Follicular Lymphoma

Current Management and Novel Approaches

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Preface

Few things strike fear into the clinician and patient as the word "cancer." Despite decades of research and countless dollars, the majority of cancers remain incurable, and therapy carries a high cost, both financially and on patients' short- and long-term quality of life. With the exception of very early-stage malignancies, most cancers carry a high risk of relapse following frontline treatment. Side effects occur often, can be severe, and are unpredictable. Fortunately, due to technical advances, emerging science, and a fundamental shift in new drug development, we are witnessing dramatic improvements in life expectancy and treatment tolerability across a spectrum of malignancies.

For years, follicular lymphoma has remained a disease with more questions than answers. The natural history can be highly variable, and clinical information at diagnosis only occasionally predicts a patient's long-term outcome. Some patients achieve spontaneous remission in the absence of therapy, while others transform or fail high-dose chemotherapy in short order. Historically, attempts to biologically define these dramatically different patient groups have been largely unsuccessful. Although the cell of origin and the hallmark mutation have been well described, very few targeted therapeutics existed. Finally, traditional chemotherapy approaches are associated with high response rates, yet nearly all patients still relapse. The reason behind many of these observations was largely unknown.

Fortunately, the last several years have been marked by a significant improvement in the understanding of the pathogenesis and biology underlying follicular lymphoma. Research into the role of the immune microenvironment have helped teams develop innovative approaches to treat follicular lymphoma. The work on key cellular pathways, novel cellular antigens, and unique genomic drivers has led to the identification of several potential therapeutic targets resulting in an explosion of active drugs and dramatically improved out-comes.

In this textbook, we will review the pathogenesis and molecular drivers of follicular lymphoma. We will also cover the history and activity of traditional therapeutic strategies and discuss many exciting advances which have recently become available for patients. In each section, the authors will also discuss the future

therapeutic role of key molecular pathways, targeted agents, immunotherapeutics, and next-generation radiotherapy approaches.

As our understanding of lymphoma continues to evolve, I am confident that we will soon discover the answers to key questions surrounding follicular lymphoma. But most importantly, ongoing and future work will lead to even better options for clinicians – eventually leading to curative, less toxic options for all patients.

Finally, whether clinician, scientist, patient, or caregiver, our time in this life is short. Never underestimate the power of small acts of kindness.

Houston, TX, USA Nathan H. Fowler

Contents

Part III Emerging Therapy in Follicular Lymphoma

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Part I
Biology and Pathogenesis of Follicular Lymphoma

Chapter 1
Follicular Lymphoma: Epidemiology, Pathogenesis and Initiating Events

Zi Yun Ng, Connull Leslie, and Chan Yoon Cheah

Epidemiology

Introduction

Follicular lymphoma (FL) is the second most common lymphoma in the United States (US) and Western Europe and the most common indolent lymphoma [1]. FL is a lymphoproliferative disorder of germinal centre B-cells with a median age of diagnosis of 58 years [1]. It is commonly associated with the inappropriate activation of *BCL2*, a proto-oncogene which is most commonly activated through the *t*(14; 18)(q32;q21) chromosomal translocation [2].

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General Trend of Incidence

In the United States, Teras et al. analysed data from the Surveillance, Epidemiology, and End Results (SEER) registries to provide estimates of the total numbers of US lymphoid neoplasm cases by subtype as well as a detailed evaluation of incidence and survival statistics. The US age-adjusted incidence rate from 2011 to 2012 for FL was 3.4 per 100,000 population. In this study, while most lymphoid malignancies showed excess risk for males, this was not seen for FL which had an incidence rate ratio (IRR) for gender of 1.18 [3]. Similarly, in the United Kingdom from 2004 to 2014, FL had a higher age-standardised sex rate ratio of 0.93 ([95% CI 0.89–0.98], $P = 0.006$), meaning there were marginally more females than males diagnosed with FL [4].

From 1992 to 2001, the incidence of FL showed a non-significant rise of 1.8% per year among the elderly [5]. However, the incidence for both genders declined from 2001 to 2012. For males with FL, the annual percentage change in incidence dropped from 4.7% in 2001–2004 to −2.2% in 2004–2012. For females, the annual percentage change declined from 3.4% to −0.8% (2001–2004) and then a further −3.6% from 2007 to 2012. It is hypothesised that the decline in incidence rates is due to declining smoking rates over this period. Gender and race did not significantly influence 2-, 5-, and 10-year survival rates [3].

International Variation

Multiple epidemiological studies have shown that FL has higher incidence in Caucasian populations compared to African or Asian [2, 5, 6]. Analysis of 19 case-control studies by the InterLymph Consortium showed that magnitudes of associations with FL according to region (Europe, North America and Australia) were mostly consistent [1]. A study of non-Hodgkin lymphoma (NHL) from 1988 to 1990 showed FL comprised a greater proportion of NHL diagnoses in North America, London and Cape Town (28%–32%) relative to other sites like Hong Kong (8%), Sweden (11%) or France (17%) [7]. Similarly, a study of 4056 cases of NHL at 13 major medical centres in Thailand from 2007 to 2014 found only 5.6% of these cases to be FL [8].

When looking at a migrant population in England from 2001 to 2007, rates of FL were lowest among Chinese and individuals of African descent, intermediate among South Asians and highest among Caucasians. There was little difference between Afro-Caribbeans and Africans, with incidence rates around 60% lower than that of Caucasians. Between the South Asian groups, Pakistanis showed the highest rates, followed by Indians and Bangladeshis (IRRs of 1.11, 0.68 and 0.54, respectively) [9]. FL is less common in India compared to Europe or America; for example, a study from Mumbai showed that FL accounted for only 12.6% of 2773 NHL cases [10].

Interestingly, investigating incidence of FL in Americans of Asian descent, Clarke et al. found the incidence was significantly lower in foreign-born Asian-Americans compared to American-born (IRR 0.57 [95% CI 0.44–0.73]), suggesting a role for environmental factors in the pathogenesis of FL [11]. Supporting this, the risk of FL seems to be lower in first-generation Asian-born Japanese and Chinese migrants compared to their descendants [12].

Genetic Factors

The InterLymph Consortium which comprised of 19 case-control studies (3530 cases and 22,639 controls) in Europe, North America and Australia showed that a family history of non-Hodgkin lymphoma in a first-degree relative confers approximately double the population background risk of FL [1]. The risk was 3.6 times higher in participants with first-degree male relatives with multiple myeloma compared to the general population. Interestingly, this was not evident if there was a first-degree female relative with myeloma. First-degree relatives with leukaemia or Hodgkin lymphoma did not seem to confer an increased risk of FL [1]. Analysis of 4455 individuals in the Swedish Family-Cancer Database found that a parental history of FL was associated with a significantly increased risk of FL (standardised incidence ratio of 6.1), while an affected sibling conferred a 2.3 times risk [13].

There have been an increasing number of genome-wide association studies (GWAS) identifying single-nucleotide polymorphisms (SNPs) associated with risk of developing FL (detailed in Table 1.1).

Certain polymorphisms of the DNA repair gene *XRCC3* may increase the risk of developing FL, especially in current smokers [20].

Environmental Factors

The aforementioned migrant studies provide some evidence that environmental factors play a role in the pathogenesis of FL [12]. Attempts to study environmental risk factors in epidemiological studies are greatly hampered by unavoidable confounders and bias. As a result, drawing firm conclusions regarding the relative contribution of specific environmental risk factors is challenging, as data from studies are often conflicting.

A number of studies have examined the association between occupation and risk of FL. A reduced risk of FL was found in bakers and millers (OR 0.51 [95% CI 0.28–0.93]) and university or higher education teachers (OR 0.58 [95% CI 0.41–0.83]) [1]. However, a separate meta-analysis showed an increased risk for NHL in teachers at all levels [21]. A small prospective study in Germany which included 92 FL patients showed significant FL risk increases for occupational groups like medical, dental and veterinary workers (OR 3.1 [95% CI 1.4–6.8]); sales workers (OR

Table 1.1 Genome-wide association studies with the relevant SNPs identified to be associated with FL

Genome-wide association studies (GWAS)	Single-nucleotide polymorphisms (SNPs) identified to be associated with FL
Conde et al. [14]	rs10484561 rs7755224 SNPs in the psoriasis susceptibility region 1 (PSORS1)
Smedby et al. [15]	rs10484561 – also associated with risk of diffuse large B-cell lymphoma (DLBCL) rs2647012
Skibola et al. [16]	rs6457327 – region of strongest association near PSORS1 locus
Vijai et al. [17]	rs4530903 rs9268853 rs2647046 rs2621416
Skibola et al. [18]	rs9275517[a] – no longer associated when its high linkage disequilibrium with rs2647012 was accounted for rs3117222[a] – correlated with increased levels of HLA-DPB1, suggesting its expression regulation as a possible disease mechanism
Skibola et al. [19]	rs17203612 rs3130437
	rs4938573[b] near CXCR5 rs4937362[b] near ETS1 rs6444305[b] in LPP rs17749561[b] near BCL2 rs13254990[b] near PVT1

[a]Inversely associated with FL
[b]SNPs in non-HLA loci

2.8 [95% CI 1.3–5.9]); machinery fitters (OR 3.4 [95% CI 1.5–7.8]); and electrical fitters (OR 3.5 [95% CI 1.5–8.4]) [22]. Risk of FL certainly significantly increased with exposure to chemical solvents such as benzene, toluene, xylene and styrene (OR 1.7 [95% CI 1.2–2.5] $P = 4 \times 10^{-7}$) [23]. Spray painters and those working with paint solvents had increased risk of FL (OR 2.66 [95% CI 1.36–5.24]) [1, 24, 25]. Medical doctors who had worked more than 10 years had a significantly elevated risk (OR 2.06 [95% CI 1.08–3.92]) based on 38 cases vs. 13 controls [1]. Employment in other occupations was not associated with risk of FL, including working/living on a farm [1]. The *t*(14;18) translocation which occurs in up to 70–90% of FL was found to be associated with certain agricultural pesticides in two studies [26, 27]. A different study found that occupational exposure to pesticides would increase BCL2-IGH prevalence together with the frequency of BCL2-IGH-bearing cells especially during periods of high pesticide use [28]. It should be noted that this translocation can be detected in healthy individuals or patients with other cancers. There were also modestly increased risks of FL related to residential proximity to a petroleum refinery (OR 1.3) or a primary metal industry (OR 1.2) [29].

Unlike other lymphomas, studies suggest that autoimmune diseases are not generally associated with an increased risk of FL with the exception of Sjögren's

syndrome (OR 3.37 [95% CI 1.23–9.19], $P = 0.024$) [1]. Rather, atopic diseases (with the possible exception of eczema) were associated with a lower risk of FL [1, 30]. Females with allergic rhinitis (OR 0.70 [95% CI 0.56–0.88], $P = 0.002$) and food allergy (OR 0.74 [95% CI 0.63–0.86], $P < 0.001$) had lower risk of FL, but this was not apparent in males. Risk for combined and individual atopic/allergic disorders showed greater reduction in Australia compared to Europe or North America [1]. A 22% lower risk of FL was noted if there was a history of a blood transfusion – with reductions in risk most notable if the transfusion was received after 55 years of age and within 40 years of FL diagnosis [1]. Smaller studies examining the impact of prior blood transfusion have suggested either no association [31] or increased risk [32, 33]. Interestingly, although acquired immunosuppression from human immunodeficiency virus (HIV) or organ transplants confer increased risk of lymphoid malignancies such as plasmablastic lymphoma, Epstein-Barr virus (EBV)-driven lymphomas and primary central nervous system (CNS) lymphoma, no increase in FL incidence has been described, suggesting a different mechanism of lymphomagenesis [34, 35].

A population-based case-control study of in-person interviews of 1593 NHL individuals from 1988 to 1995 showed that non-steroidal anti-inflammatory drug use, treatment of type 2 diabetes mellitus with oral hypoglycaemics, a history of hepatitis and three or more lifetime bee stings were inversely associated with FL. On the other hand, a history of heart disease and beta-blocker use were positively associated with FL risk. It is suggested that these conditions exert an immunomodulatory effect that influences the development of FL [36]. In the InterLymph study, positive hepatitis C virus serology was not linked with FL risk (OR 1.28 [95% CI 0.64–2.57]) [1]. Polio vaccination was associated with decreased risk, while influenza vaccination was the opposite; however, the knowledge between vaccinations and FL risk is incomplete [37].

Earlier studies indicated an increased risk of FL for current smokers compared to non-smokers [38, 39], particularly in those with more than a 36-pack-year history [40]. This effect was found in females but not males, for reasons that are unclear [1, 41, 42]. A modest risk of FL among women who ever smoked cigarettes was limited to current smokers, along with a significant positive trend for total duration of smoking. Additionally, duration, rather than frequency of cigarette smoking, appeared more important in the trend in pack-years of smoking in women [1]. The association between smoking and FL is biologically plausible given the increased risk of t(14;18) in heavy smokers [43]. However, two prospective studies showed contrary results, suggesting a lower risk of FL with current/former smokers with one showing a hazard ratio of 0.62 [95% CI 0.45–0.85] [44] and another observing a relative risk of 0.67 [95% CI 0.52–0.86] [45].

