

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BcR signaling, and a subset of those cases also belong to stereotyped subset #2 [71, 72]. Moreover, analysis of CLL with trisomy 12 found that this subgroup has a distinct methylation profile, which, together with altered chromatin activation, explains some of the biological differences of this cytogenetically defined subtype [149].

In MCL, two epigenetic subtypes were identified [150], which correspond to the clinicopathological subtypes of cMCL and leukemic nnMCL (C1 and C2 MCL, respectively). By integrating the knowledge on methylation changes in normal B cells, these two subtypes could again be related to their COO (naïve-like for C1 MCL and memory-like for C2 MCL), confirming earlier results (e.g., through IGHV sequencing) [151]. In plasma cell myeloma/multiple myeloma (PCM/MM), apart from the aforementioned global loss of methylation, specific hypermethylation of enhancer regions could be observed, as opposed to the widely reported hypermethylation of CpG islands (CpG-island methylator phenotype) reported in some solid tumors [152]. In BL and FL, despite both originating from the GC, significant differences could be observed [153]. Interestingly, as BL and FL are thought to originate from the dark zone (DZ) and light zone (LZ), respectively; several genes associated with the DZ were shown to be hypomethylated and upregulated in BL and hypermethylated and downregulated in FL, whereas other genes associated with the LZ exhibited the opposite pattern. Analysis of primary central nervous system lymphomas (PCNSL) [154] showed a distinct methylation pattern from DLBCL, underlining the previously postulated separation of PCNSL as a distinct subtype of DLBCL, rather than merely representing DLBCL with isolated CNS manifestation. Recently, DNA methylation analysis of LPL/WM identified two disease subtypes, which resemble memory B cells and plasma cells (PC-like) [155]. The two subtypes show several different clinical, morphological (plasma cell differentiation in PC-like WM), immunophenotypical, and genetic characteristics, highlighting the clinicopathological significance of these previously unknown subtypes.

To sum up, although the classical role of DNA methylation is to regulate gene expression, recent epigenetic studies in B-cell neoplasms have revealed that DNA methylation is strongly related to cellular memory, without a regulatory role, in terms of identifying the cellular origin and measuring the past proliferative history. These two components of cellular memory are variables independently associated with the clinical behavior of the patients and they could represent an important complement to the detection of genetic changes in clinical diagnostics.

### *Towards a DNA methylation–based classification of B-cell lymphomas*

DNA methylation has been successfully used to generate tumor classifiers, first for central nervous system tumors [156] and more recently for sarcomas [157]. As the characterization of B-cell malignancies (which include more than 40 different entities) is still not complete, a globally applicable classifier is not available yet. Duran-Ferrer et al. recently designed a two-step methylation classifier that accurately classified an unknown B-cell malignancy into general categories first, such as ALL, CLL, MCL, PCM/MM, and DLBCL, and their subtypes in a second step (e.g., n-CLL, i-CLL, and m-CLL) [8]. Importantly, this classifier is based on a limited number of CpG sites and therefore is amenable to be used in targeted gene panels or locus-specific assays. Looking forward, with further characterization of rare B-cell lymphomas, a pan B-cell lymphoma methylation classifier can be achieved in the coming years, offering a powerful tool to complement the traditional classification of lymphomas. Figure 1a summarizes DNA methylation changes in B-cell lymphomas and their prognostic implications.

### *Chromatin landscape of B-cell lymphomas*

Overall, the chromatin profile of B-cell lymphomas is less explored than their DNA methylome, but CLL has been the subject of several integrative chromatin studies, which have revealed important insights into deregulated genes, pathways, and transcription factors networks [10, 158–160]. Chromatin accessibility studies of CLL [161] can reproduce the subclassification of the major disease subtypes (U-CLL and M-CLL), and like in the aforementioned methylation studies, an intermediate group could also be identified, highlighting the added value of epigenetics in refining the subclassification of lymphoid malignancies. The reference epigenome of CLL [10], which includes six histone marks and chromatin accessibility besides DNA methylation and gene expression, revealed the chromatin state changes CLL cells undergo in malignant transformation, as well as subtype (U-CLL and M-CLL) specific differences. Other findings include more active and open chromatin in

