Molecular Genetics in Indolent Lymphomas

2

Jude Fitzgibbon and Oliver Weigert

2.1 Introduction

The earliest genetic maps of indolent lymphomas appear quite rudimentary today and for the most part relied almost exclusively on conventional cytogenetics and array-based profiling to document chromosomal translocations and copy number aberrations. These observations have served as an important framework upon which next-generation sequencing (NGS) tools are delivering additional insights into the genomes of these malignancies. Certainly, we have moved away from thinking of a genetic lesion as simply the presence or absence of mutation, and there is a growing emphasis now on the longitudinal and spatial profiling of these indolent lymphomas, the early and late occurrences of mutations in lymphoma evolution, the complex reciprocal interactions of lymphoma cells with components of the tumor microenvironment, and the importance of dynamic monitoring of diseases. The growing body of knowledge is providing us with an unprecedented understanding of the biology of this group of lymphomas, and with each additional layer, there remains the ever-present challenge to translate these new insights into meaningful interventions for the benefit of lymphoma patients.

J. Fitzgibbon
Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK
e-mail: j.fitzgibbon@qmul.ac.uk

O. Weigert (✉)
Department of Medicine III, LMU Hospital, Munich, Germany
e-mail: oliver.weigert@med.uni-muenchen.de

2.2 The Molecular Biology of Indolent Lymphomas: Follicular Lymphoma as a Prototypical Example

2.2.1 The Translocation t (14;18) in FL

The primary genetic event in the classical model of FL pathogenesis is the reciprocal translocation t (14;18) (q32;q21) that is detected in approximately 90% patients [1]. This rearrangement relocates the immunoglobulin heavy chain (IGH) enhancer region adjacent to the anti-apoptotic *BCL2* gene, resulting in aberrant constitutive overexpression of *BCL2*. This critical early step in the pathogenesis of FL occurs in the bone marrow in response to faulty VDJ recombination early during B-cell maturation, though the occurrence of the rearrangement in 30–50% of normal, healthy individuals supports the notion that upregulation of *BCL2* alone is not sufficient for FL development. These t (14;18) B cells are long-lived IgD^+ or IgM^+ $CD27^+$ memory B cells, which have experienced the germinal center while bypassing the usual

M. Dreyling, M. Ladetto (eds.), *Indolent Lymphomas*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-55989-2_2

physiological cell-death signaling that occurs in non-antigen-stimulated B cells, enabling repeated reentry into the GC reaction to acquire the necessary secondary hits. In a landmark study, [2]. demonstrated a clonal relationship between paired pre-diagnostic blood samples and their corresponding subsequent tumor samples, suggesting that circulating t (14;18) positive cells does indeed represent a low-risk pre-malignant precursor cell [2, 3] that can predate the disease by several years.

The reason why some individuals with t (14;18) positive circulating cells go on to develop FL while the majority do not is still debated, and while predisposing (epi-)genetic factors may offer one explanation [4, 5], it is plausible that cell intrinsic (secondary genetic hits) and extrinsic (immune microenvironmental) factors may also be of greater significance. These variables may in part explain the diversity of FL-related conditions, which include in situ follicular neoplasia [6], the highly curable pediatric-type follicular lymphoma that is typically t (14;18) negative [7], and duodenal-type FL, which bears a similar mutational profile to classical FL, yet possesses a distinctive tumor microenvironment and follows a benign clinical course [8].

2.2.2 Recurrent Genetic Alterations in FL

Molecular profiling has become synonymous with the documentation of gene mutations. Rapid advancements in the field of DNA sequencing have led to the development of cost-effective technologies capable of profiling a large series of malignancies, fueling initiatives like the Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium [9], and databases like COSMIC cataloguing somatic mutations in cancer [10]. These programs, in conjunction with stand-alone studies by single centers, have collectively succeeded in creating an encyclopedic knowledge around the coding landscape of cancers, including indolent lymphoma.

With >1000 FL tumors subjected to whole exome (WES), genome (WGS), or targeted resequencing, the coding genome and knowledge of the mutations that work in concert with the t (14;18) are nearing completion (Table 2.1). Prior to NGS, cytogenetic studies demonstrated chromosomal alterations in nearly all cases of FL with changes including translocation of *BCL2*; deletions of 1p36, 6q, and 17p; gains in chromosomes 2, 7, 8, 12, 18, and X; and copy neutral loss of heterozygosity (cnLOH) of 16p, 1p36, and 6p [45–52]. We can now point to many target genes residing within these chromosomal regions (*CREBBP, TNFRSF14, TP53,*) and throughout the genome, informed both by NGS and by functional studies confirming their role in lymphoma pathogenesis [14–16, 18, 20–22, 25, 36, 53–56].

The landscape of recurrently mutated genes in follicular lymphoma shows significant overlap with those found in other lymphoid malignancies, especially the Germinal Centre B cell (GCB) subtype of the aggressive diffuse large B-cell lymphoma (DLBCL). The FL mutational profile is most notable for the occurrence of multiple gene mutations encoding components of the epigenome, while other core processes recurrently disrupted in cancer are also affected, including B-cell receptor (BCR)-Nf-κB, (*CARD11*, *TNFAIP3*), JAK-STAT (*STAT6)*, and mTOR signaling [13, 14, 17, 27, 34, 35, 57–60]. While mTORC1 signaling is usually restrained by a scarcity of amino acids, this natural brake on cell metabolism is abrogated in ~20% of FL, by mutations in *RRAGC* and related lysosomal components *ATP6V1B2* and *ATP6AP1*, with these mutations being conspicuously unique to FL [34].

2.2.3 Mutations in Epigenetic Regulators

From the earliest NGS studies, it was apparent that mutations in genes that regulate the epigenome, particularly histone modifiers, were a hallmark of the genetic features of FL and to a lesser extent many of the other indolent lymphomas. The term *epigenetics* refers to (heritable) mechanisms involved in regulating gene expression that do not alter the underlying DNA sequence. These typically involve the dynamic

Table 2.1 Gene mutations affecting >10% of classical follicular lymphoma

Gene	Effect	Frequency (%)	Mutation	Function	Lymphoma biology
KMT2D	↓	80–90	Pastore et al. (2015) [11], Karube et al. (2018) [12], Morin et al. (2011) [13] and Green et al. (2013) [14]	Histone K4me3 methytransferase	Zhang et al. (2015) [15] and Ortega-Molina et al. (2015) [16]
CREBBP	↓	33–70	Pastore et al. (2015) [11], Pasqualucci et al. (2011) [17], Karube et al. (2018) [12], Morin et al. (2011) [13] and Green et al. (2013) [14]	Histone acetylation	Zhang et al. (2017) [18], Hashwah et al. (2017) [19], Ennishi et al. (2019) [20, 21] and Mondello et al. (2020) [22]
TNFRSF14	↓	18–50	Launay et al. (2012) [23], Cheung et al. (2010) [24], Karube et al. (2018) [12] and Green et al. (2013) [14]	Regulator inflammatory and inhibitory T-cell immune response	Boice et al. (2016) [25]
Histone linkers	↓	40	Okosun et al. (2014) [26, 27] and Karube et al. (2018) [12]	Chromatin remodeling	–
EZH2	↑	25	Pastore et al. (2015) [11], Karube et al. (2018) [12] and Bodor et al. (2013) [28]	Histone K27me3 metyltransferase	Caganova et al. (2013) [29], Béguelin et al. (2013, 2016) [30, 31] and Berg (2014) [32]
EP300	↓	10–20	Pastore et al. (2015) [11], Okosun et al. (2014) [26, 27], Pasqualucci et al. (2011) [17] and Morin et al. (2011) [13]	Histone acetyltransferase	Meyer et al. (2019) [33]
ARID1A	↓	15	Pastore et al. (2015) [11], Karube et al. (2018) [12] and Morin et al. (2011) [13]	SWI/SNF family, transcriptional regulator	–
RRAGC *ATP6V1B2,ATP6AP1*	↑	20	Okosun et al. (2016) [34] and Green et al. (2015) [35]	mTORC1 regulators	Ortega-Molina et al. (2019) [36]
MEF2B	↓	10	Pastore et al. (2015) [11], Karube et al. (2018) [12] and Morin et al. (2011) [13]	Transcription factor	Brescia et al. (2018) [37]
GNA13	↓	10	Pastore et al. (2015) [11], Karube et al. (2018) [12], Morin et al. (2011) [13] and Green et al. (2013) [14]	B-cell growth and lymphoma cell dissemination	Muppidi et al. (2014) [38]
FOXO1	↑	10	Karube et al. (2018) [12]	Transcription factor	Szydlowski et al. (2016) [39] and Kabrani et al. (2018) [40]
CARD11	↑	10	Pastore et al. (2015) [11], Okosun et al. (2014) [26, 27], Karube et al. (2018) [12] and Morin et al. (2011) [13]	NF-κB regulator	Compagno et al. (2009) [41] and Davis et al. (2010) [42]
STAT6	↑	10	Pastore et al. (2015) [11], Yildiz et al. (2015) [43] and Okosun et al. (2014) [26, 27]	JAK-STAT signalling	Yildiz et al. (2015) [43]