There is some suggestion that a diet high in vitamin D [2, 46] and linoleic acid (a polyunsaturated fatty acid) was associated with a lower risk of FL [2]. Men with a dietary pattern high in "fat and meat" (highest quartile vs. lowest) had an increased risk of FL (HR 5.16 [95% CI 1.33–20.0]) [47]. A few studies found that a diet high in vegetables and fruit was associated with a decreased risk of FL [47, 48]. An inverse relationship between FL risk and antioxidants like vitamin C, lutein +

zeaxanthin, β-cryptoxanthin, isoflavones and flavonols was observed by Frankenfeld et al. [49] Increasing nitrate intake (both plants and animals) was positively associated with FL risk, although geographic and ethnic variability as confounders cannot be excluded [2]. FL risk was modestly reduced in women (OR 0.79), but not men who ever drank alcohol, especially in current drinkers. There was no clear pattern with number of drinks per week, duration or cumulative alcohol consumption due to lack of data collected [1]. Although several studies support a higher risk in non-drinkers [45], other studies yield conflicting results [44, 50]. For example, wine consumption marginally increased the risk (OR 2.19 [95% CI 0.83–5.80]), especially if alcohol consumption started before 20 years of age (OR 4.04 [95% CI 1.19–13.76]) and if the amount exceeded 19 grams of alcohol per day (OR 4.37 [95% CI 1.04–18.45]) [2]. An increasing trend was observed for FL risk and the quantity of coffee assumption – with a doubled risk for an intake of more than four cups per day (OR 2.0 [95% CI 1.2–3.4]) and tripled for a consumption over at least 30 years (OR 3.1 [95% CI 1.7–5.6]). The effect appeared synergistic in current smokers in this Italian population-based case-control study of 161 FL cases [51]. However, a smaller Scandinavian population-based case-control study of 105 FL cases failed to confirm an association between coffee intake and risk of developing FL [48]. An increasing amount of recreational sun exposure was associated with a lower risk of FL (OR 0.7–0.78), but this was attenuated when compared with total sun exposure (OR 0.82–0.88) [1]. This association is dependent on the Ex11 + 32 T > C polymorphism in the vitamin D receptor gene. People homozygous for the C allele with <7 hours per week of sun exposure were six times more likely to develop FL compared to individuals homozygous for the T allele [52]. Examined in women only, there was no association between hair dye use (type, frequency, duration) and FL risk, except a modest increase in those who used hair dyes before 1980 (OR 1.40 [95% CI 1.10–1.78]) [1].

In a population-based control study, increased body mass index (BMI) was positively associated with risk of FL [53]. The InterLymph meta-analysis showed that being overweight or obese as a young adult conferred a higher risk of FL [54] (with 15% increase for each additional 5 kg/m^2 over BMI of 25 in young adults) [1]. Being overweight or obese as a young adult was associated with ~1.5 times risk of FL [1]. However, a population-based case-control study of 586 FL cases did not find an association between early adult weight and FL risk [55]. Like obesity, lack of physical activity has been associated with decreased immune function. A population-based case-control study suggested that total physical activity of more than 19.1 hours per week may have a protective effect, with the benefits more pronounced for women [56].

Epidemiology: Summary

FL is equally balanced among both males and females and most common in the United States and Europe. The disease arises from a complex interplay of genetic

and environmental factors, though most patients do not have clearly identifiable risk factors at presentation. A family history of NHL confers an increased risk, and GWAS have revealed SNPs in both HLA and non-HLA regions that influence this. Exposure to pesticides and chemical solvents (e.g. spray painters), Sjogren's syndrome, heavy smoking (especially in women), obesity and sedentary lifestyle have all been linked to increased risk of FL in some studies. A diet high in vitamin D, vegetables and fruit and low in fat and meat may be protective. Larger epidemiological studies are needed to answer these questions in further detail.

Follicular Lymphoma Pathogenesis

Follicular lymphoma (FL) cells have dependence on a microenvironment mimicking the normal lymph node germinal centre, as might be expected from a mature B-cell lymphoma showing germinal centre features both morphologically (neoplastic cells appear centrocyte- and centroblast-like) and immunophenotypically. Reflected in the 2016 revision of the WHO classification of lymphoid neoplasms [57], forms of follicular lymphoma not associated with the characteristic *BCL2-IGH* rearrangement, such as paediatric-type FL [58, 59] and predominantly diffuse FL with 1p36 deletion [60], are increasingly recognised as biologically and clinically distinct neoplasms. These lesions aside from the characteristic *t*(14;18) *BCL2-IGH* translocation, present in around 85% of FL cases, are recognised as the likely initial necessary although not sufficient abnormality present early in a multihit pathway which culminates in clinically overt follicular lymphoma.

Cell of Origin: First Hit

The *t*(14;18)(q32;q21) *BCL2-IGH* translocation is thought to develop early in B-cell development, during V(D)J recombination of the immunoglobulin heavy-chain locus in B-cell precursors developing within the bone marrow [61]. Low levels of translocation-carrying cells can be detected in the circulating blood of healthy individuals, with an increasing prevalence of up to 66% of individuals aged 50 years or older [62], with the vast majority not developing clinical disease. There is persistence of these *BCL2-IGH*-carrying clones over multiple years in a given person [63].

The *t*(14;18) translocation juxtapositions the *BCL2* gene with the immunoglobulin heavy-chain gene resulting in overproduction of the BCL2 protein which blocks a final common pathway for programmed cell death, preventing apoptosis [64]. In cases of follicular lymphoma which do not show BCL2 protein expression by immunohistochemistry, a subset may show false-negative staining due to mutations in the *BCL2* gene [65].

There is a proven clonal relationship between detected *t*(14:18)-carrying cells and subsequently developed follicular lymphoma and a link between prevalence of such cells and higher risk of subsequent follicular lymphoma [66]. Evidence that circulating translocation-carrying cells are not naive B-cells but germinal centre-experienced and expanded clones suggests clinical disease requires further mutational events as the germinal centre entry of *t*(14;18)-carrying B-cells appears an insufficient event to trigger pre-FL to FL progression [63]. As further events in a multihit pathway will not happen at once, the presence of early FL precursors blurs the distinction between healthy individuals and subclinical patients.

Recognising that within the germinal centre environment *t*(14:18)-mediated anti-apoptotic BCL2 protein expression provokes persistence of such cells by rescue from induced apoptosis, these will be cells with only low-affinity B-cell receptors allowing a larger spectrum of antigen cross-reactivity. Such cells will likely undergo repetitive rounds of expansion within germinal centres during the numerous antigenic challenges faced by the immune system. These cells are at subsequent increased risk of acquiring oncogenic mutations due to repeated exposure to the activation-induced cytidine deaminase (AID) "mutator" inducing somatic hypermutation in germinal centre B-cells. Although most of the randomly occurring chromosomal alterations will be a selective disadvantage over subsequent iterative cycles (noting that FL prevalence increases with age), there would be further accumulation of chromosomal lesions, some of which provide selective advantage and malignant progression [67]. There is evidence from mouse models that AID is required for germinal centre-derived lymphomagenesis [68], supporting the concept that AID-mediated modification contributes to pathogenesis of follicular lymphoma (Fig. 1.1.)

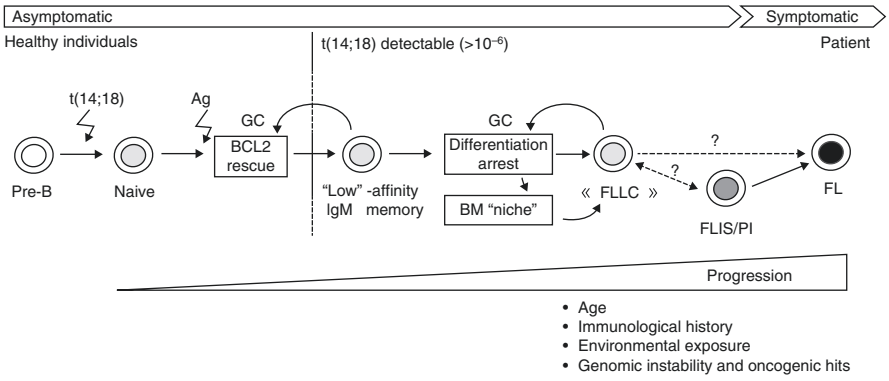

Fig. 1.1 A protracted model of multihit FL genesis. FLLC follicular lymphoma-like B-cell clones, FLIS in situ follicular lymphoma, PI follicular lymphoma with partial involvement [67]. (Reprinted from Roulland et al. [67], © 2011, with permission from Elsevier)

Microenvironment

Follicular lymphoma is characterised by numerous closely associated non-malignant immune cells, appreciated in diagnostic samples as nodular morphology representing the expanded follicular dendritic cell (DC) network and the usually numerous small host T-cells (Fig. 1.2). These non-neoplastic immune cells appear to influence disease behaviour, with gene expression profiling studies showing differences between FL which transformed to diffuse large B-cell lymphoma (DLBCL) and FL that did not (within the 7-year follow-up period) being genes involved in T-cell function; and rapidly transforming FL appeared more similar to reactive follicular hyperplasia, while non-transforming FL resembled non-activated lymphoid tissue [69].

The role of the microenvironment in FL appears to simultaneously support growth and survival of the neoplastic cells and suppress the antitumour immune response (Fig. 1.3). Follicular dendritic cells contribute to B-cell receptor (BCR) signalling and higher levels of sustained signalling eventually supporting survival of FL neoplastic cells [70]. Expression of IL-12 by neoplastic B-cells has also been shown to induce functional intratumoural T-cell exhaustion by promoting TIM-3 expression, similar to changes seen in chronic viral infection [71]. Elevated num-

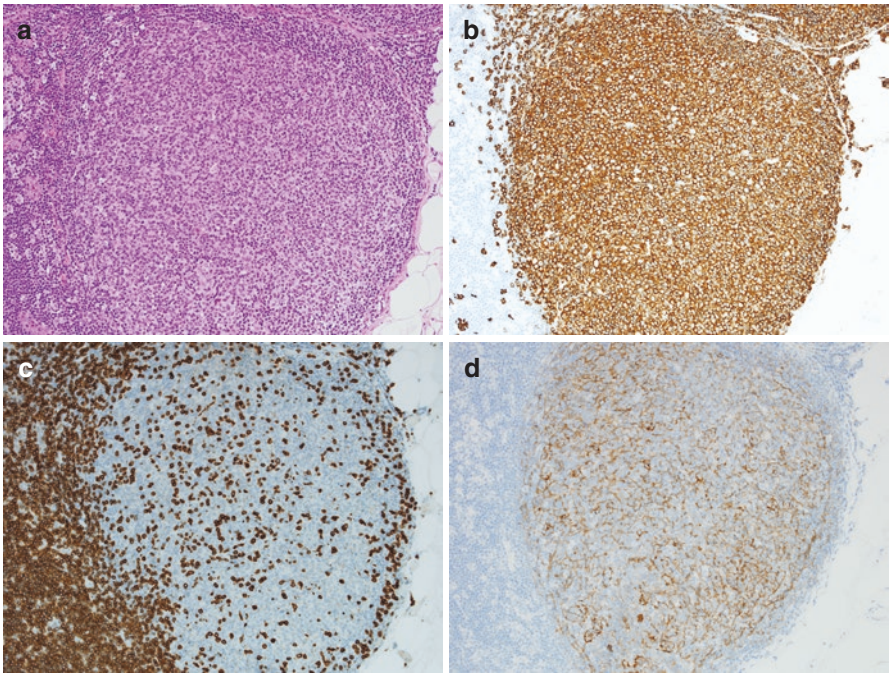

Fig. 1.2 Neoplastic nodules in follicular lymphoma (2A – H+E) include the clonal B-cells (2B – CD20) with admixed host T-cells (2C – CD3) and an expanded distorted follicular dendritic cell network (2D – CD21)

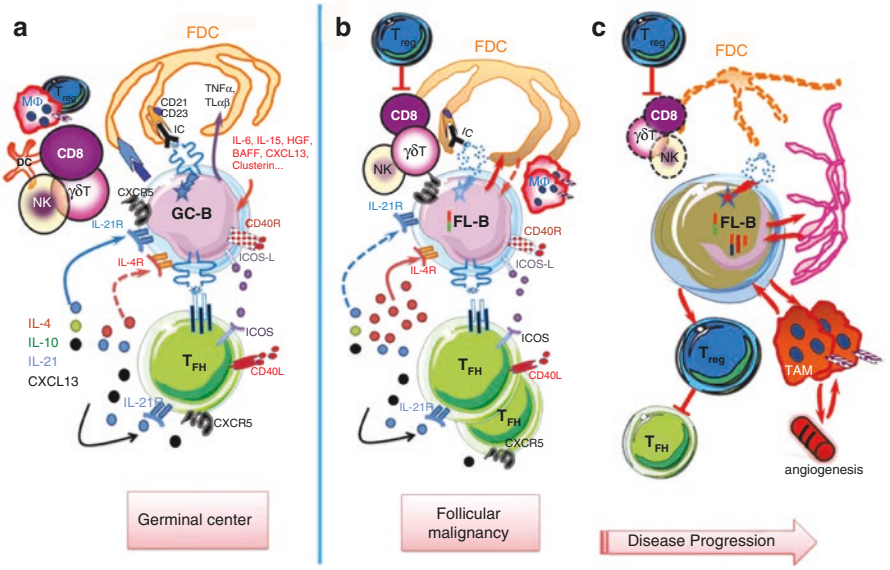

Fig. 1.3 Schematic illustration of interactions between B-cells and their microenvironment in the context of the normal germinal centre (GC) reaction and the follicular lymphoma niche. (**a**) To make high-affinity, class-switched antibody, B-cells must receive cognate help from T follicular helper (T_{FH}) cells during the GC reaction leading to maturation of activated B-cells along with production of memory B-cells and plasma cells. In the absence of T-cell help during B-cell priming by dendritic cells (DCs) followed by follicular dendritic cells (FDCs), B-cells are driving to apoptosis. A range of cytokines including CD40L, IL-21 and IL-4 produced by T_{FH} cells can direct antibody class switching. Moreover, T_{FH} cells produce high levels of chemokine CXCL13 along with FDCs allowing the B-cell migration within an appropriate GC area where rescued B-cells undergo final maturation. In the opposite, B-cells produce inducible T-cell co-stimulator ICOS-L which engages ICOS-driving production of cytokines in T_{FH} cells. The next critical cell in the development of the GC reaction is the FDC that produces under specific and coordinated signalling from immune accessory cells and B-cells themselves a wide range of factors which support recruitment and survival of B-cells. FDCs also concentrate antigen as immune complexes on their surface bridging B-cell receptor (BCR) on B-cells leading to a specific B-cell signalling involved in the cell activation and maturation. Several other hematopoietic cells are present during the GC reaction holding specific functions such as antigen presentation for DCs and macrophages; innate immune response for macrophages, natural killer (NK) cells and γδ-T cells; and adaptive immune response for CD8+ and T regulatory (T_{reg}) cells. (**b**) Early in FL emergence, specific changes take place in the microenvironment induced either directly by the BCL2-translocated B-cells (represented by nuclear green-red bar code) or indirectly by emerging cell subsets including T_{reg} cells which attenuate CD8+ T-cell function. T_{FH} cells are highly represented in the FL tumour, and they up-regulate IL-4 production sustaining B-cell survival. FDCs modify released factors in response to cross-talk modifications along with FL B-cells but also through other cell subsets such as macrophages which show significant perturbation. BCL2-translocated FL B-cells present specific modifications including the BCR membrane complex and its secondary signalling. (**c**) Progressed FL disease shows large modification of the tumour landscape. B-cells present genetic instability (represented by several nuclear bar codes) driving several cell function modifications including a constitutive BCR signal (red star). During progression, cells seen in the normal GC reaction are vanishing (T_{FH} cells, FDCs, CD8+ T-cells, and others), while follicular reticular cell-like cells (pink stromal cells) along with tumour-associated macrophages (TAMs) appear in response to stress signals building a microenvironment specific of tumour aggressiveness including angiogenesis promotion [75]. (Reprinted from de Jong and Fest [75], © 2011, with permission from Elsevier)

bers of infiltrating macrophages associated with increased neovascularisation through angiogenic sprouting have been associated with poor prognosis [72].