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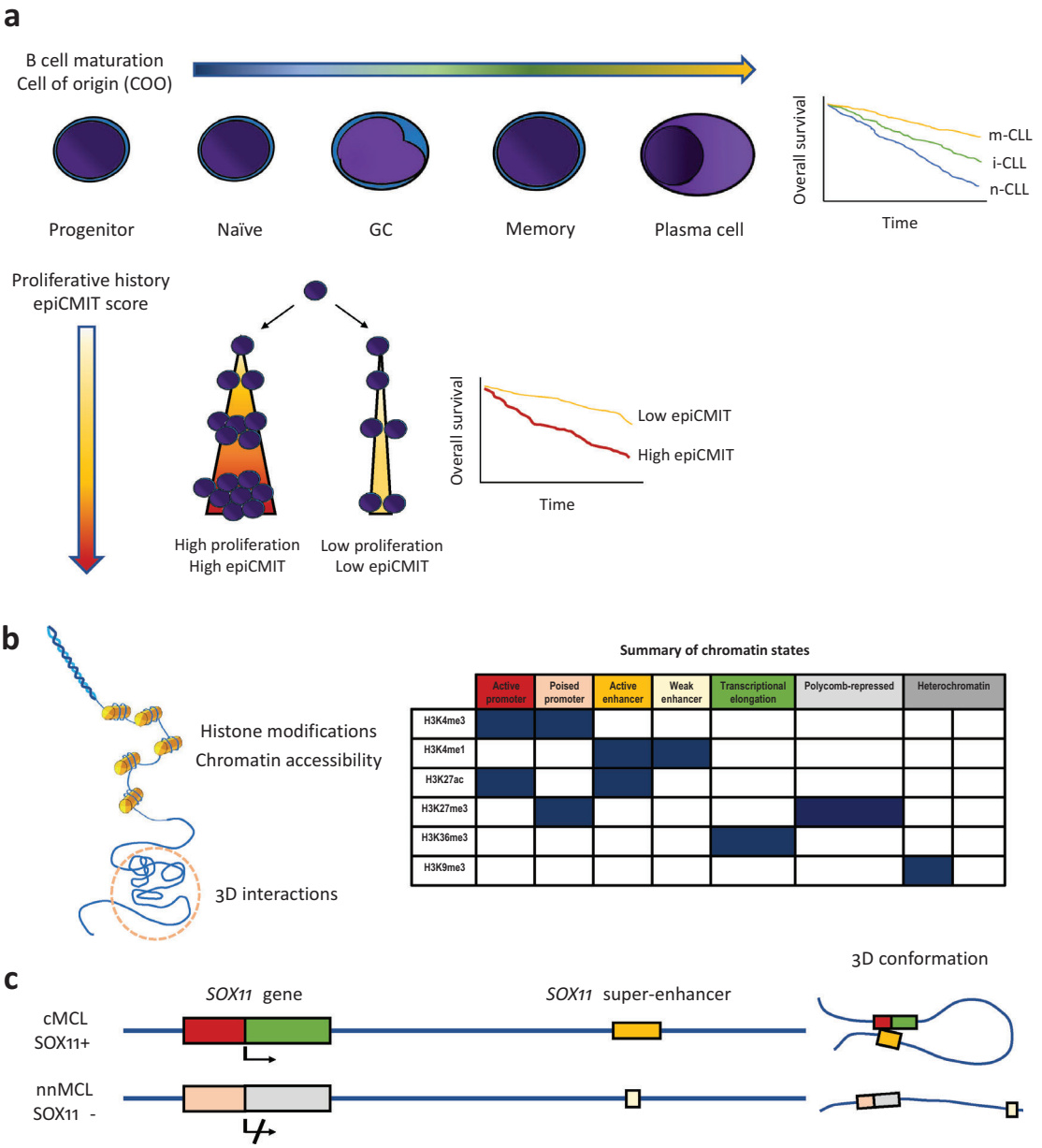


**Fig. 1** *Epigenetics in B-cell malignancies. (a) DNA methylation changes during B-cell maturation (upper panel); in B-cell neoplasms, DNA methylation imprints of normal B-cell maturation allow classification of them into different clinicobiological subtypes (the Kaplan–Meier curve on the right shows chronic lymphocytic leukemia [CLL] as an example). Cell proliferation is also imprinted into the DNA methylome, which allows to determine the past proliferative history (epigenetically determined cumulative mitoses score), whose magnitude also has important prognostic value in B-cell malignancies (lower panel). (b) Histone modifications, chromatin accessibility, and 3D genome interactions are important epigenetic marks (left). The combination of different histone marks allows identification of segments of the genome with different functions, the so-called chromatin states (right). (c) Example of multilayered epigenetic analysis for SOX11 in mantle cell lymphoma (MCL). Chromatin state analysis in SOX11 positive MCL shows active promoter/distal super-enhancer regions, which are in close contact in the 3D space (chromatin loop). SOX11 negative MCL has a poised promoter and a small weak enhancer, which are maintained distant in the 3D space (no chromatin loop).*

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the clinically more aggressive U-CLL subgroup, de novo activation of regions enriched for several transcription factor binding sites (*NFAT*, *FOX*, and *TCF/LEF*), and some associations of chromatin configurations with genetic alterations (e.g., *MYD88* mutated CLL and cases with trisomy 12). Interestingly, differential methylation in trisomy 12 CLLs is linked to differential chromatin activation in these cases [149]. Figure 1 (b) illustrates the different aforementioned epigenetic layers and a summary of chromatin states based on histone marks.