↑ Gain, ↓ loss of function (Table modified from Carbone et al. [44])

addition or removal of chemical groups to histones or DNA by enzymes known as writers or erasers, thereby altering the access of transcription factors and the expression levels of affected genes [61, 62]. In FL, the most prevalent mutations affect histone methyltransferases (*KMT2D*, *EZH2*), acetyltransferases (*CREBBP, EP300*), and chromatin structure (*ARID1A,* histone linkers, e.g., *HIST1H1E*) [13, 17, 26, 57, 63, 64], with these "epimutations" co-occurring in up to 80% cases [65, 66]. These somatic mutations are predominantly inactivating, with the exception of *EZH2,* and alter chromatin state to condensed transcriptionally repressed heterochromatin. These lesions are well established as early, disease-initiating events in low-grade FL [14, 26, 35, 57, 60], and their lymphoma-promoting functional consequences have emerged, such as the GC expansion and terminal differentiation block induced by KMT2D loss [15, 16] and immune evasion through MHC class I/II downregulation seen with *CREBBP* [35], and *EZH2* mutations [21]. Critically, we are lacking an understanding of why FL (and other indolent lymphomas to a lesser extent) are addicted to mutations affecting components of the epi-machinery and how coexisting epi-mutations may cooperate over the course of lymphomagenesis.

2.2.4 Molecular Genetics and the Role of the Tumor Microenvironment

The importance of the immune microenvironment in FL is highlighted by the fact that in vitro growth of tumors is challenging [67]. Molecular studies employing gene expression profiling of bulk tumor samples have played an important role in characterizing the tumor microenvironment, complementing efforts to enumerate immune cell populations by immunohistochemistry or flow cytometry [68] seminal study described two prognostic gene expression signatures that were crucially defined not by tumor cell characteristics but by the dominant cellular composition of the microenvironment. Although these signatures appear to lose prognostic significance in cohorts treated with rituximab-containing therapy (thus highlighting the changing fortunes of such prognostic markers as therapy evolves), the importance of the immune microenvironment composition to outcome constituted a core principle for subsequent studies [69].

An important new area of study is an understanding of how specific genetic lesions enable FL tumor cells to co-opt the microenvironment for their benefit, with several individual examples emerging. One early link was the observation that unusually high numbers (~80%) of FL cases carry novel sites for immunoglobulin N-glycosylation, as a result of activation-induced cytidine deaminase (AID)–driven somatic hypermutation affecting heavy chain variable (V_H) genes [70] and with functional studies showing the ability of the resulting glycosylated immunoglobulin to bind dendritic cell and macrophage-expressed lectins, thus stimulating BCR signaling [71, 72]. TNFRSF14, a cell surface receptor involved in T-cell signaling and loss of *TNFRSF14* through mutations, deletions (1p36), and cnLOH in approximately 20–40% FL cases, has been linked to a tumor-supportive microenvironment with an increase in stroma-activating cytokines and T follicular helper (T_{FH}) cells. Intriguingly, TNFRSF14 function could be restored after the administration of soluble TNFRSF14 protein via specifically engineered chimeric antigen receptor T cells in a mouse xenograft model [25].

The impact of epimutations on the immune microenvironment is likely to proceed by multiple mechanisms. *CREBBP* and *EZH2* mutation likely contribute to immune evasion through class I/II downregulation [21, 35], while recent functional work showed that activating, *EZH2* mutations skewed GC B-cell dependence away from the normal T_{FH} cell signal support and toward lymphotoxin β-mediated dendritic cell (DC) support, with *EZH2*-mutant FL pathology samples intriguingly showing increased follicular dendritic cell networks [56]. It is likely that we are only scratching the surface of the microenvironmental effects of the epimutations and that novel approaches such as single-cell transcriptomics combined with improved disease models will constitute fertile ground for further discovery.

2.2.5 Histological Transformation of Follicular Lymphoma

There is considerable interest in understanding the biology of histological transformation (HT) of indolent lymphoma to an aggressive subtype, usually DLBCL, with HT representing the leading cause of follicular lymphoma-related mortality [73]. While the examination of serial FL–HT biopsies has been invaluable, research programs have been hampered by the paucity of available biopsies, leading to all cases of HT being combined together to adequately power genetic observations. It would be preferable to examine the genetics of HT in discrete subgroups, whereby distinctions may exist between transformations following chemotherapy relative to treatment-naïve HT [74, 75], differing time to HT, number of preceding episodes of FL, and prior therapies. We also need to be mindful that most genetic studies predate the use of anti-CD20 therapy rituximab, which has reduced HT rate and undoubtably impacted tumor evolution and will need renewed validation.

Accepting these limitations, studies have described an increased genomic complexity and mutational burden accompanying HT [57, 60], while recurrent events associated with transformation include disruption to DNA damage response and cell cycle regulation through *CDKN2A/B* loss and *TP53* mutation/deletion, and increased activity of key GC cell cycle regulator MYC through translocation, amplification, and/or mutation [26, 57, 60, 76, 77]. The molecular heterogeneity between HT cases is considerable however, and indeed most HT-associated events occur in pre-transformation samples at lower incidence. These obstacles to curating a discrete genetic signature for HT emphasizes the importance of pursuing parallel investigative approaches, including gene expression profiling and immune microenvironment characterization.