Stromal cells within the germinal centre provide signals for malignant cells in two general ways: recruitment to the germinal centre and mediation of growth and survival [73]. While there is evidence that stromal cells in germinal centres (fibroblastic reticular cells) interact with FL B-cells using cross-talk mechanisms similar to those used by reactive B-cells [74], the specific migratory drivers of neoplastic cells compared to the normal counterpart, which lead to re-entry of FL clones to germinal centres with maturation arrest and subsequent amplified gene instability, are an issue requiring further investigation.

Numerous studies have examined the relationship between non-neoplastic immune cells and outcome in follicular lymphoma with contradictory results [75, 76]. Given the evidence that classes of non-malignant cells may have therapeutic implications, there has been interest in objective measurement of these populations by computer-assisted scoring [77] although neither this approach nor other quantifications of the tumour microenvironment are currently in general diagnostic use.

In FL cells with impaired checkpoint selection, the BCR has only loose affinity to any specific antigen. In place of antigen affinity as a driver of cell survival, in some FL cases, a sequence motif introduced during somatic hypermutation characteristic of an N-glycosylation site is present, a finding not seen in normal B-cells or other lymphomas characterised by mutated B-cell subsets [78]. The glycan added to these sites shows unusual termination with high mannose, with evidence that macrophages in FL tissue have up-regulated mannose-binding lectins, which in co-location with surface immunoglobulin has an anti-apoptotic effect [79].

Both the constitutional up-regulation of BCL2 and the acquisition of highly mannosylated BCR appear to be critical steps in lymphomagenesis and substitute for antigen affinity in maintaining FL cells in the germinal centre environment [80]. Disrupting such interactions within the microenvironment may be therapeutic opportunities.

Early Lesions

The term “in situ follicular neoplasia” (ISFN) should be applied to lymph nodes in which abnormal bright BCL2 expression (associated with the characteristic *BCL2-IGH* translocation) is seen in follicle centre B-cells where there is preservation of normal lymph node architecture and associated non-neoplastic reactive germinal centres [81] (Fig. 1.4). In the updated (2016) WHO classification, these changes have been renamed ISFN (previously “follicular lymphoma in situ”) to recognise the low rate of progression to clinically overt disease [57].

Such alterations may be seen in patients with synchronous or subsequent clinically evident follicular lymphoma and if seen require clinical assessment, although these are also seen in patients who do not subsequently developed clinically evident follicular lymphoma. In the former cases, the ISFN likely reflects spreading and

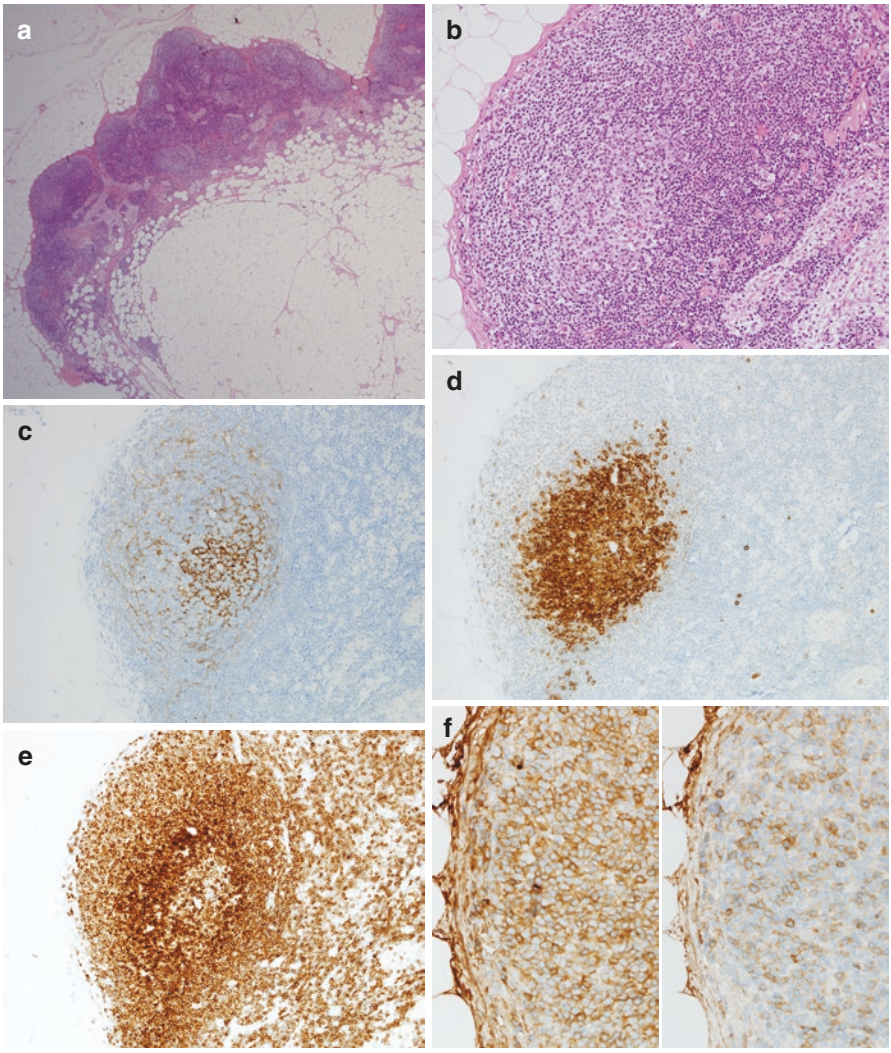

Fig. 1.4 In situ follicular neoplasia (ISFN) is usually an incidental finding noted in lymph nodes with a "reactive" architecture (4A – H+E ×2) in which follicles are mildly expanded (4B – H+E ×20) and there is an associated follicular dendritic cell network (4C – CD21). The neoplastic cells show strong expression of CD10 (4D) in keeping with germinal centre type, with aberrant expression of anti-apoptotic protein BCL2 (4E) and kappa light-chain restriction (4F)

homing of neoplastic cells to reactive germinal centres of adjacent or distant lymph nodes; however, in the latter, it appears to represent a pre-malignant finding. In several case series, ISFN has been seen in association with a second B-cell lymphoma of other types [82, 83] suggesting increased risk for B-cell neoplasms, although such patients would also have had reason for lymph node excision and hence increased likelihood of incidental detection of ISFN.

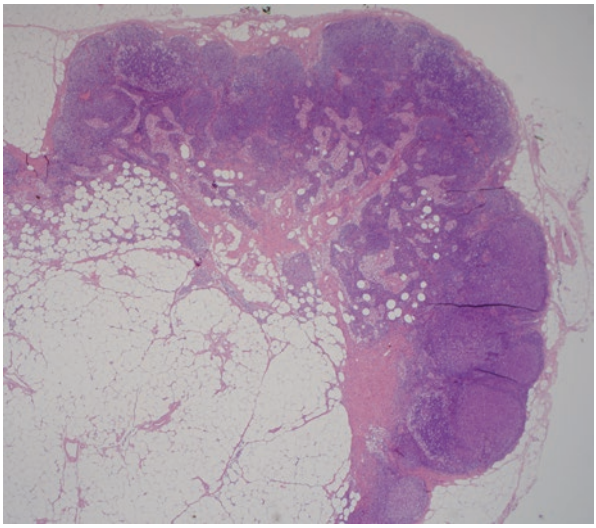

Fig. 1.5 The node is only partially replaced by follicular lymphoma (bottom right) where there is expansion of neoplastic follicles and spillover of neoplastic cells into interfollicular zones, with adjacent quiescent or benign reactive follicles in the remainder of the node (top left)

Partial involvement of a node by follicular lymphoma identifies patients at greater risk of subsequent clinical follicular lymphoma than ISFN and is identified by altered architecture, expanded follicle size, blurred edge to germinal centre, variable and weaker expression of BCL2 and CD10 and neoplastic cells outside the expanded germinal centre [82] (Fig. 1.5). In contrast to this architecturally abnormal node, ISFN likely represents tissue counterpart of FL-like cells in the peripheral blood of healthy people which have seeded reactive hyperplastic germinal centres and expanded in an antigen-dependent manner. The low risk of progression suggests these cells lack additional mutations required for malignant transformation.

Disease Evolution and Clonal Variation

As a germinal centre lymphoma with high expression of AID, follicular lymphoma cells will show ongoing somatic hypermutation of immunoglobulin loci following entry into a germinal centre, and this may occur well before clinically malignant transformation. This enables detailed tracking of neoplastic subclonal evolution, as in a pool of tumour cells each clone can be detected by a unique somatic hypermutation fingerprint. In the small number of examined cases, modelling indicates that FL clones may expand within lymph nodes, then migrate to the bone marrow and stay quiescent for long periods before again expanding lymph nodes with less mutated "founder" FL cells [84]. Under such a model, these clonally related bone marrow-resident "in situ follicular neoplasia" cells cause the relapse of FL after treatment, although this will not show the same linear accumulation of somatic mutations which may have been present in the initial disease presentation [85].

The evolution of minor subclones within an FL may be more complex than initially appreciated as indicated by a case example involving a donor-recipient pair

who both developed follicular lymphoma 7 years after allogeneic transplantation [86]. Both patients harboured identical *BCL2-IGH* translocations and the same *V(D)J* rearrangement, as well as 15 further shared somatic mutations, indicating these were present at least 7 years before clinical presentation. There were an additional six mutations detected in one or other of the lymphomas indicating acquisition following clonal divergence. This example and other studies of paired diagnosis/relapse samples [87] support the existence of an FL clonal hierarchy, which can branch at various stages of development, and indicate that despite complete clinical response to therapy, self-renewing tumour cell precursors may not be eradicated.

The progression from early genetic hits to a dynamic evolution of subclones, in which subsequent genetic hits may be variably seen in clinically evident clonal expansions, "in situ" disease or sequestration within "niches" such as the bone marrow, with subclones re-emerging following therapy or resulting in transformation to high-grade lymphoma, is a model which is undergoing further investigation (Fig. 1.6). Transformed follicular lymphoma most commonly shows a germinal

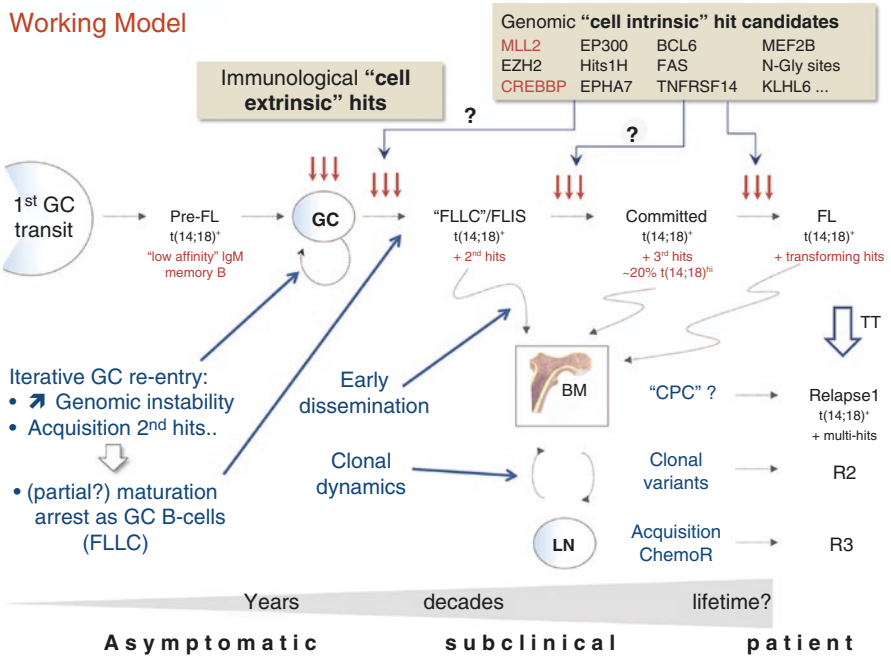

Fig. 1.6 A working model of follicular lymphoma (FL) genesis. Progression proceeds from the very early steps to a dynamic 'Darwinian-like' subclonal evolution, with some variants acquiring selective advantages for germinal centre (GC) re-entry leading to AID-mediated off-target mutagenesis. Mutations specifically involved and required for committed precursor cell formation and commitment to FL development are not yet known. Candidate early hits are indicated in red and together with B-cell receptor signalling through N-glycosylation sites/lectins are likely to participate in committed FL precursor clone (CPC) genesis and early FL progression. BM bone marrow, LN lymph node, FLIS in situ FL, FLLC FL-like t(14;18)+ B cell, TT treatment, ChemoR chemotherapy + rituximab [80]. (Reprinted from Ghia et al. [80], with permission from John Wiley and Sons)

centre phenotype, although a significant minority (around 18%) have an activated B-cell phenotype; these cases are more often negative for *BCL2* translocation and arise in *BCL2* translocation-negative follicular lymphomas, further indicating heterogeneity in follicular lymphomagenesis [88].

There are experimental limitations hampering investigation of FL pathogenesis, such as lack of a B-cell line reflecting the untransformed indolent stage of FL and the high propensity of primary FL cells to undergo apoptosis due to inability to maintain fully functional human follicular dendritic cell networks in culture. Better understanding of issues such as the drivers of germinal centre re-entry of early FL clones, the nature of the impaired BCR signalling and the complex tumour microenvironment may present targets for innovative therapeutic strategies.