In MCL, through analysis of chromatin states and chromatin conformation [150], a distant *SOX11* enhancer could be identified, which is active only in SOX11 positive MCL, shedding light on the mechanisms that lead to the expression of this important oncogene (Fig. 1c). These results have been recently confirmed, including demonstration of physical proximity by FISH analysis and identification, within the enhancer element, of a small accessible region that is exclusively active in SOX11 expressing MCL [162]. Recent work on the genome-wide 3D genome architecture of CLL and MCL [163] highlighted changes in active and inactive compartments during B-cell development and lymphomas, as well as identified an intermediate compartment that is enriched in poised and polycomb repressed chromatin. In PCM/MM, the chromatin landscape has been characterized and revealed the presence of a widespread activation of regulatory elements leading to upregulation of members of the NOTCH, NF-$\kappa$B, MTOR signaling, and TP53 pathways as well as other processes such as osteoblast differentiation and response to oxidative stress [164].

### New technologies for precision medicine in lymphoma

As elaborated in the previous sections, bulk genomic, epigenomic, and transcriptomic studies have provided a comprehensive and unbiased view of lymphoid malignancies. Integration of the unbiased functional screen by CRISPR-Cas9 based methods has also been useful for the identification of druggable vulnerabilities in lymphoma cell lines [49, 165]. However, bulk sequencing or screen of cell lines offers limited insight into the clonal composition of tumors, a key factor in treatment resistance and tumor progression [166]. Another missing element of bulk analysis is the tumor microenvironment (TME), which closely interacts with malignant cells [167] and is especially relevant with the advent of immunotherapies, such as immune checkpoint inhibitors. The rise of single-cell technologies has opened new possibilities, allowing capture of both tumoral heterogeneity and the microenvironment in one single experiment [168], even combining different information layers (multi-omics) [169] and spatial characterization of cell populations in tissue samples [170]. The Human Cell Atlas (HCA) project leverages these new technologies and aims to provide a comprehensive catalog of cells in human tissues and organs [171], including transcriptomic, epigenetic, and spatial characteristics. Thus far, HCA projects have already investigated several tissues and organs, some of them with prominent immune cell populations. For instance, the thymus cell atlas [172] provides a comprehensive map of the cellular composition of this lymphoid organ and especially of T-cell development. Several studies have investigated the single-cell composition of the liver [173–175], including the hepatic immune microenvironment and fetal hepatic hematopoiesis. As expected, the normal human heart contains only a relatively modest amount of immune cells, mainly myeloid and T cells [176]. The lung on the other hand is constantly challenged by potential infectious agents and therefore relies on well-functioning mucosal immunity, especially rich in alveolar macrophages [177]. Upon completion, the HCA will provide a comprehensive map of the normal cell populations of different organs, allowing to better interpret changes observed in the disease. Other single-cell studies have looked specifically at the B-cell lineage, elucidating the maturation process and different cell subtypes/states [178–182]. In the study by King et al. [178], tonsillar B cells were sequenced, characterizing the maturation process from naïve B cell to plasmablast. Interestingly, a previous unappreciated heterogeneity of memory B cells could be shown, as well as comparable SHM rates between memory B cells and plasmablasts. In mice [179], two different subsets of memory B cells were identified, which were shown to arise from activated B cells and GC B cells. Detailed analysis of GC B cells [180] expanded the traditional DZ and LZ subdivision of GC B cells, defining four DZ states, six intermediate states, an LZ state, as well as premem-ory and plasmablast states. Using mass cytometry, surface molecules of B cells from various sources (blood, bone marrow, lymph node, and tonsil) were characterized [181], proposing classification into 12 different B-cell subsets (immature/transitional, two naïve, GC, six memory, plasma cell, and tonsil-specific subsets). Similarly, using single-cell

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transcriptomics [182] of fluorescence-activated cell sorting (FACS)-sorted peripheral blood mononuclear cells, 10 different B-cell clusters were defined, expanding classic B-cell characterization by traditional flow sorting. These studies provide a framework for the interpretation of changes in malignant transformation of B cells and allow a novel transcriptomic-based refined COO classification.