2.2.6 Classifying Mutations

The application of NGS tools has facilitated the sequencing of each unique DNA position hundreds and thousands of times, improving the sensitivity for detecting low-variant allele frequency (VAF) variants that may reflect either a low proportion of tumor cells within a biopsy sample or a "subclonal" variant that is present in only a fraction of tumor cells. It is worth emphasizing that several important variables, including depth of sequencing, the biopsy tumor content, and the occurrence of sequencing artifacts, impact the confidence in detecting subclonal variants. Mutations can also be defined by their computationally modeled or experimentally validated pathogenic potential (Table 2.1), with driver events viewed as mutations that confer a fitness advantage to the tumor cell within the context of a given microenvironment, while passenger mutations seemingly play no pathogenic role [78, 79]. Thus, it is important to note that defining a driver mutation is often inferred from surrogate factors, such as its clonal prevalence and postulated biologic effect, and not always confirmed by experimental evidence, although many of the landscape mutations in FL have indeed been shown to drive lymphomagenesis (Table 2.1). Driver mutations, somewhat surprisingly, may occur both clonally and in rare subclones in tumors with patterns varying according to the tumor type; *TP53* mutations, for example, are predominantly clonal in follicular lymphoma [80], but may be subclonal in chronic lymphocytic leukaemia (CLL), with a shift toward clonality over time [81]. Strikingly, the impact of a mutation may vary depending on when it arises during development, with studies by [15] demonstrating that conditional deletion of *Kmt2d* early during B-cell development, but not after the initiation of the GC reaction, results in an increase in GC B cells and enhances B-cell proliferation.

2.2.7 Temporal and Spatial Evolution in FL and Evidence for a Common Progenitor Cell (CPC)

Concepts of tumour evolution stem from the seminal work in the 1970s by Peter Nowell and John Cairns [82, 83], with early theories focussed on

the linear acquisition of mutations, each providing a growth and proliferation advantage driving malignant transformation. In recent years, high-throughput sequencing of sequential and spatial biopsies and more recently cell-free DNA studies have led to a much greater appreciation of the clonal and subclonal structure of tumors, revealing a previously underappreciated degree of genetic and evolutionary complexity [84, 85]. The multiple relapsing indolent haematologic malignancies, including CLL [81, 86], and FL [14, 26, 35, 57, 60] have provided some of the earliest models for examining tumor heterogeneity and clonal evolution, revealing both patterns of early and late divergence at relapse and evidence of intraclonal competition within the unique ecosystem of the tumor microenvironment.

In GC lymphomas the most comprehensive analysis of temporal clonal evolution has been performed in FL. Initial studies in paired FL and HT cases analyzed somatic hypermutation patterns in the immunoglobulin heavy-chain variable region (*IGH-VH*), which uniquely tag each clone [87–89]. Although both linear (evolved directly from the diagnostic sample) and branching (evolved separately from an earlier CPC) evolutionary patterns were described, the predominant mechanism was one of early branching evolution. Moreover, the molecular analysis of donor-derived examples of FL offers further support for this notion of a progenitor cell population occurring many years prior to clinically detectable disease, reminiscent of work on comparisons of paired pre-diagnostic peripheral blood and subsequent FL tumor discussed previously [2]. In both published donor-derived cases, the patients underwent a bone marrow transplant for another hematological malignancy, after which both the donor and the recipient developed a clonally related FL, between 3 and 11 years posttransplant [87, 90].

In more recent studies, NGS has been leveraged to better characterize the CPC and the genetic changes driving transformation and progression [14, 26, 35, 57, 58, 60]. Described phylogenetic trees comprising a predominant branched evolutionary pattern, where truncal mutations shared between separate disease episodes were enriched for the epigenetic regulators *CREBBP* and *KMT2D* and allowed inference that these genetic events are likely to characterize this CPC population of cells. These findings have been built on by other groups, most notably by [57], who performed a similar temporal study in a larger cohort of FL–HT and FL–FL cases and undertook a detailed analysis of clonal dynamics. Using ultra-deep sequencing, they demonstrated that HT events were predominantly composed of clones that were not detectable even at low levels at diagnosis, suggesting later acquisition and selection, while cases with relapsed or progressive FL were characterized by the expansion of preexisting subclones, pointing to markedly different evolutionary mechanisms underpinning both these processes. Indeed, it is now apparent that virtually all tumor types, including FL, comprise a mixture of subclones, each with a distinct mutation profile, driven by their inherent genetic instability and providing a rich substrate for Darwinian natural selection. Tumor progression and treatment resistance are reliant on this genetic plasticity, with evidence to suggest that increased intratumor heterogeneity at diagnosis predicts a more aggressive disease course and poorer prognosis. Meanwhile, spatial profiling of FL biopsies has demonstrated the marked genetic heterogeneity that may coexist within the same patient at the same timepoint [91].

Together, these models may have significant implications for treatment, raising pertinent questions regarding the appropriateness of tailoring therapy to the molecular profile of a single biopsy, the need to target subclonal mutations, the possible role of chemotherapy in driving the selection of more aggressive subclones, and critically the nature of the CPC B cells that potentially serve as a reservoir for subsequent disease episodes. These challenges are not unique to FL but indeed all forms of indolent lymphomas, discussed in this book.

2.3 Molecular Genetics in Indolent Lymphomas: A Clinical Perspective

Despite our increasing understanding of the molecular biology of these lymphomas, translation into clinical practice is still lagging.

Ultimately, the biggest challenge for any genetic test is to demonstrate clinical utility. The revised WHO classification acknowledges the evolving role of genetics in the classification of lymphoid malignancies, complementing clinical, morphologic, and immunophenotypic features [92, 93]. The field is rapidly evolving, and genetics holds great promise to improve diagnostic accuracy, to serve as robust prognostic and predictive biomarkers, and ultimately to guide and personalize treatment.

Methods used in clinical practice include karyotyping (conventional and metaphase cytogenetics), fluorescent in situ hybridization (FISH), polymerase chain reaction (genomic and reverse transcriptase (RT) PCR), array technology (comparative genomic hybridization, single-nucleotide polymorphism, and gene expression arrays), massive-parallel sequencing of DNA and RNA (often referred to as next-generation sequencing, NGS), and analysis of circulating tumor cells and cell-free DNA (often referred to as liquid biopsies). Appropriate molecular workup is best performed in specialized centers and laboratories, with expertise in deciding if, when, and what assay to perform for which patient and sample; by understanding the benefits but also limitations of each test; and through interpretation of the data. As of today, only few tests are mandatory, but an increasing number is optional or recommended, and numerous promising assays are in development and clinical evaluation. The following section will briefly describe current and evolving standards for genetic testing in patients with different subtypes of indolent lymphoma.

2.3.1 Follicular Lymphoma

Clonality tests (immunoglobulin rearrangements) are usually not required to make the diagnosis and should be restricted to samples with diagnostic difficulties, such as inconclusive morphology or suspected lymphoma despite reactive morphology. The EuroClonality (BIOMED-2) consortium has developed a standardized multiplex PCR assay, which detects most but not all clonal *IGH* rearrangements. False negative results may result from rare rearrangements, which are not covered, and from sequence variants interfering with primer binding in regions affected by somatic hypermutation.

Molecular genetics can help confirm the presence of clonal *BCL2* rearrangements, a hallmark of FL (see the previous section). Karyotype analysis is capable of detecting the prototypical t (14;18) (q32;q21) [*IGH*/*BCL2*] translocations as well as other less-common translocations. More commonly, the FISH technology with *BCL2* break-apart or fusion probes is used to detect 18q21/*BCL2* rearrangements irrespective of their translocation partners [94]. However, this is rarely needed to make the diagnosis of FL in routine practice. Of note, most FL negative for the *BCL2* rearrangements still aberrantly express BCL2 protein by immunohistochemistry (IHC). Furthermore, despite distinct differences in their molecular profiles, the absence of the BCL2 translocation has not yet been shown to impact outcome of patients with grade 1, 2, and 3A FL who received standard treatment [95]. Rare variants, such as pediatric-type FL—a clinically highly indolent subtype—typically lack *BCL2* rearrangements [7].