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Chapter 2
Pathologic Features, Grading, and Variants of Follicular Lymphoma

Ali Sakhdari and Roberto N. Miranda

General Features

Follicular lymphoma (FL) is an indolent, mature B-cell neoplasm, characterized by neoplastic germinal centers composed predominantly of centrocytes and centroblasts, and the main underlying mechanism is the gene fusion of *IGH-BCL2* that codes for a chimeric protein that inhibits apoptosis [1, 2].

After recognition of Hodgkin lymphoma in the mid-nineteenth century by Hodgkin and Wilks, FL was one of the earliest non-Hodgkin lymphomas (NHL) that was described. Due to its similarities to the more common reactive lymph node conditions, Brill and Symmers, the first descriptors of the entity, initially considered it as a generalized giant lymph node hyperplasia [3, 4]. For many years, even after full recognition of its malignant nature, many clinicians and pathologists believed that FL was mainly an intermediary stage between different other types of diseases of the hematopoietic system having the potential to transform to other subtypes [5, 6]. It was not until the landmark publication by Henry Rappaport in 1956 that FL was considered as a separate entity in the realm of "malignant lymphomas" [7].

The field of lymphoma has seen many different and at times revolutionary classifications. Gall and Mallory's [8], Rappaport's [7], Kiel's [9], Lukes-Collins' [10], and more recently the working formulation [11], REAL [12], and finally the World

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Health Organization (WHO) classification [13] have been published and widely utilized around the world in the past 70 to 80 years. Follicular lymphoma has been recognized by all of these major classifications, albeit named differently: poorly differentiated lymphocytic lymphoma (Rappaport's), centroblastic-centrocytic lymphoma (Kiel), follicular center cell (Lukes-Collins, working formulation, REAL), and follicular lymphoma (WHO) [2].

The etiology of follicular lymphoma is largely unknown, although there are few genetic associations and extrinsic factors that have been identified as potential culprits, including history of lymphoma in family members, smoking, certain occupations, and some autoimmune disorders [14].

Macroscopic Features

FL is a lymph node-based lymphoma; thus, the diagnosis can be initially suspected because of enlarged lymph nodes. On sections, a vague or subtle nodularity can be observed (Fig. 2.1); occasionally, there is irregular sclerosis. Nearly any extranodal site can be affected by FL, and the appearance is that of a mass or small nodules. In the duodenum, it appears as small nodules in the mucosa and in the spleen as a miliary enlargement of the white pulp or as distinct large nodules or masses.

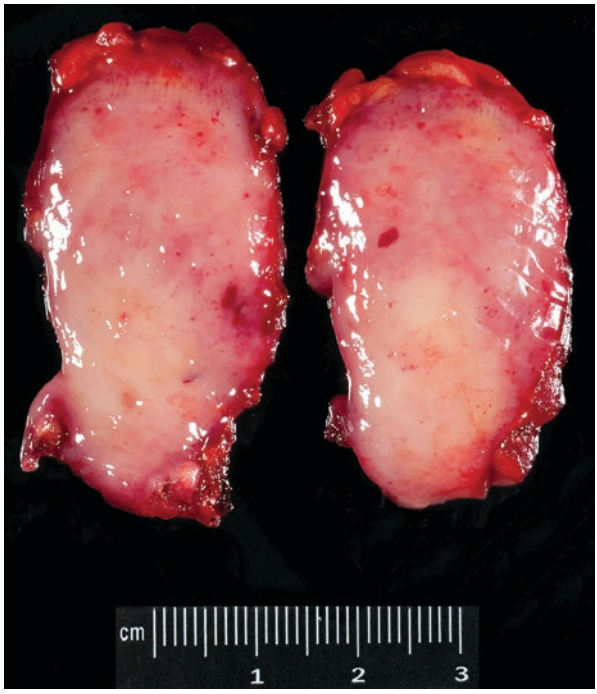

Fig. 2.1 Gross appearance of enlarged lymph node involved with follicular lymphoma (FL). Focal areas show subtle vague nodularity

Histologic Features

Lymph nodes can show partial or complete effacement of the architecture due to numerous back-to-back neoplastic follicles. They are to a great extent uniform, with round edges and frequent attenuation of the normal mantle zone of the follicles (Fig. 2.2a). These neoplastic follicles appear homogeneous with loss of polarity and are composed of variable proportions of small centrocytes and large centroblasts (Fig. 2.2b). These features contrast with follicular lymphoid hyperplasia with hyperplastic germinal centers that show widely spaced follicles (Fig. 2.2c), and the reactive follicles show distinct mantle zones and polarity of germinal centers (Fig. 2.2d). Lymphoma cells commonly permeate into interfollicular areas in small numbers, but can noticeably expand these areas to constitute a diffuse component. Involvement of medullary sinuses inside the lymph node as well as extension to the extracapsular

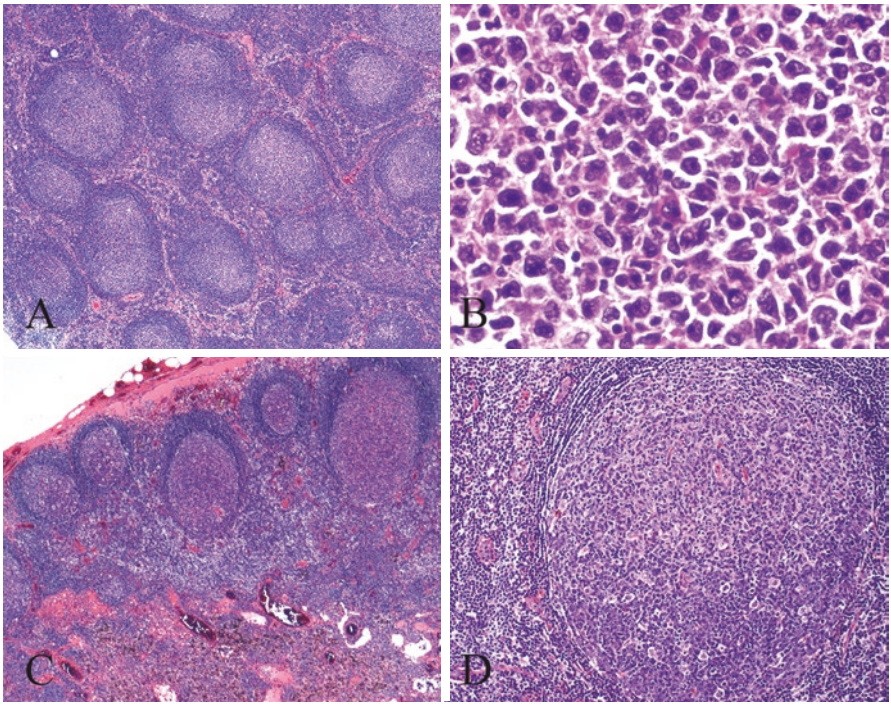

Fig. 2.2 Follicular lymphoma and follicular lymphoid hyperplasia. (**a**) Low magnification shows numerous neoplastic follicles with a uniform cell population, lack of polarity, and blurry interface between mantle zone and germinal centers. (**b**) High magnification shows a predominance of small centrocytes and scattered large centroblasts. (**c**) Low magnification of lymph node with follicular lymphoid hyperplasia shows widely spaced lymphoid follicles with polarity, surrounded by distinct mantle zones with polarity, being wider toward the capsule and thinner toward the medullary region. (**d**) High magnification of hyperplastic germinal center with polarity of germinal center cells, dark with a starry sky at the bottom, clear and uniform at the top

soft tissue fat is commonly observed [1, 13, 14]. Follicular lymphoma also has a tendency to involve the surrounding tissues and structures.

The neoplastic centrocytes are approximately 2 diameters larger than reactive small mature lymphocytes (Fig. 2.2b). The centrocytes show imperceptible cytoplasm and a homogeneous population of hyperchromatic, indented, or twisted nuclei without nucleoli. In contrast, the neoplastic centroblasts are 3–4 diameters as large as small mature lymphocytes and display an oval to round and vesicular nuclei with one to three distinct, mostly membrane-bound, basophilic nucleoli (Fig. 2.2b). They have moderately abundant cytoplasm which is characteristically clear on routine histologic sections or basophilic on Wright-Giemsa stain [1].

In addition to the neoplastic lymphocytes, there are nonneoplastic cells in the background that are commonly seen admixed with the neoplastic centrocytes and centroblasts. Reactive T-lymphocytes (Fig. 2.3a), histiocytes, and follicular dendritic cells (Fig. 2.3b) are almost invariably seen in all cases of follicular lymphoma, although in variable proportions and with some prognostic importance shown in major studies [14, 15]. Rare cases show plasmacytic differentiation of neoplastic cells, while reactive plasma cells are rarely seen in the background of FLs [16].

FL can infiltrate and present in almost any organ, and although a disease mainly of adults, children can also be affected. For some locations and age groups, there are clinical, genetic, and immunophenotypic features that differ from typical nodal-based FL. Variants of FL or specific clinicopathologic entities based on FL include FL of the testis, duodenum, and skin (cutaneous) and the pediatric variant. Follicular lymphoma and large cell lymphoma with *IRF4* rearrangement are considered as distinct entities and discussed below.

Bone marrow examination is part of the staging of patients with FL. FL in the bone marrow is characterized by paratrabecular aggregates (Fig. 2.4a); the aspirate smears demonstrate lymphocytosis, with a predominance of small lymphocytes that

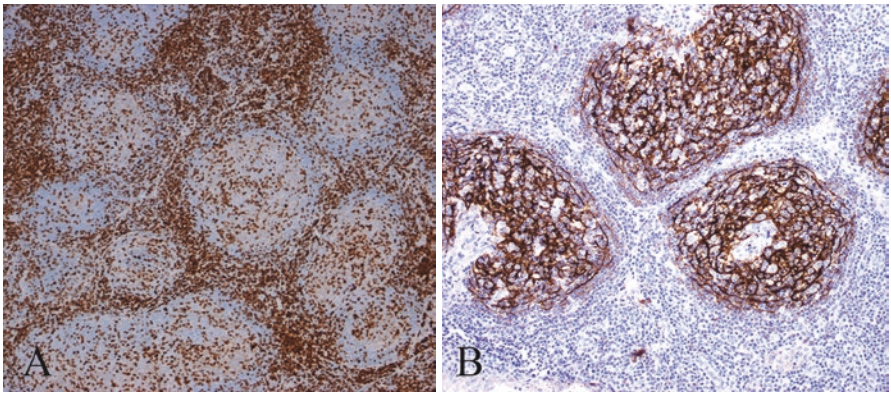

Fig. 2.3 Follicular lymphoma immunohistochemistry. (**a**) Immunohistochemistry for the T-cell marker CD3 shows many small lymphocytes admixed in germinal centers and in the interfollicular areas. (**b**) Immunohistochemistry for the follicular dendritic cell marker CD21 highlights meshworks underlying neoplastic lymphoid follicles

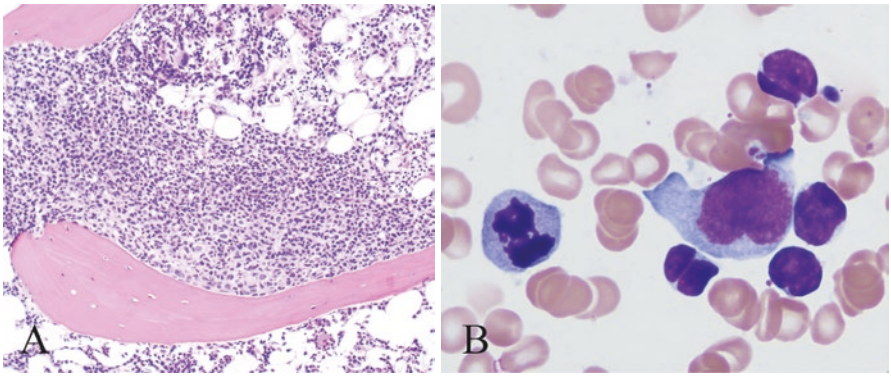

Fig. 2.4 Follicular lymphoma involvement of the bone marrow. (**a**) Paratrabecular lymphoid aggregate is characteristic of FL involvement of bone marrow. The infiltrate may be more subtle or replace entirely the marrow space. (**b**) Bone marrow aspirate smear shows the variability of FL cells and includes small lymphocytes with hyperchromatic nuclei, with irregular nuclear contours and indented nuclei

occasionally show an indentation or apparent bi-lobation (Fig. 2.4b). The diagnosis can be supported by the demonstration of B-cell markers; however, it is common to observe the loss of CD10 and BCL6 expression. The extent of bone marrow involvement is variable and ranges from single or multiple paratrabecular aggregates to diffuse involvement. The sensitivity of detecting minor populations of FL in the bone marrow can be enhanced using flow cytometry immunophenotype or polymerase chain reaction; however, the significance of findings of such minute numbers of FL cells in the bone marrow is uncertain. On the other hand, the lymphoma can be detected histopathologically, but because of its focal nature is not detected by flow cytometry or molecular techniques. Grading in the bone marrow is similar to what is applied to lymph nodes; however, most cases show low-grade morphology, occasionally with concurrent high-grade morphology in the lymph node, in what is commonly referred to as "discordant histology." There is no correlation between the extent of bone marrow involvement and peripheral lymphocytosis. Although it is common to see low levels of follicular lymphoma lymphocytes in the peripheral blood [1], only rare cases of leukemic phase have been reported. The prognostic significance of this presentation is controversial. Post-therapy lymphoid aggregates are sometimes challenging to define. Since morphologically they can be suspicious, further investigation is recommended. Benign cases are generally composed only or predominantly of T-lymphocytes, admixed with rare B-lymphocytes.

FL of the spleen has two main patterns of infiltration, one along the white pulp that is expanded and grossly appears with a miliary pattern and another where large single or multiple masses destroy the splenic architecture [17]. FL in the liver is recognized by the presence of neoplastic follicles in the portal tracts. FL in the gastrointestinal tract beyond duodenal FL represents dissemination of disease and commonly involves all layers of the intestine. FL in the orbit can be

confused with extranodal marginal zone lymphoma (mucosa-associated lymphoid tissue [MALT] lymphoma) [18].