CLL has been more extensively studied with single-cell technologies [183–189], focusing on tumoral heterogeneity, clonal evolution, and changes after treatment. Integration of single-cell genomics and transcriptomics identified two new disease drivers (*LCP1* and *WNK1*), thanks to the precise determination of tumor subclones [183]. One study focused on the effects of *SF3B1* mutations on signaling pathways and alternative splicing, providing insight into the mechanistic effects of this important gene in CLL [186]. A thorough case study of one CLL patient looked at cytogenetic, transcriptomic, and genomic changes over a period of 29 years, highlighting the clonal changes in different disease phases [189]. Gaiti et al. applied a combination of single-cell transcriptomics and methylation analysis to explore intratumoral heterogeneity in the evolution of CLL (including after treatment with ibrutinib), finding considerable heterogeneity after initial malignant transformation and clones that preferentially exited the lymph node upon ibrutinib exposure [187]. Another study also analyzed the changes CLL cells undergo when treated with ibrutinib, this time considering single-cell transcriptomics and chromatin accessibility, uncovering NF-$\kappa$B signaling downregulation, a quiescence-like gene signature, and changes in chromatin accessibility (e.g., decreased accessibility for CLL-specific enhancers) upon exposure [184]. Recently, Penter et al. leveraged (naturally *in vivo* occurring) mitochondrial DNA mutations to obtain a precise tracking of CLL clones, which was then used to study differences in chromatin states and transcription [188], showing how mitochondrial DNA mutations are powerful *in vivo* markers of (sub)clones.

In the first study that used single-cell technology in FL [190], the transcriptional heterogeneity was determined and compared to different GC B-cell states. First, characterization of normal GC B cells allowed definition of three main cell states (DZ, intermediate zone, and LZ), which lie in a continuum and show a nuanced synchronous expression of gene clusters. When comparing FL cases with these normal GC B-cell states, these synchronized gene clusters could not be observed and FL B cells were not found to be blocked in a specific GC stage, pointing to a desynchronization of the normal GC specific expression program. In a later study on FL [191], it was found that patient samples include multiple malignant subclones at the transcriptional level, which seem to be at least in part caused by mutational heterogeneity, although it is apparent that other (unknown) drivers could also be responsible for transcriptional heterogeneity. Furthermore, tumor-infiltrating CD4+ Treg cells with coexpression of immune checkpoint genes were identified, highlighting the utility of the simultaneous profiling of the TME in single-cell studies. Another interesting FL study [192] characterized tumor samples from different anatomical sites of the same patient using a multi-omics approach. Subclones were identified (leveraging BcR sequencing) and, interestingly, were shown to sometimes coexist in both locations and other times to be location specific, suggesting divergent evolution, which might be responsible for treatment resistance. In the aforementioned study by Holmes et al. that characterized the GC into novel cell subpopulations [180], DLBCL samples were studied using a single-cell RNA sequencing (scRNA-seq) defined COO classification (based on normal GC data) and compared to the classical GCB versus ABC COO model. Around 80% of DLBCL cases could be assigned to a specific B-cell subpopulation; it was found that a majority of GCB DLBCL fall between an intermediate LZ-like and early LZ stage, most ABC DLBCL are related to late GC and prememory B cells (rather than plasmablasts), and interestingly several DLBCL cases that displayed a DZ signature were enriched for *MYC* and *BCL2* translocations (double hit lymphomas), reinforcing the view that these lymphomas represent a separate entity (HGBCL with *MYC* and *BCL2* and/or *BCL6* translocation). Roider et al. exploited single-cell sequencing to dissect both the tumor and microenvironment heterogeneity of four FL, two transformed FL, and three DLBCL clinical samples in an interesting proof-of-concept study for the use of single-cell technology in precision oncology [193]. Different proportions of T-cell subtypes were shown to populate the TME of different lymphomas (e.g., expected higher proportion of Tfh cells in FL). All examined samples had at least two transcriptional distinct populations of malignant cells and three cases were studied in depth, including *in vitro* drug-response assays, which revealed

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different sensitivity of subclones to chemotherapeutic and targeted agents. In a more recent study, 17 DLBCL samples were analyzed by scRNA-seq in depth, and 74 gene expression programs were identified from the malignant B cells, illustrating high degrees of inter- and intratumor heterogeneity (Ye and Pan-Hammarström et al., manuscript submitted). Furthermore, eight nonmalignant B-cell subclusters, 16 T-cell subclusters, and six myeloid subpopulations were characterized, and more than 2700 pairs of cell–cell interactions were predicted, indicating a complex and highly dynamic TME in DLBCL.