The clinical impact of gene mutations in FL is a rapidly evolving research field [96]. Mutations in few individual genes have been associated with outcome in patients with FL. Most notably, *TP53* mutations (present in <5% of newly diagnosed FL) predicted inferior outcome both in pre-rituximab and rituximab eras [11, 80]. Gain-of-function mutations in *EZH2* (seen in up to 25% of newly diagnosed FL; also see the previous section) have been consistently associated with favorable treatment outcome in studies of homogenously treated patients who received frontline cyclophosphamide, hydroxydaunorubicin (doxorubicin), oncovin (vincristine), and prednisone- and cyclophosphamide, vincristine, and prednisone-based immunochemotherapy for advanced, symptomatic FL [97, 98]. Accordingly, *EZH2* mutations hold promise to serve both as a biomarker and as a therapeutic target.

Prognostic risk models integrating gene mutations [11, 99], copy number alterations [100], and gene expression data have shown promising results, but further optimization, standardization,

validation, and exploration in additional cohorts is needed before they can be recommended for routine clinical use.

Finally, minimal residual disease (MRD) assessments hold promise to increasingly personalize patient management in FL, but also in other lymphomas. Clonal markers, such as chromosomal rearrangements and/or somatic mutations, can be identified in many (but not all) lymphomas and quantified in circulating tumor cells and/or cell-free DNA from peripheral blood and bone marrow samples (or other patient materials) by various techniques, including polymerase chain reaction–based methods and NGS [101]. MRD results can provide real-time information about tumor burden and response to therapy, noninvasive genomic profiling, and monitoring of clonal dynamics. However, MRD assessment is not (yet) a clinical standard and should be further validated in clinical trials to determine how to best incorporate MRD testing into routine practice and whether MRD-directed therapies improve treatment outcome.

2.3.2 Marginal Zone Lymphoma (MZL)

The detection of *IgH* gene rearrangements (e.g., by PCR) can be helpful in distinguishing extranodal MZL of mucosa-associated lymphoid tissue (MALT lymphoma) from reactive proliferations. Although not diagnostic, the detection of recurrent chromosomal translocations involving *MALT1* (t (11;18) (q21;q21)), *BCL10* (t (1;14) (p22;q32)), or *FOXP1* (t (3;14) (p13;q32)) strongly supports the diagnosis of MALT lymphoma. Furthermore, approximately half of the cases show trisomies of 3/3q or 18/18q or deletions of 6q23 [102]. All these alterations can be detected by karyotyping or FISH testing. Chromosomal studies may be useful to identify patients with gastric MALT lymphoma who are less likely to benefit from *H. pylori* eradication, including presence of a t (11;18) translocation or trisomy 3/3q [103, 104]. Furthermore, MALT lymphomas harboring a t (3;14) translocation seem to have a higher risk of histologic transformation to high-grade tumors.

Nodal marginal zone lymphomas (NMZL) typically lack recurrent chromosomal translocations, but often harbor numerical abnormalities similar to those seen in MALT lymphomas, such as trisomies 3/3q, 18/18q, as well as deletions of 6q23. Likewise, splenic marginal zone lymphomas (SMZL) also usually do not carry chromosomal translocations, but often have abnormal karyotype, including deletion of 7q31 or 8p, or complex chromosomal alterations [105]. Molecular studies have identified recurrent mutations in *NOTCH2* and *KLF2* (in up to 40% of cases of SMZL) and in genes of the NF-KB pathway (including *MALT1*, *CARD11*, *TNFAIP3*, and *MYD88*); however, the data on their clinical impact is still controversial and an area of active research [106–108].

2.3.3 Waldenström's Macroglobulinemia

Waldenström's macroglobulinemia (WM) or lymphoplasmocytic lymphoma (LPL) with IgM paraprotein carries highly recurrent somatic mutations, including activating mutations in MYD88 (>90% of cases [109]) and in CXCR4 (approximately 30% of cases [110]). As these mutations virtually all cluster in hotspots (MYD88 L265P point mutations and nonsense (NS) or frameshift (FS) mutations within the C-terminus of CXCR4 (so-called "warts, hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome-like" mutations), they can easily be detected by PCR-based assays [111]. Determining the MYD88 and CXCR4 mutation status is not only helpful in distinguishing WM from other B-cell lymphomas with indolent morphology, but also provides important prognostic and predictive information. Patients with $MYD88^{L265P}$/$CXCR4^{WHIM/NS}$ often present with more aggressive disease and high tumor burden. $CXCR4^{WHIM/FS}$ mutation status has been associated with high IgM levels, including hyperviscosity crisis [112]. Importantly, response to the BTK inhibition is adversely impacted by $MYD88^{wild\text{-}type}$ and $CXCR4^{WHIM}$; hence, determining

the mutation status of these genes before the initiation of ibrutinib treatment should now be considered the standard of care [111]. Whether mutations in other genes like *BTK* or downstream *CARD11* or *PLCG2* are also involved in mediating treatment resistance is an area of active research. In that regard, WM can serve as a prime example, illustrating that response to molecular targeting treatments may be particularly predictable by gene mutation status. As the field is increasingly shifting toward cytotoxic-free therapies, a broader mutational analysis will probably be required to personalize treatment approaches in the very near future. Other aberrations have also been shown to be associated with poor outcome in patients with WM, including deletions of 6q and 11q, as well as 17p/*TP53*, but validation of these results with modern therapies is pending [113].

2.3.4 Mantle Cell Lymphoma (MCL)

Almost all cases of MCL harbor the t (11;14) [*IGH*/*CCND1*] translocation, which can easily be detected by FISH or, with a lower sensitivity, by PCR. However, similar to FL, the detection of this hallmark translocation is required to make the diagnosis only if routine morphological workup (including IHC for CCND1 (cyclin D1)) yields inconclusive results. In cyclin D1-negative MCL, *CCND2* and, rarely, *CCND3* translocations have been described [114]. Most MCLs have a pre-germinal center phenotype with unmuted *IGVH* regions and aggressive clinical course. However, a subset of MCLs have mutated *IGVH* genes and a more indolent clinical course, including non-nodal/leukemic presentation and splenomegaly. Chromosomal abnormalities, such as tetraploidy or complex karyotype, and deletions/losses of 17p13/*TP53* and 9p21/*CDKN2A* have been associated with inferior treatment outcome, even in the context of dose-intensified regimens [115]. Similarly, mutations in *TP53* as well as *NOTCH1* and *NOTCH2* have been shown to be associated with blastoid morphology, highly aggressive clinical course, and dismal treatment outcome [116, 117]. Similar to FL, prognostic and predictive models integrating gene mutations [118], gene expression data [119, 120], or miRNA profiles [121] have been proposed but require further validation in additional cohorts.

2.3.5 Hairy Cell Leukemia (HCL)

More than 95% of cases of hairy cell leukemia (HCL) carry the somatic, activating BRAF mutation V600E [122]. As the HCL clone can be small, sensitive molecular assays, such as allele-specific oligonucleotide (ASO)-PCR, digital droplet PCR (ddPCR), or NGS, are required to detect the mutation in peripheral blood and/or bone marrow samples. Although not specific, the presence of the mutation can be helpful in distinguishing HCL from other B-cell lymphoproliferative disorders [123]. Rare variants of HCL lack the BRAF V600E mutation (HZL-v), many of which haboring mutations in *MAP2K2* instead [124], particularly cases with IGHV4–34 immunoglobulin rearrangements. With the availability of BRAF inhibitors and other molecular-targeting therapeutics, testing for these mutations is expected to be increasingly relevant in the clinical setting. Numerous other genetic and karyotypic abnormalities (e.g., chromosome 5 abnormalities in approximately 40% cases) have been described [125]; however, none of these have been incorporated into the diagnostic criteria for HCL yet.