Morphologic and Cytologic Variability

There is a wide range of morphologic changes associated with FL; these changes include the following:

1. *Stromal sclerosis* is a common finding and on occasion can be extensive. Sclerosis is usually seen in the interfollicular areas surrounding the neoplastic follicles, and it can obscure the neoplastic lymphocytes particularly in small biopsies of the retroperitoneum or abdomen (Fig. 2.5a). In some cases, the sclerosis is within the follicles, and in rare cases there is massive deposition of amor-

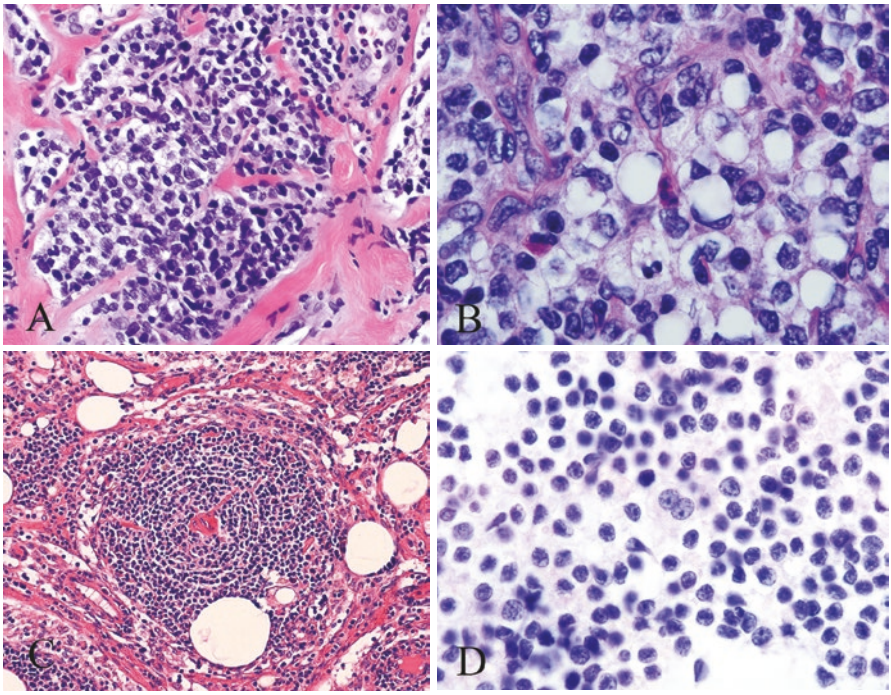

Fig. 2.5 Variability of histologic and cytologic features of FL. (**a**) Sclerosis is common in FL, and it can be interstitial around individual lymphocytes or around clusters of cells or markedly irregular. (**b**) Signet ring cell appearance can occur in cases of FL, and it may create confusion with signet ring cell adenocarcinoma. (**c**) Neoplastic follicle of FL reminiscent of Castleman disease shows a hyalinized vessel in the center. (**d**) Cytologic specimen from lymph node involved by FL shows a uniform population of small, round lymphocytes with hyperchromatic nuclei; this finding may be surprising considering the marked irregularity of centrocytes observed in tissue sections. A follicular dendritic cell is seen at the center of the field

phous pink material among the neoplastic cells giving the large cells a spindle-shaped morphology [1, 19].

2. *Signet ring cell morphology* occurs rarely. In these cases, lymphoma cells accumulate intracytoplasmic immunoglobulin; and the neoplastic lymphocytes acquire a signet ring morphology by pushing the nuclei to an eccentric location, and the cytoplasm appears as clear and vacuolated (Fig. 2.5b). These changes need to be distinguished from signet ring adenocarcinoma; otherwise, cases of FL do not have prognostic significance [1, 20, 21].
3. *Marginal zone differentiation* occurs rarely; and sections show lymphoma lymphocytes at the interfollicular areas or surrounding the residual follicles, usually appearing as a marginal zone, including the lymphocytes with a monocytoid appearance. These lymphocytes are clonally related with the follicular lymphoma cells in germinal centers. Some reports show a poorer prognosis for cases with marginal zone differentiation [1, 22, 23].
4. *Floral change* is the appearance of neoplastic follicles with an effect that is seen in hyperplastic follicles with follicle lysis or progressive transformation of germinal centers. The finding is defined by small lymphocytes from the mantle zone which infiltrate into the germinal center and produce an appearance of fragmentation of the follicle, producing small round islands of centrocytes or centroblasts that are seen from low magnification as "petals." Interestingly, most reports of floral change describe a high-grade morphology [14, 24–26], but we have noted a similar change in cases of low-grade FL.
5. *Castleman-like change* indicates that neoplastic follicles show germinal centers with a concentric arrangement of centrocytes, usually with concurrent sclerosis or a distinctive vessel within the germinal center, reminiscent of hyaline-vascular Castleman disease (Fig. 2.5c) [27]. These cases otherwise should fulfill all criteria for a diagnosis of FL.
6. *Plasmacytic differentiation* occurs rarely in FL [16]. Both lymphocytes and plasma cells carry the *IGH-BCL2* rearrangement. These cases may be confused with marginal zone lymphoma with plasmacytic differentiation.

Cytologic Features

The diagnosis of FL can be rendered with cytologic specimens such as those obtained with fine needle aspiration. Cytologic specimens are cellular and show a spectrum of cell sizes consistent with the grading. Interestingly, small centrocytes are markedly irregular on tissue sections and can be rather round on cytologic specimens. Some cases have a nuclear indentation that makes nuclear appear as bilobed, but this feature can be found only in rare lymphocytes (Fig. 2.5d). Large cells and follicular dendritic cells that appear as large cells with twin nuclei are also found. Macrophages with tingible bodies can be found in both FL and follicular lymphoid hyperplasia, but are more consistently found in hyperplasia. This finding can further complicate making a definitive diagnosis as both are common in a reactive

component and in the background or microenvironment of FL. This difficulty occurs both in low-grade and in high-grade FL, and it is not unusual to miss the diagnosis of FL when assessment is based entirely on the morphologic features of cytologic specimens.

The concomitant use of flow cytometry immunophenotyping in cytologic specimens is essential and allows assignment of the B-cell lineage and associated FL markers. Flow cytometry also allows demonstration of surface immunoglobulin light-chain restriction favoring FL. Immunohistochemistry on smears or cell blocks can also help to define FL in cytologic specimens. In many instances, the cytologic specimens along with flow cytometry analysis can predict histologic findings and tumor grading accurately. However, discrepancies may occur between histology and cytology diagnoses. We therefore recommend tissue biopsies for initial diagnosis and grading of FL. For follow-up, cytologic specimens may be sufficient depending on the clinical indication for the biopsy; if transformation is suspected, biopsy is recommended, particularly if the diagnosis or grading is discordant with the clinical suspicion.

Grading

The current grading system for follicular lymphoma recommended by the WHO is based on the long-held system of enumerating the absolute number of centroblasts in the neoplastic follicles. The recommendation is to average the number of centroblasts per high-power field (×400 magnification) in ten random neoplastic follicles. The average number of the centroblasts per high-power field has traditionally defined grades 1, 2, and 3. Grade 1 FLs have up to 5 centroblasts per high-power field (Fig. 2.6a); and grade 2 have 6 to 15 centroblasts per high-power field (Fig. 2.6b). Since grade 1 and 2 FLs appear to have the same prognostic significance, the current recommendation is to lump them as "low-grade FL." Grade 3 FL has more than 15 centroblasts per high-power field. Grade 3 FL is divided into grade 3A (when centroblasts are admixed with scattered neoplastic centrocytes) (Fig. 2.6c) and grade 3B (when centroblasts in the follicle lack admixed centrocytes, albeit rare reactive small round lymphocytes, likely T-cells, may be present) (Fig. 2.6d). Grade 3A and 3B FLs are lumped as "high-grade FL" (Table 2.1).

The growth pattern is based on the extent of neoplastic follicles in the pathologic specimen, and it is included when classifying FL (Table 2.2). FL follicular pattern indicates that >75% of the tissue section is occupied by neoplastic follicles (Fig. 2.2a). The recommendation is thus to include the percentage of neoplastic follicles as compared with diffuse areas. It is common that neoplastic cells are interspersed in the interfollicular areas in otherwise FL with entirely follicular pattern, and this is not considered diffuse pattern. An area of diffuse follicular lymphoma is diagnosed if diffuse sheets of neoplastic B-cells are present without a clear-cut follicular growth pattern, irrespective of the size of the area (as little as 10% to more than 90%). Simple broadening of interfollicular areas is not considered sufficient for designation as a diffuse area [28].

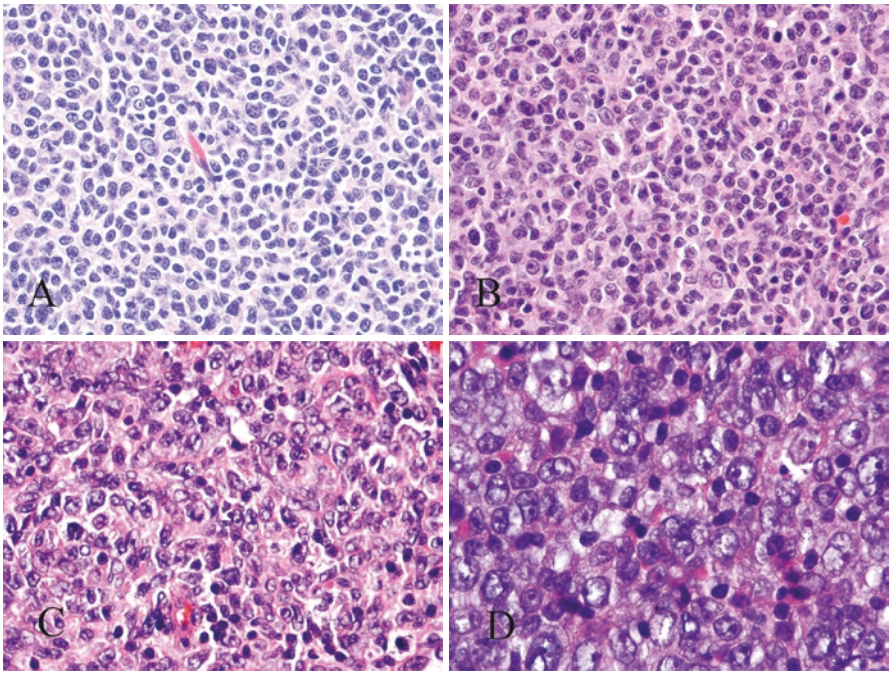

Fig. 2.6 Grading of follicular lymphoma. (**a**) Grade 1 FL is characterized by the predominance of small centrocytes and up to 5 large centroblasts per high-power field. (**b**) Grade 2 FL is characterized by the predominance of small centrocytes and 6–15 large centroblasts per high-power field. (**c**) Grade 3A FL is characterized by the mixture of small centrocytes and >15 large centroblasts per high-power field. (**d**) Grade 3B FL is characterized by the absence of small centrocytes and >15 large centroblasts per high-power field. The small lymphocytes observed in this field are round and likely reactive T-lymphocytes

Table 2.1 Criteria for grading of follicular lymphoma [2]

Grading	Definition
Low grade	0–15 centroblasts per high-power field (×400)
Grade 1	0–5 centroblasts per high-power field
Grade 2	6–15 centroblasts per high-power field
High grade	>15 centroblasts per high-power field (×400)
Grade 3A	Small centrocytes admixed with large centroblasts
Grade 3B	Large centroblasts, the only neoplastic cell component

Reproduced with permission from Jaffe et al. [2], Table 13.17

Table 2.2 Extent of follicular pattern in reporting follicular lymphoma [2]

Pattern	Extent of follicle formation
Follicular	>75%
Follicular and diffuse	25–75%
Predominantly diffuse	1–25%
Diffuse	0%

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This distinction may not be relevant in low-grade FL, but is relevant with grade 3A or 3B FL. The presence of any diffuse area of cytological grade 3A or 3B FL should be reported as diffuse large B-cell lymphoma (DLBCL) [29]. Low-grade FL with an entirely diffuse pattern is rare and may represent "sampling error" in needle or core biopsies or post-therapy cases when sclerosis is extensive; however, true diffuse FL of low grade has been reported and considered as a variant of FL.

Assigning grading and growth pattern is usually not problematic in lymph node excision specimens, but it may be confusing when handling needle or core biopsies and only a limited number of germinal centers are available, as there is more restriction to apply criteria for diffuse pattern. The current system of grading can occasionally be difficult to reproduce, and there are no specific recommendations for cases with predominance of large centrocytes [30] or cases with predominance of small centroblasts in the decision for grading. Furthermore, there is no consideration for the size of the follicle; this is particularly noticeable in cases with small germinal centers, and despite a predominance of large centroblasts, the counts may not reach to >15 centroblasts per high-power field.