cHL is another ideal candidate for single-cell sequencing, due to the low tumor cell content and a prominent and heterogeneous inflammatory TME. Indeed, scRNA-seq of 22 cHL [194] cases revealed a novel subset of *LAG3* expressing T cells, which locate closer to the tumoral cells, are associated with loss of MHC-II expression, and might represent a novel treatment target. In MM/PCM, malignant clones are readily identified at the single-cell transcriptional level, including small residual clones after treatment, and transcriptionally different tumor subclones could be identified in a third of cases in one study [195] and all cases in another [196]. Zavidij et al. [197] concentrated on the TME in PCM/MM and found an increase of NK, T, and CD16+ cells and a decrease of plasmacytoid DC and CD14+ monocytes in comparison to normal samples. A detailed look at T-cell subsets specifically highlighted an increase in Tregs and a switch from memory to effector cytotoxic cells during disease progression. Analyzing PCM/MM patients enrolled in a clinical trial [198], several genes (implicated in mitochondrial, endoplasmic reticulum, and oxidative stress) associated with therapy resistance were identified, which, combined in a signature, were shown to predict outcome better than traditional cytogenetic models. One particular identified overexpressed gene was demonstrated to confer resistance to proteasome inhibitors and shown to be targetable with cyclosporin A *in vitro*. Leveraging single-cell resolution and multiple samplings during treatment, different clonal dynamics were observed in patients, with some being primarily sensitive or resistant and others showing the emergence of new resistant subclones during treatment. PCM/MM studies highlight the potential of single-cell technologies since it is still an incurable disease in which treatment resistance develops, at least in part due to tumor heterogeneity.

Thus far, two studies have tackled MCL using single-cell technologies. In the first [199], bone marrow cells from a single patient were sequenced, describing four transcriptional malignant subclones as well as the TME, but conclusions from this study are limited due to only analyzing one case. The second study sequenced samples from five patients at different timepoints, of which three were ibrutinib responsive and two were nonresponsive [200]. Using this approach, a 17q gain (which, among other genes, includes *BIRC5*) was identified in one ibrutinib-resistant case. The overexpression of survivin (encoded by *BIRC5*) was then validated in a patient-derived xenograft model and an independent series of ibrutinib-treated MCLs, and was shown to be an actionable target to overcome ibrutinib resistance in *in vitro* models (cell lines).

In summary, single-cell studies of lymphomas highlight both tumor cell heterogeneity (which might explain treatment resistance), potential new actionable targets, as well as the cellular composition of the TME.

## Concluding remarks

By applying high-throughput genomic, transcriptomic, and epigenomic technologies in the last 10 years, new biomarkers with clinical impact have been identified in most B-cell malignancies. While the number of diagnostic and prognostic markers is steadily increasing, there are still relatively few markers that can be used to predict therapy response at the individual patient level. Hopefully, with comprehensive molecular profiling, as part of clinical trials, we will discover new predictive markers that can be applied to tailor the patient's treatment and follow-up. Although an increasing number of targeted drugs are available for patients with lymphoid malignancies, at least for selected entities, very few precision medicine trials exist worldwide that include patients with lymphoid tumors. In the coming years, the implementation of broad gene panel sequencing in the diagnostic workup of lymphoid tumors will result in improved patient subcategorization and this will hopefully prompt the initiation of new precision medicine studies. Finally, even though new technologies, such as single-cell sequencing and spatial transcriptomics, have not yet entered clinical practice, they are very promising and could be a key factor in unlocking the potential of precision medicine in the treatment of lymphoid malignancies. Tumor heterogeneity and the TME have long been recognized as

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crucial factors of tumor biology, but only with the advent of single-cell sequencing can they be studied with sufficient depth and efficiency, particularly in patients treated with targeted therapies. This may lead to the development of new strategies and approaches for personalized treatment and care for patients with lymphoid malignancies.


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### Author contributions

Marco M. Bühler: conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). José I. Martin-Subero: conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). Qiang Pan-Hammarström: conceptualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). Elias Campo: conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). Richard Rosenquist : conceptualization (lead); writing – original draft (lead); writing – review & editing (lead).

### Conflict of interest

Richard Rosenquist has received honoraria from Abbvie, AstraZeneca, Janssen, Illumina, and Roche. Elias Campo has received honoraria from Roche, NanoString, Takeda, Eusapharma, Illumina, AbbVie, and AstraZeneca.

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