2.3.6 Chronic Lymphocytic Leukemia

Understanding the prognostic and predictive value of specific cytogenetic and molecular findings in CLL is increasingly guiding treatment decisions in clinical practice (see Chap. 13). Briefly, interphase FISH (e.g., for del (17p), del (11q), and del (13q)) and determining the mutation status of *IGVH* and *TP53* are widely considered standard clinical tests [126, 127]. Unmutated *IGVH* regions are found in approximately half of CLL and have invariably been linked with inferior treatment outcome, including higher risk of relapse and shorter overall survival [128, 129].

Cytogenetic abnormalities are present in >80% of CLL. The single most common alteration is deletion of 13q (present in up to 50% of CLL), which has historically been associated with a favorable clinical course [130]. Deletions of 11q22–23 occur in up to 20% CLL, typically involve *ATM* and sometimes *BIRC3*, and are often linked to refractoriness to chemotherapy [131]. Patients with deletions of 17p and/or inactivating mutations in *TP53* often have complex karyotype, aggressive clinical course, and poor response to standard chemotherapy and should be prioritized for TP53-independent therapies, such as ibrutinib, idelalisib, and venetoclax [132] (and *ESMO guidelines eUpdate from June 27, 2017: Chronic Lymphocytic Leukaemia Treatment Recommendations*). Interestingly, the mutation frequency of *TP53* increases over time, from approximately 5% at initial diagnosis to about 40% at the time of refractory disease [133]. Hence, testing for *TP53* mutations is recommended for all patients before the start of any new therapy. Also, deep sequencing rather than Sanger sequencing is recommended (capturing at least exons 4–9), as subclonal *TP53* mutations (i.e., <1%) seem to have the same unfavorable clinical impact as *TP53* mutations present in the major clone. Emerging data indicates that other recurrent gene mutations, such as *SF3B1* and *NOTCH1* mutations, are associated with more aggressive disease [131], but further validation in additional cohorts is needed, especially in the context of novel therapies. Furthermore, screening for genetic treatment resistance is a rapidly developing field, as exemplified by ibrutinib resistance mediated by the C481S point mutation in *BTK* or activating mutations in downstream *PLCG2* [134].

2.3.7 Indolent T-Cell Lymphomas

T-cell lymphomas (TCL) are much less common and—with the exception of cutaneous TCL (see Chap. 14) and large granular lymphocytic (LGL) lymphoma (see Chap. 15)—mostly have an aggressive clinical course. Rearrangements of T-cell receptor (*TCR*) genes are frequently seen and can be diagnostically helpful [135]. The aforementioned BIOMED-2 multiplex PCR assay captures the most common *TCR* rearrangements (mostly *TCRγ* and *TCRβ*, less commonly *TCRα*). However, clinicians should be aware of false positive results, as clonal T-cell clones can be detected with increasing frequency in elderly subjects [136]. Translocations affecting the *TCR* genes are much less common compared to B-cell lymphomas. Other genetic tests are not routinely performed in indolent TCL, but defining the molecular landscapes of these diseases is an area of highly active research.

2.4 Perspective

At the time of writing, genetics has become synonymous with coding mutations, and we have an unparalleled understanding of the coding genome and genes that are subject to recurring gene mutation in indolent lymphomas. This is in contrast with the relatively scant information gleaned thus far on how individual gene mutations work together, if all mutations in the same genes behave the same, and if the impact of specific mutation is maintained throughout the course of the disease. The portrait of these indolent lymphomas will change significantly as we incorporate knowledge on genetic susceptibility, the non-coding (epi) genome, and decide whether collectively these can unlock insights into the personalities of these lymphoma that will pave the way to improvements in the overall management of our patients, for their life both with and after lymphoma.

References

1. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 1985;228(4706):1440–3.
2. Roulland S, Kelly RS, Morgado E, Sungalee S, Solal-Celigny P, Colombat P, et al. T (14;18) translocation: a predictive blood biomarker for follicular lymphoma. J Clin Oncol. 2014;32(13):1347–55.
3. Hirt C, Camargo MC, Yu KJ, Hewitt SM, Dolken G, Rabkin CS. Risk of follicular lymphoma associated with BCL2 translocations in peripheral blood. Leuk Lymphoma. 2015;56(9):2625–9.

4. Skibola CF, Bracci PM, Halperin E, Conde L, Craig DW, Agana L, et al. Genetic variants at 6p21.33 are associated with susceptibility to follicular lymphoma. Nat Genet. 2009;41(8):873–5.
5. Conde L, Halperin E, Akers NK, Brown KM, Smedby KE, Rothman N, et al. Genome-wide association study of follicular lymphoma identifies a risk locus at 6p21.32. Nat Genet. 2010;42(8):661–4.
6. Schmidt J, Ramis-Zaldivar JE, Bonzheim I, Steinhilber J, Muller I, Haake A, et al. CREBBP gene mutations are frequently detected in in situ follicular neoplasia. Blood. 2018;132(25):2687–90.
7. Louissaint A Jr, Schafernak KT, Geyer JT, Kovach AE, Ghandi M, Gratzinger D, et al. Pediatric-type nodal follicular lymphoma: a biologically distinct lymphoma with frequent MAPK pathway mutations. Blood. 2016;128(8):1093–100.
8. Hellmuth JC, Louissaint A Jr, Szczepanowski M, Haebe S, Pastore A, Alig S, et al. Duodenal-type and nodal follicular lymphomas differ by their immune microenvironment rather than their mutation profiles. Blood. 2018;132(16):1695–702.
9. International Cancer Genome C, Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, et al. International network of cancer genome projects. Nature. 2010;464(7291):993–8.
10. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, Beare D, et al. COSMIC: mining complete cancer genomes in the catalogue of somatic mutations in Cancer. Nucleic Acids Res. 2011;39(Database issue):D945–50.
11. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16(9):1111–22.
12. Karube K, Enjuanes A, Dlouhy I, Jares P, Martin-Garcia D, Nadeu F, et al. Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. Leukemia. 2018;32(3):675–84.
13. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476(7360):298–303.
14. Green MR, Gentles AJ, Nair RV, Irish JM, Kihira S, Liu CL, et al. Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. Blood. 2013;121(9):1604–11.
15. Zhang J, Dominguez-Sola D, Hussein S, Lee JE, Holmes AB, Bansal M, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. Nat Med. 2015;21(10):1190–8.
16. Ortega-Molina A, Boss IW, Canela A, Pan H, Jiang Y, Zhao C, et al. The histone lysine methyltransferase KMT2D sustains a gene expression program that represses B cell lymphoma development. Nat Med. 2015;21(10):1199–208.
17. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet. 2011;43(9):830–7.
18. Zhang J, Vlasevska S, Wells VA, Nataraj S, Holmes AB, Duval R, et al. The CREBBP acetyltransferase is a haploinsufficient tumor suppressor in B-cell lymphoma. Cancer Discov. 2017;7(3):322–37.
19. Hashwah H, Schmid CA, Kasser S, Bertram K, Stelling A, Manz MG, et al. Inactivation of CREBBP expands the germinal center B cell compartment, down-regulates MHCII expression and promotes DLBCL growth. Proc Natl Acad Sci U S A. 2017;114(36):9701–6.
20. Ennishi D, Jiang A, Boyle M, Collinge B, Grande BM, Ben-Neriah S, et al. Double-hit gene expression signature defines a distinct subgroup of germinal center B-cell-like diffuse large B-cell lymphoma. J Clin Oncol. 2019;37(3):190–201.
21. Ennishi D, Takata K, Beguelin W, Duns G, Mottok A, Farinha P, et al. Molecular and genetic characterization of MHC deficiency identifies EZH2 as therapeutic target for enhancing immune recognition. Cancer Discov. 2019;9(4):546–63.
22. Mondello P, Tadros S, Teater M, Fontan L, Chang AY, Jain N, et al. Selective inhibition of HDAC3 targets synthetic vulnerabilities and activates immune surveillance in lymphoma. Cancer Discov. 2020;10(3):440–59.
23. Launay E, Pangault C, Bertrand P, Jardin F, Lamy T, Tilly H, et al. High rate of TNFRSF14 gene alterations related to 1p36 region in de novo follicular lymphoma and impact on prognosis. Leukemia. 2012;26(3):559–62.
24. Cheung KJ, Johnson NA, Affleck JG, Severson T, Steidl C, Ben-Neriah S, et al. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. Cancer Res. 2010;70(22):9166–74.
25. Boice M, Salloum D, Mourcin F, Sanghvi V, Amin R, Oricchio E, et al. Loss of the HVEM tumor suppressor in lymphoma and restoration by modified CAR-T cells. Cell. 2016;167(2):405–18 e13.
26. Okosun J, Bodor C, Wang J, Araf S, Yang CY, Pan C, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. Nat Genet. 2014;46(2):176–81.
27. Okosun J, Packham G, Fitzgibbon J. Investigational epigenetically targeted drugs in early phase trials for the treatment of haematological malignancies. Expert Opin Investig Drugs. 2014;23(10):1321–32.
28. Bodor C, Grossmann V, Popov N, Okosun J, O'Riain C, Tan K, et al. EZH2 mutations are frequent and represent an early event in follicular lymphoma. Blood. 2013;122(18):3165–8.
29. Caganova M, Carrisi C, Varano G, Mainoldi F, Zanardi F, Germain PL, et al. Germinal center dysregulation by histone methyltransferase