Immunophenotype

Follicular lymphoma cells express the pan-B-cell antigens CD19, CD20 (Fig. 2.7a), CD22, CD79a, and PAX5. The centrocytes or centroblasts are negative for pan-T-cell markers CD2, CD3, CD5, and CD7; however, background lymphocytes that constitute most of the microenvironment are highlighted with T-cell markers such as CD3 (Fig. 2.3a). The majority of cases are positive for the germinal center B-cell antigens CD10, BCL6 (Fig. 2.7b), LMO2, GCET1/GCET2, and STMN1. Commonly, the neoplastic cells also downregulate the expression of both CD10 and BCL6 in the interfollicular areas [31]. Flow cytometry immunophenotype allows

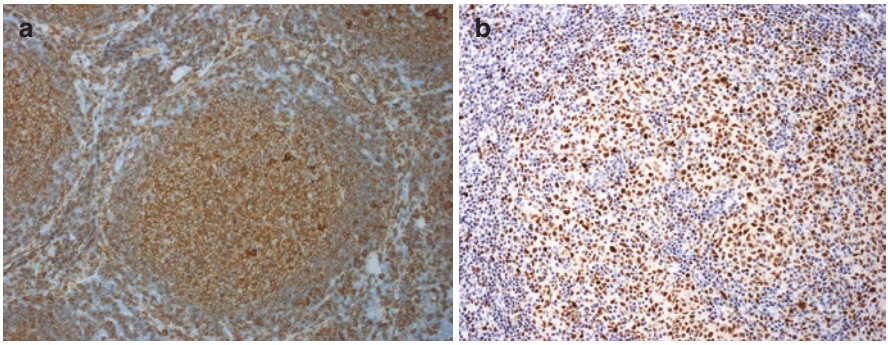

Fig. 2.7 Immunohistochemistry of FL. (**a**) FL consistently expresses the B-cell marker CD20. A large neoplastic follicle is composed predominantly of CD20 positive cells. (**b**) The neoplastic follicles of FL are consistently positive for BCL6

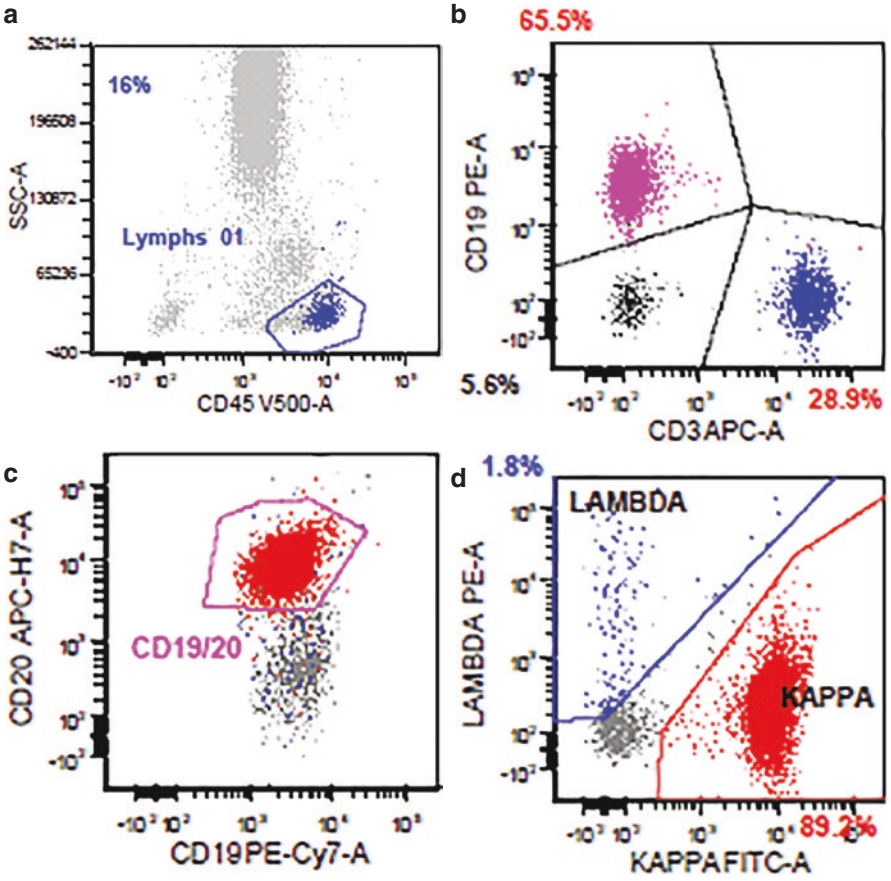

Fig. 2.8 Flow cytometry immunophenotype of FL. (**a**) The strategy to identify abnormal populations of lymphocytes starts with the analysis of the leukocyte marker CD45 (*x*-axis) vs. side scattered (granularity and nuclear complexity, *y*-axis). The lymphocytes are gated (encircled) and in this case represent 16% of all cells in a bone marrow specimen. (**b**) Analysis of the gated lymphocyte population reveals the presence of B-lymphocytes detected with CD19 (y-axis) and T-lymphocytes detected with CD3 (*x*-axis). In this particular case, 65% of lymphocytes are B-cells (CD19+), and 28.9% of lymphocytes are T-cells (CD3+). (**c**) Gating on the CD19+ population reveals that these lymphocytes also express the pan-B-cell marker CD20 (*y*-axis). (**d**). Gating on the CD19+ population reveals that most (89.2%) B-lymphocytes express kappa and few (1.8%) express lambda, with a kappa/lambda ratio = 49.5; this is an excess of kappa+ cells, a surrogate for B-cell clonality, and therefore supports a diagnosis of B-cell lymphoma. The normal/reactive kappa/lambda ratio is 2–3

determining that the lymphoma cells express B-cell markers as well as monotypic surface immunoglobulin light chain (Fig. 2.8); most cases express surface immunoglobulin IgM.

Almost all low-grade (grades 1 and 2) FL cases express the BCL2 protein. BCL2 is expressed less frequently in high-grade FL (grade 3A or 3B). A subset of FL cases

which are negative for the common clone 100/D5 of BCL2 due to mutation of BCL2 can be positive with using the E17 clone [13, 14]. There are certain cases of FL that may overlap morphologically with follicular hyperplasia, and BCL2 expression is helpful to establish a diagnosis, since the germinal centers in follicular hyperplasia lack BCL2 expression (Fig. 2.9a, b); this distinction may be more difficult if the FL case truly lacks BCL2 expression.

Follicular dendritic cells can be preserved, expanded, or partially depleted in the neoplastic follicles of FL; they are visualized with one or more follicular dendritic cell markers CD21 (Fig. 2.3b), CD23, CD35, CXCL13, D2-40, clusterin, or EGFR [1].

The Ki-67 proliferation index generally correlates with the histologic grades in FL. Low-grade FL typically has a low proliferation rate, generally less than 30%, while grade 3A FL or grade 3B FL has a high proliferation rate, generally more than 40%. The proliferation rate of germinal center cells can be used for distinguishing FL from follicular hyperplasia that has an extremely high proliferation rate or sometimes shows a polarization pattern (Fig. 2.9c, d). Although rare, there are occasional

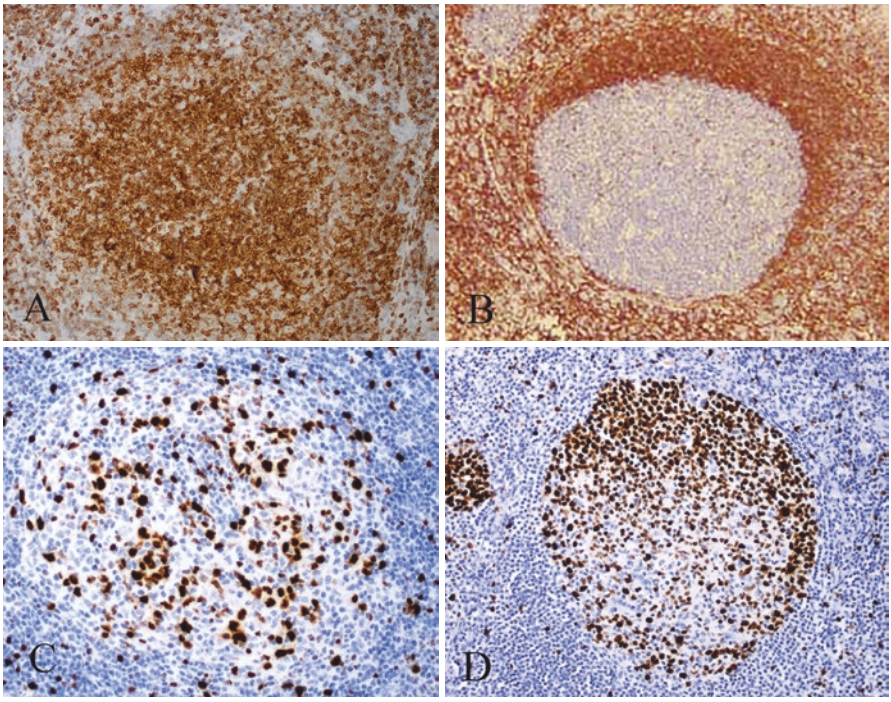

Fig. 2.9 Immunohistochemical markers useful to distinguish between FL and follicular lymphoid hyperplasia. (**a**) BCL2 is strongly expressed in the germinal center cells of FL. (**b**) BCL2 is negative in the germinal center follicular lymphoid hyperplasia. This feature is very useful in clinical practice. Note that interfollicular lymphocytes are positive for BCL2 and likely correspond to T-lymphocytes that normally express BCL2; similarly, reactive mantle zone lymphocytes normally express BCL2. (**c**) The proliferation marker Ki-67 highlights a subset (~30%) of follicular center lymphocytes in this case of FL. (**d**) The proliferation marker Ki-67 highlights almost all follicular center lymphocytes in this case of follicular lymphoid hyperplasia; the reactivity is stronger toward the top of the follicle and faint at the bottom, denoting polarity, which is a feature of reactive follicles

cases of low-grade FL which show high proliferation rate as determined by Ki-67, and it has been proposed that this finding may have more aggressive clinical features [1, 32]. There are also some cases that fulfill morphologic criteria for low-grade FL; however, the cells overlap with small centroblasts, and these cases tend to have higher proliferation rate and behave more aggressively.

Rare cases of FL express CD5 [33] or harbor Epstein-Barr virus in neoplastic cells [34].

Cytogenetics and Molecular Genetics

Follicular lymphoma in its classic form characteristically shows t(14;18) translocation between *BCL2* gene at 18q21 and *IGH* gene at 14q32 [35, 36] (Fig. 2.10). This translocation is seen in almost all the low-grade FL cases, while its frequency drops in higher-grade FL [37].

Other less common cytogenetic abnormalities in follicular lymphomas include translocation of *BCL6* gene at 3q27 [38] and copy number changes or mutations of *TNFRSF14* on chromosome 1p36. Loss of 1p, 6q, 10q, and 17p or gains of chromosomes 6p, 12q, and 18q are much less common [39–41]. In addition to these cytogenetic changes, other recurrent molecular genetic changes observed in FL are shown in Table 2.3.

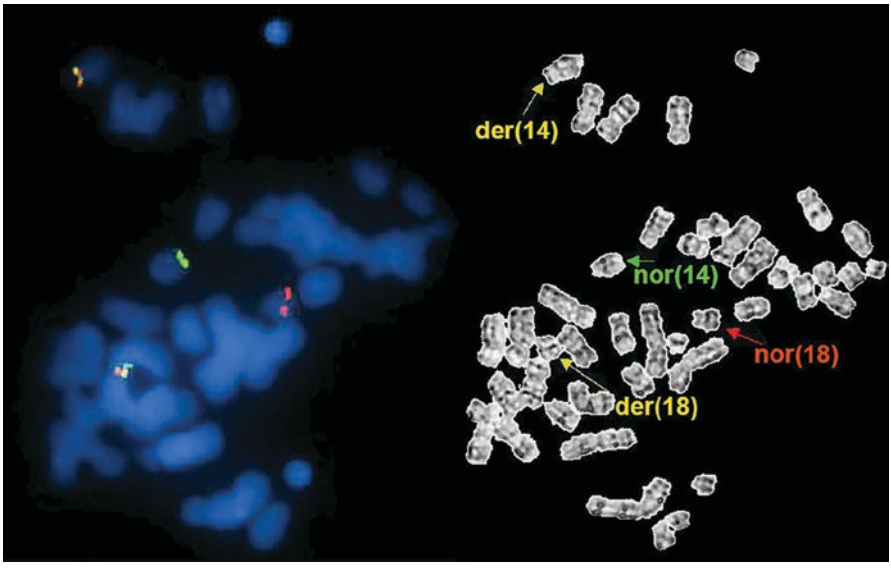

Fig. 2.10 Fluorescence in situ hybridization (FISH) analysis with *IGH* (green)/*BCL2* (red) dual-color dual-fusion rearrangement probes on a G-banded metaphase shows two fusion (yellow) signals on derivative chromosomes 14 and 18, respectively, indicating *IGH-BCL2* rearrangement. (Courtesy: Dr. Guilin Tang)

Table 2.3 Genetic abnormalities in follicular lymphoma [2]

Gene	Name	Chromosome	Function	Abnormality	Percentage in FL
BCL2	B-cell lymphoma 2	18q21	Apoptosis regulator	Translocation, mutation	85
KMT2D	Lysine methyltransferase 2D	12q31	Histone methyltransferase	Mutation	85
TNFRSF14	TNF receptor 14	1p36	Signal transduction	Mutation, deletion	45
EZH2	Enhancer of zeste 2 polycomb repressive complex 2	7q36	Transcription co-repressor	Mutation	60
EPHA7	Eph receptor A7	4	NA	Mutation	70
CREBBP	CREB binding protein	16p13	Coactivation of transcription factors	Mutation, deletion	33
BCL6	B-cell lymphoma 6	3q27	Transcription co-repressor	Mutation, translocation	45
MEF2B	Myocyte enhancer factor	19p13	Gene expression regulator	Mutation	15
EP300	E1A binding protein 300	22q13	Histone acetyltransferase	Mutation, deletion	10
TNFAIP3	TNF-alpha-induced protein 3	6q23	Inhibits NF-κB	Mutation, deletion	20
FAS	TNF receptor	10q24	Promotes apoptosis (DISC)	Mutation	5
TP53	Tumor protein 53	17p13	Tumor suppressor protein	Mutation, deletion	<5
MYC	Myc proto-oncogene	8q24	Oncogene	Translocation, gain	<5

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The immunoglobulin heavy and light chains are rearranged in FL cells and show intraclonal variation of the variable regions, known as somatic hypermutations which indicate ongoing mutations in the germinal center cells [42].

Variants of Follicular Lymphoma

As previously mentioned, FL can have a variety of presentations and can occur at multiple sites. Although the diagnosis of FL can be established following outlined criteria, some subtypes may differ in clinical behavior, outcomes following therapy, and frequency of *IGH-BCL2* translocation.

In Situ Follicular Neoplasia

Follicular lymphoma in situ refers to an acquired single neoplastic follicle replacing the germinal center of an otherwise hyperplastic lymphoid follicle, in the context of a hyperplastic lymph node. The lymph node architecture is preserved, and at first sight the neoplastic follicle is usually imperceptible and difficult to distinguish from a normal germinal center (Fig. 2.11a). Identification of the affected follicle is defined by the identification of a uniform population of neoplastic small centrocytes [1, 14]. The suspicion can be further confirmed by immunohistochemistry with a germinal center marker such as CD10 (Fig. 2.11b). BCL2 immunohistochemistry shows strong reactivity in the involved germinal center in contrasts with the surrounding hyperplastic follicles that display germinal centers that are negative with anti-BCL2 (Fig. 2.11c). The neoplastic follicles of in situ follicular neoplasia are composed almost purely of small centrocytes [43]. This finding of in situ FL is rare and commonly comes unexpectedly; therefore, some pathologists perform Bcl-2 on many cases with follicular lymphoid hyperplasia, with the fear of missing in situ follicular neoplasia, a response that may need assessment and validation.