EZH2 promotes lymphomagenesis. J Clin Invest. 2013;123(12):5009–22.
30. Beguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, et al. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. Cancer Cell. 2013;23(5):677–92.
31. Beguelin W, Teater M, Gearhart MD, Calvo Fernandez MT, Goldstein RL, Cardenas MG, et al. EZH2 and BCL6 cooperate to assemble CBX8-BCOR complex to repress bivalent promoters, mediate germinal center formation and lymphomagenesis. Cancer Cell. 2016;30(2):197–213.
32. Berg T, Thoene S, Yap D, Wee T, Schoeler N, Rosten P, et al. A transgenic mouse model demonstrating the oncogenic role of mutations in the polycomb-group gene EZH2 in lymphomagenesis. Blood. 2014;123(25):3914–24.
33. Meyer SN, Scuoppo C, Vlasevska S, Bal E, Holmes AB, Holloman M, et al. Unique and shared epigenetic programs of the CREBBP and EP300 acetyltransferases in germinal center B cells reveal targetable dependencies in lymphoma. Immunity. 2019;51(3):535–47. e9
34. Okosun J, Wolfson RL, Wang J, Araf S, Wilkins L, Castellano BM, et al. Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. Nat Genet. 2016;48(2):183–8.
35. Green MR, Kihira S, Liu CL, Nair RV, Salari R, Gentles AJ, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. Proc Natl Acad Sci U S A. 2015;112(10):E1116–25.
36. Ortega-Molina A, Deleyto-Seldas N, Carreras J, Sanz A, Lebrero-Fernandez C, Menendez C, et al. Oncogenic rag GTPase signaling enhances B cell activation and drives follicular lymphoma sensitive to pharmacological inhibition of mTOR. Nat Metab. 2019;1(8):775–89.
37. Brescia P, Schneider C, Holmes AB, Shen Q, Hussein S, Pasqualucci L, et al. MEF2B instructs germinal center development and acts as an oncogene in B cell lymphomagenesis. Cancer Cell. 2018;34(3):453–65. e9
38. Muppidi JR, Schmitz R, Green JA, Xiao W, Larsen AB, Braun SE, et al. Loss of signalling via Galpha13 in germinal centre B-cell-derived lymphoma. Nature. 2014;516(7530):254–8.
39. Szydlowski M, Kiliszek P, Sewastianik T, Jablonska E, Bialopiotrowicz E, Gorniak P, et al. FOXO1 activation is an effector of SYK and AKT inhibition in tonic BCR signal-dependent diffuse large B-cell lymphomas. Blood. 2016;127(6):739–48.
40. Kabrani E, Chu VT, Tasouri E, Sommermann T, Bassler K, Ulas T, et al. Nuclear FOXO1 promotes lymphomagenesis in germinal center B cells. Blood. 2018;132(25):2670–83.
41. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature. 2009;459(7247):717–21.
42. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature. 2010;463(7277):88–92.
43. Yildiz M, Li H, Bernard D, Amin NA, Ouillette P, Jones S, et al. Activating STAT6 mutations in follicular lymphoma. Blood. 2015;125(4):668–79.
44. Carbone A, Roulland S, Gloghini A, Younes A, von Keudell G, López-Guillermo A, Fitzgibbon J. Follicular lymphoma. Nat Rev Dis Primers. 2019;5(1):83. PMID 31831752.
45. Cheung KJ, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, et al. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. Blood. 2009;113(1):137–48.
46. Cheung MC, Bailey D, Pennell N, Imrie KR, Berinstein NL, Amato D, et al. In situ localization of follicular lymphoma: evidence for subclinical systemic disease with detection of an identical BCL-2/IGH fusion gene in blood and lymph node. Leukemia. 2009;23(6):1176–9.
47. Johnson NA, Al-Tourah A, Brown CJ, Connors JM, Gascoyne RD, Horsman DE. Prognostic significance of secondary cytogenetic alterations in follicular lymphomas. Genes Chromosomes Cancer. 2008;47(12):1038–48.
48. Fitzgibbon J, Iqbal S, Davies A, O'Shea D, Carlotti E, Chaplin T, et al. Genome-wide detection of recurring sites of uniparental disomy in follicular and transformed follicular lymphoma. Leukemia. 2007;21(7):1514–20.
49. Katzenberger T, Ott G, Klein T, Kalla J, Muller-Hermelink HK, Ott MM. Cytogenetic alterations affecting BCL6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B-cell component. Am J Pathol. 2004;165(2):481–90.
50. O'Shea D, O'Riain C, Gupta M, Waters R, Yang Y, Wrench D, et al. Regions of acquired uniparental disomy at diagnosis of follicular lymphoma are associated with both overall survival and risk of transformation. Blood. 2009;113(10):2298–301.
51. Schwaenen C, Viardot A, Berger H, Barth TF, Bentink S, Dohner H, et al. Microarray-based genomic profiling reveals novel genomic aberrations in follicular lymphoma which associate with patient survival and gene expression status. Genes Chromosomes Cancer. 2009;48(1):39–54.
52. Viardot A, Moller P, Hogel J, Werner K, Mechtersheimer G, Ho AD, et al. Clinicopathologic correlations of genomic gains and losses in follicular lymphoma. J Clin Oncol. 2002;20(23):4523–30.
53. Yap DB, Chu J, Berg T, Schapira M, Cheng SW, Moradian A, et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood. 2011;117(8):2451–9.