In contrast with in situ follicular neoplasia, follicular lymphoma with partial involvement of the lymph node is characterized by follicular hyperplasia with partial

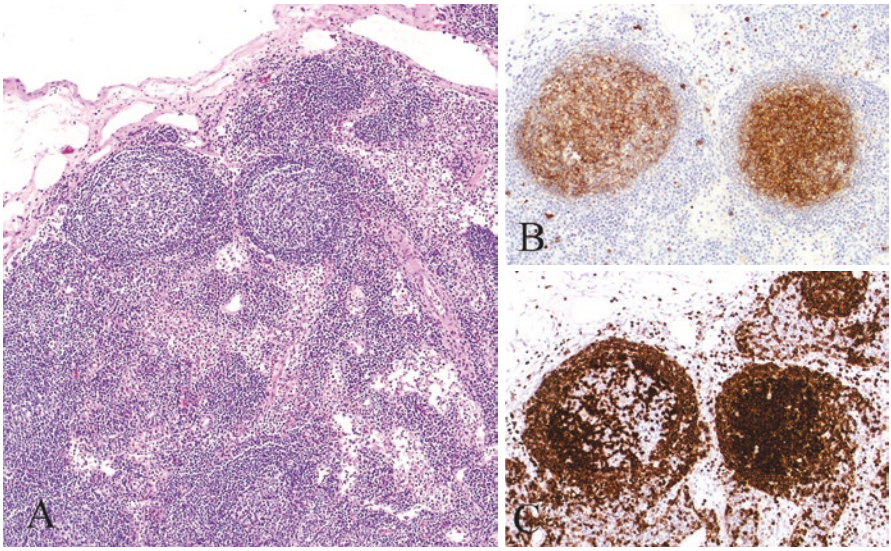

Fig. 2.11 Follicular lymphoma in situ. (**a**) Lymph node with reactive features shows widely spaced lymphoid follicles, open sinuses, and thin capsule. (**b**) Immunohistochemistry for CD10 highlights the germinal centers of two lymphoid follicles; note that the follicle on the right has brighter expression of CD10. (**c**) Immunohistochemistry for BCL2 is negative on the follicle at the left, consistent with reactive follicle, while it is strongly positive on the follicle at the right, supporting FL. FL in situ denotes the presence of an isolated neoplastic follicle amidst a hyperplastic lymph node

effacement of the lymph node architecture, i.e., some follicles are clearly hyperplastic, while other follicles appear clearly neoplastic, although subtle changes can occur. The morphologic distinction between reactive follicles and neoplastic follicles is based on multiple parameters. The presence of compact follicles, with uniform centrocytes or centroblasts, with few or no mitoses, surrounded by faint or poorly defined mantle zones, favors FL.

Duodenal-type follicular lymphoma often occurs at a limited stage and is mainly found in the second portion of the duodenum. Multiple series suggest that the entity has a low risk of distant organ involvement [44, 45]. Histologically, the neoplastic follicles are confined to the mucosa that displays expanded villi with non-confluent lymphoid follicles with distinctive germinal centers (Fig. 2.12a); the germinal centers are composed predominantly of small centrocytes. Immunophenotypically and genetically, it mimics other typical nodal or extranodal FLs. The neoplastic cells are brightly positive for CD10 and BCL2. In almost all cases, the *IGH-BCL2* can be demonstrated by FISH or by PCR, while other genetic abnormalities are found at

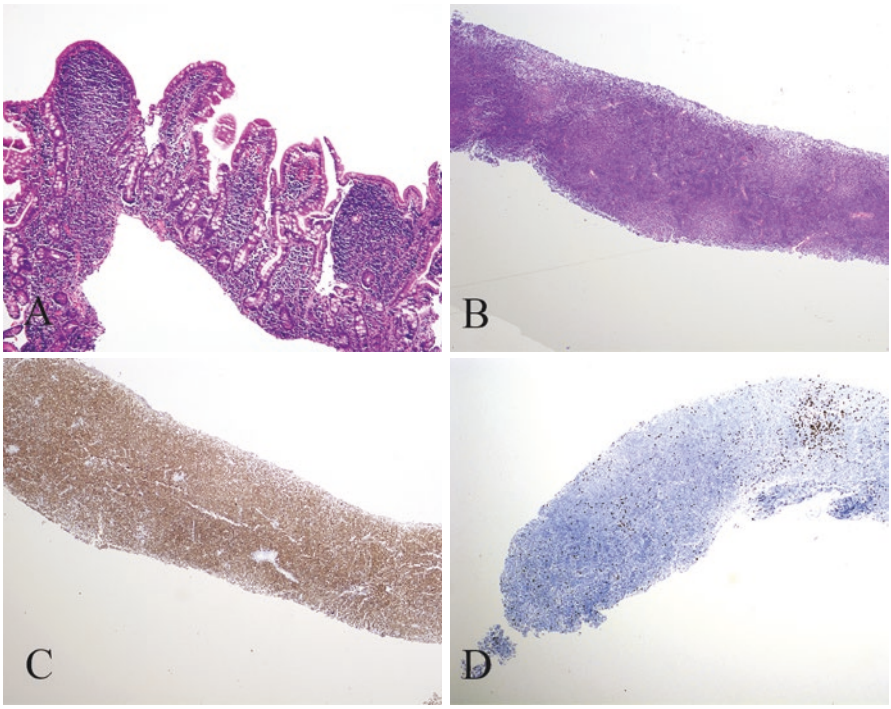

Fig. 2.12 Follicular lymphoma variants. (**a**) Duodenal-type follicular lymphoma is characterized by scattered large neoplastic follicles expanding individual villi of the duodenal mucosa. (**b**) Diffuse FL is illustrated in this field; there is diffuse growth of lymphocytes. (**c**) Immunohistochemistry with the B-cell marker PAX5 highlights uniformly this biopsy specimen involved by diffuse FL. (**d**) Immunohistochemistry for the proliferation marker Ki-67 shows that only rare lymphocytes are highlighted, consistent with a low-grade lymphoma. Focal vague nodularity is also observed toward the right and still consistent with diffuse

lower frequencies [46]. Some studies suggest similarities between gene expression patterns of this type of FL with that of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) [1, 14, 47].

Pediatric-Type Follicular Lymphoma (PTFL)

Pediatric-type FL is predominantly seen in children and young adults. The disease has a marked predilection for males at the head and neck region [21, 48]. Pathologically, almost all cases resemble a high-grade nodal FL with large, expansive, and serpiginous neoplastic follicles which are primarily composed of large centroblasts. The diagnosis is excluded if diffuse areas/DLBCL is detected [14]. Immunophenotypically, the neoplastic cells express CD10, BCL6, and often MUM1 but characteristically are negative or faintly positive for BCL2 protein. Generally, PTFLs are almost always low stage and do not harbor rearrangement involving *BCL2*, *BCL6*, or *IRF4/MUM1* [49]. Mutations of *TNFRSF14* and *MAP2K1* are common [1, 14, 50, 51].

Testicular Follicular Lymphoma

Primary FL of the testis is rare and more common in children. Almost in all instances, these lymphomas are limited stage and limited to the testis and epididymis. Typically, they lack *BCL2* rearrangement and BCL2 protein expression. Morphologically, a monotonous population of medium- to large-size centroblasts is observed. Outcomes in most patients are good with a high cure rate following surgical resection [1, 52, 53].

Diffuse Follicular Lymphoma with del1p36/TNFRSF14

Diffuse follicular lymphoma with del1p36/*TNFRSF14* has an entirely diffuse pattern. The diagnosis requires an excisional rather than a needle biopsy since diffuse areas in a needle biopsy may only reflect focal diffuse areas in an otherwise typical follicular lymphoma. Only few series of diffuse FL have been reported, but it appears that at least in a subset of cases, there is deletion or mutation of *TNFRSF14* gene located in chromosome 1p36 [54]. These neoplasms tend to occur in younger patients and are more common in the inguinal region [55]. Morphologically, there is a diffuse growth pattern (Fig. 2.12b) of lymphocytes that express B-cell markers such as CD20 or PAX5 (Fig. 2.12c) and low proliferation rate (Fig. 2.12d). Small follicles called microfollicles can exist in the background [21]. The neoplastic cells are positive for CD10 and CD23 and at least partially for BCL2, mostly in the diffuse areas. Most of the cases reported in the literature are negative for t(14;18), and a subset carry mutations of *STAT6* [54, 56].

Large B-Cell Lymphoma (Follicular Lymphoma) with IRF4/MUM1 *Rearrangement*

Large B-cell lymphoma (follicular lymphoma) with *IRF4/MUM1* rearrangement has similarities to pediatric-type FL; however, it includes cases with diffuse large cell component [57]. The lymphoma is more common in children and young adults and commonly involves Waldeyer's ring and lymph nodes in the head and neck. Morphologically, it has a predominant population of large neoplastic centroblasts. Although most instances show a diffuse growth pattern, at least partial or total follicular pattern of growth is also observed. Unlike pediatric-type FL, the entity lacks starry-sky pattern or a serpiginous configuration. BCL2 is expressed in approximately two-thirds of cases; however, these cases do not harbor the *IGH-BCL2* rearrangement. Unlike typical FL, expression of IRF4/MUM1 nuclear protein is very consistent [14, 58, 59]. This lymphoma carries a translocation between *IRF4* gene at 6p25.6 and *IGH* gene at 14q32. Unlike pediatric-type FL, the entity is diagnosed almost equally in males and females; commonly, they express BCL2 protein. Morphologically, also they rarely show the florid serpiginous pattern of growth or starry-sky pattern commonly seen in the PTFL. These lymphomas are often diagnosed at limited stage and tend to have a favorable prognosis [57, 60].

The Microenvironment of Follicular Lymphoma

Although centrocytes and centroblasts define FL, there is a significant number of immune cells and stromal cells intimately associated that define the microenvironment in FL [60]. Examples of benign effector cells include T-cells (Fig. 2.3a), histiocytes and macrophages, follicular dendritic cells, and stromal cells. The T-cells include CD4+, CD8+, as well as subsets of immunoregulatory CD4+ cells. The number and distribution of cells in the microenvironment of FL have been analyzed on their influence on diagnosis and prognosis. Published gene expression profiling of non-tumor cells in FL suggests that two main populations with prognostic significance exist in FL, one with a predominance of T-lymphocytes with a better outcome and another with monocytes and dendritic cells with a worst outcome [15]. However, the complex interactions of the microenvironment and their prognostic impact are not easy to replicate or assimilate, and reporting the microenvironment is not included routinely in the diagnosis of FL [61].

Transformation of Follicular Lymphoma

Approximately 20–25% of FL cases transform to a high-grade B-cell lymphoma. In most instances, this transformation is to diffuse large B-cell lymphoma (DLBCL)

with centroblastic morphology and in most cases shows the same neoplastic clone as the follicular lymphoma. Less frequently, other types of DLBCL occur, and some cases transform to high-grade B-cell lymphoma with *MYC* translocation and more rarely to lymphoblastic lymphoma [62, 63]. The outcome of transformed FL is heterogeneous with conventional chemotherapy [64]. One of the genes that has been implicated in transformation of follicular lymphoma to a more aggressive neoplasm (e.g., DLBCL) is *TP53* [65]. Although the presence of any type of abnormality in *TP53* gene is much more rare in diagnostic samples (<5% of all FLs), the transformed follicular lymphomas are particularly enriched (25–30%) for these mutations [64, 66]. These mutations presumably lead to inactivation of p53 tumor suppressor gene. The presence of *TP53* mutations is correlated with shortened disease-free and overall survival in follicular lymphoma regardless of transformation [67]. Studies [68] also showed significant prognostic value of expression of p53 protein in follicular lymphoma [69, 70]. This is not surprising as there is usually a positive correlation between *TP53* mutation and p53 expression [71]. MYC gene translocation is rare in FL but may occur more frequently during transformation [64, 68]. This molecular change leads to activation of a putative proto-oncogene (c-Myc); it is usually an indication of an aggressive behavior and a need for more intensive standard treatment [72]. Although the fourth revised edition of the WHO monograph has not included FL with *MYC* translocation under the newly introduced "high-grade B-cell lymphoma with *MYC* and *BCL2/BCL6* translocation," our understanding is that these tumors are as aggressive as diffuse large B-cell lymphoma harboring these two translocations (so-called double-hit lymphoma) [14, 72]. A large proportion of DLBCL with MYC gene rearrangement has history of FL [73]. At the protein level, c-Myc expression is rare in low-grade FL, while its expression proportionally increases with increase in the grade [74]. There is no unequivocal association between MYC protein expression and the aggressiveness of the tumor although studies in this regard are limited [70]; however, the presence of high Myc protein expression is predictive of *MYC* gene breakpoint in most instances [74].

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Chapter 3
Genomic Drivers in Follicular Lymphoma

Saber Tadros and Michael R. Green

Overview

The future of follicular lymphoma (FL) diagnosis and treatment lies in personalized medicine. Patient-tailored management should target the unique pathways underlying lymphomagenesis. Undoubtedly, the recent exponential increase in our knowledge of the cancer genome in lymphoma will enable clinicians to rationally target tumors and raise the potentials for a cure. Since the microenvironment is central to disease etiology and affects responses to therapy, the advances in identifying non-malignant immune cells infiltrating the tumors and characterizing their role open new horizons for development of targeted therapy.

The most frequent genetic event in FL is the t(14;18)(q32;q21) translocation that places *BCL2* under control of the immunoglobulin heavy-chain enhancer. Although it is identified in 90% of FL patients [1], it is also observed in some healthy individuals [2]. Certainly, those individuals with translocation-positive cells showed increased risk of FL development, but the majority of them never develop FL [3]. In mice with a *Bcl-2*-Ig transgene, mature B-cells have a proliferative advantage, but this does not induce a high penetrance of lymphoma [4, 5]. These data show that additional genetic alterations are required, in addition to *BCL2* translocation, to induce FL. The acquisition of secondary mutations occurs in a serial fashion, with "early drivers" and "late drivers" cooperating to promote lymphomagenesis (Fig. 3.1). Here we will discuss the genetic landscape of FL and provide some insight into the mechanism by which mutations cause disease genesis and progression.