54. Jiang Y, Ortega-Molina A, Geng H, Ying HY, Hatzi K, Parsa S, et al. CREBBP inactivation promotes the development of HDAC3-dependent lymphomas. Cancer Discov. 2017;7(1):38–53.
55. Garcia-Ramirez I, Tadros S, Gonzalez-Herrero I, Martin-Lorenzo A, Rodriguez-Hernandez G, Moore D, et al. Crebbp loss cooperates with Bcl2 overexpression to promote lymphoma in mice. Blood. 2017;129(19):2645–56.
56. Beguelin W, Teater M, Meydan C, Hoehn KB, Phillip JM, Soshnev AA, et al. Mutant EZH2 induces a premalignant lymphoma niche by reprogramming the immune response. Cancer Cell. 2020;37(5):655–73 e11.
57. Kridel R, Chan FC, Mottok A, Boyle M, Farinha P, Tan K, et al. Histological transformation and progression in follicular lymphoma: a clonal evolution study. PLoS Med. 2016;13(12):e1002197.
58. Bouska A, Zhang W, Gong Q, Iqbal J, Scuto A, Vose J, et al. Combined copy number and mutation analysis identifies oncogenic pathways associated with transformation of follicular lymphoma. Leukemia. 2017;31(1):83–91.
59. Krysiak K, Gomez F, White BS, Matlock M, Miller CA, Trani L, et al. Recurrent somatic mutations affecting B-cell receptor signaling pathway genes in follicular lymphoma. Blood. 2017;129(4):473–83.
60. Pasqualucci L, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, et al. Genetics of follicular lymphoma transformation. Cell Rep. 2014;6(1):130–40.
61. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011;21(3):381–95.
62. Plass C, Pfister SM, Lindroth AM, Bogatyrova O, Claus R, Lichter P. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. Nat Rev Genet. 2013;14(11):765–80.
63. Pasqualucci L. The genetic basis of diffuse large B-cell lymphoma. Curr Opin Hematol. 2013;20(4):336–44.
64. Green MR, Vicente-Duenas C, Romero-Camarero I, Long Liu C, Dai B, Gonzalez-Herrero I, et al. Transient expression of Bcl6 is sufficient for oncogenic function and induction of mature B-cell lymphoma. Nat Commun. 2014;5:3904.
65. Araf S, Okosun J, Koniali L, Fitzgibbon J, Heward J. Epigenetic dysregulation in follicular lymphoma. Epigenomics. 2016;8(1):77–84.
66. Lunning MA, Green MR. Mutation of chromatin modifiers; an emerging hallmark of germinal center B-cell lymphomas. Blood Cancer J. 2015;5:e361.
67. Johnson PW, Watt SM, Betts DR, Davies D, Jordan S, Norton AJ, et al. Isolated follicular lymphoma cells are resistant to apoptosis and can be grown in vitro in the CD40/stromal cell system. Blood. 1993;82(6):1848–57.
68. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004;351(21):2159–69.
69. Huet S, Tesson B, Jais JP, Feldman AL, Magnano L, Thomas E, et al. A gene-expression profiling score for prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts. Lancet Oncol. 2018;19(4):549–61.
70. Zhu D, McCarthy H, Ottensmeier CH, Johnson P, Hamblin TJ, Stevenson FK. Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. Blood. 2002;99(7):2562–8.
71. Coelho V, Krysov S, Ghaemmaghami AM, Emara M, Potter KN, Johnson P, et al. Glycosylation of surface Ig creates a functional bridge between human follicular lymphoma and microenvironmental lectins. Proc Natl Acad Sci U S A. 2010;107(43):18587–92.
72. Amin R, Mourcin F, Uhel F, Pangault C, Ruminy P, Dupre L, et al. DC-SIGN-expressing macrophages trigger activation of mannosylated IgM B-cell receptor in follicular lymphoma. Blood. 2015;126(16):1911–20.
73. Sarkozy C, Maurer MJ, Link BK, Ghesquieres H, Nicolas E, Thompson CA, et al. Cause of death in follicular lymphoma in the first decade of the rituximab era: a pooled analysis of French and US cohorts. J Clin Oncol. 2019;37(2):144–52.
74. Link BK, Maurer MJ, Nowakowski GS, Ansell SM, Macon WR, Syrbu SI, et al. Rates and outcomes of follicular lymphoma transformation in the immunochemotherapy era: a report from the University of Iowa/MayoClinic specialized program of research excellence molecular epidemiology resource. J Clin Oncol. 2013;31(26):3272–8.
75. Rusconi C, Anastasia A, Chiarenza A, Marcheselli L, Cavallo F, Rattotti S, et al. Outcome of transformed follicular lymphoma worsens according to the timing of transformation and to the number of previous therapies. A retrospective multicenter study on behalf of Fondazione Italiana Linfomi (FIL). Br J Haematol. 2019;185(4):713–7.
76. Elenitoba-Johnson KS, Gascoyne RD, Lim MS, Chhanabai M, Jaffe ES, Raffeld M. Homozygous deletions at chromosome 9p21 involving p16 and p15 are associated with histologic progression in follicle center lymphoma. Blood. 1998;91(12):4677–85.
77. Sander CA, Yano T, Clark HM, Harris C, Longo DL, Jaffe ES, et al. p53 mutation is associated with progression in follicular lymphomas. Blood. 1993;82(7):1994–2004.
78. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009;458(7239):719–24.
79. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546–58.
80. O'Shea D, O'Riain C, Taylor C, Waters R, Carlotti E, Macdougall F, et al. The presence of TP53 mutation at diagnosis of follicular lymphoma identifies

a high-risk group of patients with shortened time to disease progression and poorer overall survival. Blood. 2008;112(8):3126–9.
81. Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell. 2013;152(4):714–26.
82. Cairns J. Mutation selection and the natural history of cancer. Nature. 1975;255(5505):197–200.
83. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976;194(4260):23–8.
84. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92.
85. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. Cell. 2017;168(4):613–28.
86. Schuh A, Becq J, Humphray S, Alexa A, Burns A, Clifford R, et al. Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns. Blood. 2012;120(20):4191–6.
87. Carlotti E, Wrench D, Matthews J, Iqbal S, Davies A, Norton A, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma may occur by divergent evolution from a common progenitor cell or by direct evolution from the follicular lymphoma clone. Blood. 2009;113(15):3553–7.
88. Eide MB, Liestol K, Lingjaerde OC, Hystad ME, Kresse SH, Meza-Zepeda L, et al. Genomic alterations reveal potential for higher grade transformation in follicular lymphoma and confirm parallel evolution of tumor cell clones. Blood. 2010;116(9):1489–97.
89. Ruminy P, Jardin F, Picquenot JM, Parmentier F, Contentin N, Buchonnet G, et al. S (mu) mutation patterns suggest different progression pathways in follicular lymphoma: early direct or late from FL progenitor cells. Blood. 2008;112(5):1951–9.
90. Weigert O, Kopp N, Lane AA, Yoda A, Dahlberg SE, Neuberg D, et al. Molecular ontogeny of donor-derived follicular lymphomas occurring after hematopoietic cell transplantation. Cancer Discov. 2012;2(1):47–55.
91. Araf S, Wang J, Korfi K, Pangault C, Kotsiou E, Rio-Machin A, et al. Genomic profiling reveals spatial intra-tumor heterogeneity in follicular lymphoma. Leukemia. 2018;32(5):1261–5.
92. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. In: IARC, editor. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: WHO Press; 2008.
93. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
94. Dreyling M, Ghielmini M, Rule S, Salles G, Vitolo U, Ladetto M, et al. Newly diagnosed and relapsed follicular lymphoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2016;27(suppl 5):v83–90.
95. Leich E, Hoster E, Wartenberg M, Unterhalt M, Siebert R, Koch K, et al. Similar clinical features in follicular lymphomas with and without breaks in the BCL2 locus. Leukemia. 2016;30(4):854–60.
96. Weigert O, Weinstock DM. The promises and challenges of using gene mutations for patient stratification in follicular lymphoma. Blood. 2017;130(13):1491–8.
97. Huet S, Xerri L, Tesson B, Mareschal S, Taix S, Mescam-Mancini L, et al. EZH2 alterations in follicular lymphoma: biological and clinical correlations. Blood Cancer J. 2017;7(4):e555.
98. Stevens WBC, Mendeville M, Redd R, Clear AJ, Bladergroen R, Calaminici M, et al. Prognostic relevance of CD163 and CD8 combined with EZH2 and gain of chromosome 18 in follicular lymphoma: a study by the Lunenburg lymphoma biomarker consortium. Haematologica. 2017;102(8):1413–23.
99. Jurinovic V, Kridel R, Staiger AM, Szczepanowski M, Horn H, Dreyling MH, et al. Clinicogenetic risk models predict early progression of follicular lymphoma after first-line immunochemotherapy. Blood. 2016;128(8):1112–20.
100. Qu X, Li H, Braziel RM, Passerini V, Rimsza LM, Hsi ED, et al. Genomic alterations important for the prognosis in patients with follicular lymphoma treated in SWOG study S0016. Blood. 2019;133(1):81–93.
101. Herrera AF, Armand P. Minimal residual disease assessment in lymphoma: methods and applications. J Clin Oncol. 2017;35(34):3877–87.
102. Zucca E, Bertoni F. The spectrum of MALT lymphoma at different sites: biological and therapeutic relevance. Blood. 2016;127(17):2082–92.
103. Taji S, Nomura K, Matsumoto Y, Sakabe H, Yoshida N, Mitsufuji S, et al. Trisomy 3 may predict a poor response of gastric MALT lymphoma to helicobacter pylori eradication therapy. World J Gastroenterol. 2005;11(1):89–93.
104. Liu H, Ruskon-Fourmestraux A, Lavergne-Slove A, Ye H, Molina T, Bouhnik Y, et al. Resistance of t (11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to helicobacter pylori eradication therapy. Lancet. 2001;357(9249):39–40.
105. Rinaldi A, Mian M, Chigrinova E, Arcaini L, Bhagat G, Novak U, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. Blood. 2011;117(5):1595–604.
106. Rossi D, Trifonov V, Fangazio M, Bruscaggin A, Rasi S, Spina V, et al. The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. J Exp Med. 2012;209(9):1537–51.
107. Clipson A, Wang M, de Leval L, Ashton-Key M, Wotherspoon A, Vassiliou G, et al. KLF2 mutation is the most frequent somatic change in splenic marginal zone lymphoma and identifies a subset with distinct genotype. Leukemia. 2015;29(5):1177–85.
108. Parry M, Rose-Zerilli MJ, Ljungstrom V, Gibson J, Wang J, Walewska R, et al. Genetics and prognos-

tication in splenic marginal zone lymphoma: revelations from deep sequencing. Clin Cancer Res. 2015;21(18):4174–83.
109. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med. 2012;367(9):826–33.
110. Hunter ZR, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood. 2014;123(11):1637–46.
111. Kastritis E, Leblond V, Dimopoulos MA, Kimby E, Staber P, Kersten MJ, et al. Waldenstrom's macroglobulinaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29(Suppl 4):iv270.
112. Schmidt J, Federmann B, Schindler N, Steinhilber J, Bonzheim I, Fend F, et al. MYD88 L265P and CXCR4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. Br J Haematol. 2015;169(6):795–803.
113. Nguyen-Khac F, Lambert J, Chapiro E, Grelier A, Mould S, Barin C, et al. Chromosomal aberrations and their prognostic value in a series of 174 untreated patients with Waldenstrom's macroglobulinemia. Haematologica. 2013;98(4):649–54.
114. Jares P, Colomer D, Campo E. Molecular pathogenesis of mantle cell lymphoma. J Clin Invest. 2012;122(10):3416–23.
115. Delfau-Larue MH, Klapper W, Berger F, Jardin F, Briere J, Salles G, et al. High-dose cytarabine does not overcome the adverse prognostic value of CDKN2A and TP53 deletions in mantle cell lymphoma. Blood. 2015;126(5):604–11.
116. Bea S, Valdes-Mas R, Navarro A, Salaverria I, Martin-Garcia D, Jares P, et al. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. Proc Natl Acad Sci U S A. 2013;110(45):18250–5.
117. Dreyling M, Klapper W, Rule S. Blastoid and pleomorphic mantle cell lymphoma: still a diagnostic and therapeutic challenge! Blood. 2018;132(26):2722–9.
118. Ferrero S, et al. *KMT2D* mutations and *TP53* disruptions are poor prognostic biomarkers in mantle cell lymphoma receiving high-dose therapy: a FIL study. Haematologica. 2020;105(6):1604–12. https://doi.org/10.3324/haematol.2018.214056.
119. Holte H, Beiske K, Boyle M, Troen G, Blaker YN, Myklebust J, et al. The MCL35 gene expression proliferation assay predicts high-risk MCL patients in a Norwegian cohort of younger patients given intensive first line therapy. Br J Haematol. 2018;183(2):225–34.
120. D'Agaro T, Zucchetto A, Vit F, Bittolo T, Tissino E, Rossi FM, et al. A B-cell receptor-related gene signature predicts response to ibrutinib treatment in mantle cell lymphoma cell lines. Haematologica. 2019;104(9):e410–e4.
121. Iqbal J, Shen Y, Liu Y, Fu K, Jaffe ES, Liu C, et al. Genome-wide miRNA profiling of mantle cell lymphoma reveals a distinct subgroup with poor prognosis. Blood. 2012;119(21):4939–48.
122. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011;364(24):2305–15.
123. Grever MR, Abdel-Wahab O, Andritsos LA, Banerji V, Barrientos J, Blachly JS, et al. Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. Blood. 2017;129(5):553–60.
124. Waterfall JJ, Arons E, Walker RL, Pineda M, Roth L, Killian JK, et al. High prevalence of MAP 2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. Nat Genet. 2014;46(1):8–10.
125. Haglund U, Juliusson G, Stellan B, Gahrton G. Hairy cell leukemia is characterized by clonal chromosome abnormalities clustered to specific regions. Blood. 1994;83(9):2637–45.
126. Parikh SA, Strati P, Tsang M, West CP, Shanafelt TD. Should IGHV status and FISH testing be performed in all CLL patients at diagnosis? A systematic review and meta-analysis. Blood. 2016;127(14):1752–60.
127. International CLLIPIwg. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. Lancet Oncol. 2016;17(6):779–90.
128. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V (H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94(6):1848–54.
129. Ritgen M, Lange A, Stilgenbauer S, Dohner H, Bretscher C, Bosse H, et al. Unmutated immunoglobulin variable heavy-chain gene status remains an adverse prognostic factor after autologous stem cell transplantation for chronic lymphocytic leukemia. Blood. 2003;101(5):2049–53.
130. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26):1910–6.
131. Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2017;92(9):946–65.
132. Eichhorst B, Robak T, Montserrat E, Ghia P, Hillmen P, Hallek M, et al. Chronic lymphocytic leukaemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26(Suppl 5):v78–84.
133. Campo E, Cymbalista F, Ghia P, Jager U, Pospisilova S, Rosenquist R, et al. TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. Haematologica. 2018;103(12):1956–68.
134. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014;370(24):2286–94.

135. Aisenberg AC, Krontiris TG, Mak TW, Wilkes BM. Rearrangement of the gene for the beta chain of the T-cell receptor in T-cell chronic lymphocytic leukemia and related disorders. N Engl J Med. 1985;313(9):529–33.
136. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 concerted action BMH4-CT98-3936. Leukemia. 2003;17(12):2257–317.