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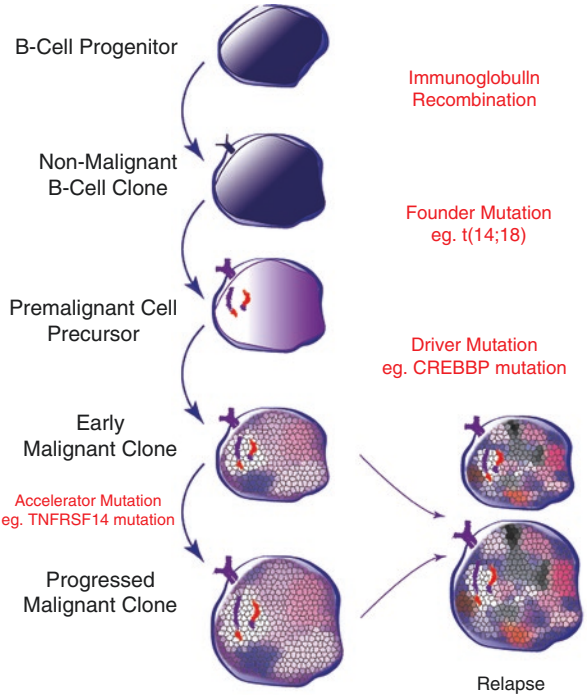

Fig. 3.1 FL genetic evolution**.** A diagram represents a suggested model of FL development. A B-cell clone is selected after immunoglobulin recombination, followed by acquisition of the t(14;18)(q32;q21) founder *IGH-BCL2* translocation yielding a premalignant tumor cell precursor. This precursor acquires one or more "driver" mutations, such as in *CREBBP*, yielding an early malignant clone. Later, "accelerator" mutations are acquired, such as *TNFRSF14*, resulting in a progressed malignant clone. Relapses may originate from either an early malignant clone or a progressed malignant clone

Chromatin Modifying Gene (CMG) Mutations

Histones and their posttranslational modifications are critically important epigenetic mechanisms controlling gene expression. Histone modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation can mediate their regulatory effects either directly by modifying the physical properties of the chromatin or indirectly by recruiting "reader" proteins. FL acquires mutations in genes with a role in catalyzing the posttranslational modifications of histones and controlling higher-order chromatin structure. Since Morin et al. showed that FL exhibits a significant enrichment for mutations in chromatin modifying genes (CMGs), the recurrent mutation of these genes has emerged as a hallmark of FL [6] (Fig. 3.2).

KMT2D/KMT2C

The *KMT2D* gene (aka *MLL2* or *MLL4*) encodes a SET domain-containing lysine methyltransferase that is part of the lysine methyltransferase 2 (KMT2, aka the myeloid/lymphoid or mixed-lineage leukemia (MLL)) family. *KMT2D* and its family member, *KMT2C*, were first found to be in nuclear receptor coactivator

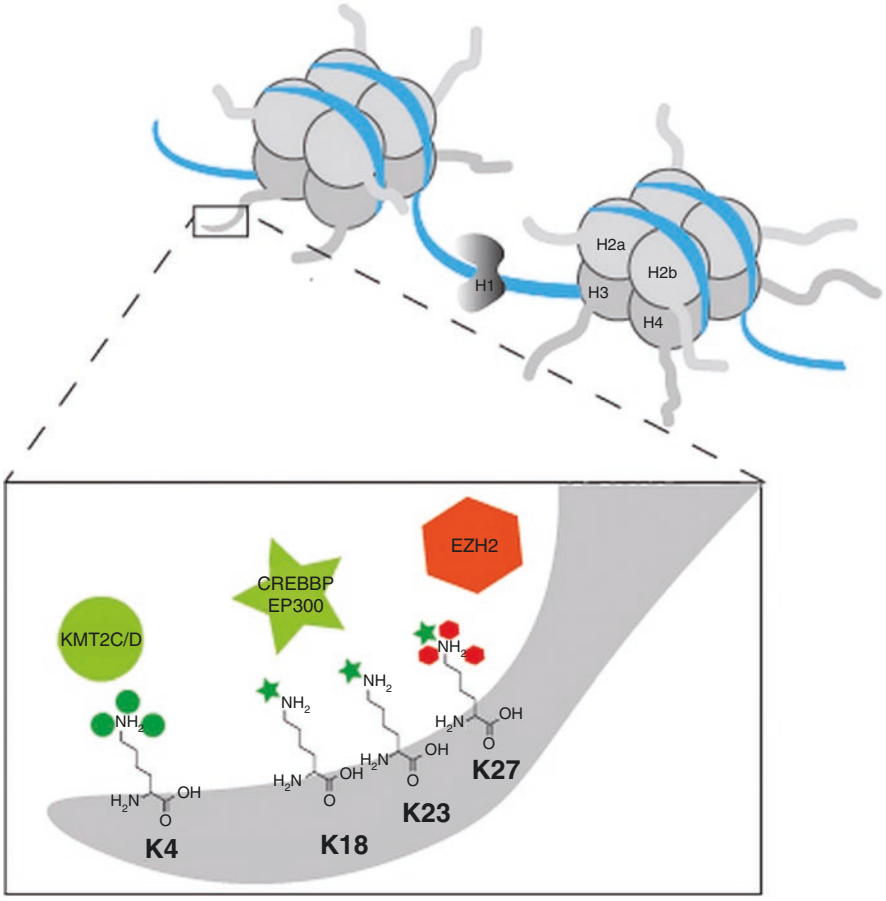

Fig. 3.2 Chromatin modifying complex. Function of chromatin modifying genes that are mutated in follicular lymphomas. A diagrammatic representation shows the nucleosome structure that is composed of DNA wrapped around histone octamers, consisting of histones H2a, H2b, H3, and H4. Linker DNA between nucleosomes is bound by histone H1. A magnified schematic of the tail of histone H3 shows the addition of activating H3K4me3 (green circles) by KMT2C/KMT2D with subsequent acetylating (green stars) to multiple residues on the H3 tail by recruitment of the CREBBP/EP300 complex that has an activation role. Activating H3K4me3 and acetylation marks antagonizes the inhibitory H3K27me3 effect (red polygon) that is written by the PRC2 complex that includes EZH2

complexes initially termed the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation. They catalyze H3K4 monomethylation at enhancer elements independently or as part of multi-protein COMPASS-like complexes that are associated with development-specific genes and principally contribute to mono- and dimethylation of H3K4 [7, 8]. The sites of H3K4me1 enrichment are known as enhancers, and the enrichment of H3K4me1 in the absence of H3K4me3 is now considered a defining

characteristic of active enhancers. It was thought because of the homology between *KMT2C* and *KMT2D* that they may function redundantly. Although knockout studies suggested partial genetic redundancy, some data showed that they bind to a unique set of target genes [9, 10].

The *KMT2D* gene is the most recurrently mutated gene in FL (~72% of cases) and is also recurrently mutated in a lower frequency of DLBCL (~24% of cases). Mutations are predominantly represented by premature stop codons, frameshift insertions or deletions, and splice-site mutations that result in truncated proteins that are functionally defective due to the loss of the catalytic SET domain and reduced methyltransferase activity or complete loss of protein expression [11]. Murine models of *Kmt2d* loss showed no obvious changes in normal B-cell development; but it provides proliferative advantage for GC B-cells and leads to reduced levels of H3K4me1, H3K4me2, and H3K4me3. Additionally, it cooperates with *Bcl2* deregulation to facilitate the development of GC B-cell malignancies [11, 12].

Mutations of *KMT2C* are detected in 13% of FLs. Notably, mutations of *KMT2C* and *KMT2D* are not mutually exclusive suggesting nonredundancy in their functions in FL. Interestingly, a recent study [13] presented evidence that both KMT2C and KMT2D function as long-range coactivators independent of their catalytic activity. It is therefore unclear whether missense mutations that reduce KMT2C/KMT2D methyltransferase activity, but maintain the protein expression, would have the same functional consequence as nonsense or frameshift mutations that result in a loss of protein.

EZH2

Mutations of *EZH2* were the first recurrent CMG mutation to be reported in FL [14]. The majority of these mutations are missense mutations of tyrosine 641 (Y641) within the SET domain of *EZH2*, which result in hypermorphic activity. Compared to the wild-type (WT) protein, mutant *EZH2* has reduced activity for H3 monomethylation, but increased activity for the rate-limiting trimethylation step [15, 16]. In patient tumors, the mutant allele is always found associated with a wild-type allele (heterozygous). It is believed that there are coordinated activities of H3K27 monomethylating enzyme (WT *EZH2*) and the mutant *EZH2* for augmented conversion of H3K27 to the trimethylated form.

The enhancer of zeste homolog 2 (*EZH2)* gene encodes a lysine methyltransferase enzyme that catalyzes trimethylation of H3K27 (H3K27me3) as part of the polycomb repressor 2 (PRC2) complex. PRC2 complex has three other protein components, namely, EED, SUZ12, and RpAp46/RpAp48. *EZH2* is the catalytic subunit of this complex [17]. The PRC2 complex contributes to a chromatin modification pattern that is highly enriched in embryonic stem cells, consisting of large regions of "repressive" Lys27 methylation harboring smaller regions of "activating" Lys4 methylation, referred to as "bivalent domains" [18]. *EZH2* and other components of the PRC2 complex are highly expressed in GC B-cells [19–21] and contribute to setting groups of genes into a bivalent state in this context also.

Conditional deletion of *Ezh2* within this compartment resulted in a significant reduction in the number of splenic GC B-cells, accompanied by impaired immunoglobulin affinity maturation with reduction in formation of high-affinity antibodies [22]. Within early GC B-cells, the silencing of these genes by *EZH2* and *BCL6* is essential to maintain the GC phenotype [23]. These genes which are highly expressed in naïve B-cells include those involved in terminal differentiation such as *PRDM1*, *IRF4*, and *XBP1* and negative regulators of the cell cycle such as *CDKN1A* and *CDKN1B* [22, 24]. A recent study showed that *EZH2*-mediated silencing of cyclin-dependent kinase inhibitor *CDKN1A*, and release of cell cycle checkpoints, is particularly important for GC formation [25]. Murine models of *Ezh2* Y461 have presented strong evidence for its role in promoting B-cell lymphoma. However, clonal evolution studies characterized these mutations as being predominantly subclonal events at diagnosis and showed that they remain subclonal at the relapse [26–28]. This therefore suggests that EZH2 mutations are "late drivers" or "accelerators" of lymphomagenesis.

CREBBP

The *CREBBP* gene (aka *CBP*) encodes a protein that has intrinsic histone acetyltransferase activity and acetylates both histone and non-histone proteins. Histone acetylation alters their charge and loosens their association with DNA to make it more accessible to transcription factors. The acetyl marks added by *CREBBP* are also recognized by "readers," such as bromodomain-containing proteins, that facilitate promoter-enhancer looping and transcriptional activation [29].

Mutations of *CREBBP* occur in 65% of FLs and 11% of DLBCLs [30]. Approximately 80% of the mutations observed in FL create missense changes in the KAT domain, with 26% of all mutations altering a single KAT domain amino acid (R1446) [26, 31, 32]. Analysis of the hierarchy somatic mutations arising during tumor evolution identified *CREBBP* mutations as early events during FL disease genesis [26, 27]. Interestingly, a recent study detected *CREBBP* mutation in the HSPC compartment in one patient whose lymphoma carried the same mutation [33], though this requires validation. Transcriptome and flow cytometry analysis of primary human FL showed significantly reduced expression of MHC class II in *CREBBP* mutant tumors compared to WT tumors [26]. This highlighted a potential role for *CREBBP* mutations in promoting immune evasion via reduced antigen presentation.

Transgenic murine models have shown that loss of *Crebbp* induces lymphomagenesis, especially in combination with *Bcl2* overexpression. This occurred in part via reduced histone acetylation at distal enhancer regions and reduced MHC class II expression. However, change in MHC class II was not robust as in primary human FLs [32, 34–36]. In addition, there was overlap between the regions of reduced H3K27Ac associated with *Crebbp* loss and the enriched loci that are bound by BCL6. Considering that CREBBP is a positive regulator of transcription and BCL6 is a transcription repressor, those observations suggested that

CREBBP has a counterbalance effect to BCL6 and its loss facilitates oncogenic gene repression by BCL6.

In addition to changes in histone acetylation, mutation of *CREBBP* may also promote lymphoma via reduced acetylation of non-histone targets such as TP53 and BCL6. The role of CREBBP in acetylation of these proteins was tested in lymphoma cell line models of *CREBBP* mutation [31] and was recently expanded upon using a *Crebbp* knockout mouse model [33]. Mutation of *CREBBP* decreases p53 acetylation in response to DNA damage and reduces DNA damage response. Recently, it was also shown that loss of *CREBBP* was associated with induction of *MYC* expression, but the mechanism for this remains to be defined [32].

HIST1H1

The linker histone, H1, interacts with linker DNA between nucleosomes and functions in the compaction of chromatin into higher-order structures. Mutations in linker histone H1 B, C, D, and E genes were detected in 27% of a cohort of 114 FLs [37]. The majority of *HIST1H1 B–E* mutations were missense changes in the C-terminal portion of these proteins, which were found to reduce their interaction with DNMT3B [37]. Although the function of these mutations needs to be addressed in future studies, they are likely to have significant cross talk with mutations in genes that control histone posttranslational modification. Recently, a study showed that modifications such as acetylation by CREBBP of linker histone weaken H3 tail-linker DNA interactions and control chromatin function [38], and alternative findings suggest that linker histone H1 determines the substrates for EZH2 [39].

Signaling Mutations

Surface Immunoglobulin (sIg)

Expression of sIg, consisting of immunoglobulin heavy (IgH) chains paired with light (IgL) chains, is a critical checkpoint in B-cell development. All classes of immunoglobulin heavy chains (IgM, IgD, IgG, IgA, and IgE) can be expressed on the B-cell surface [40] as part of the B-cell receptor (BCR) complex that comprises sIg in assembly with the Igα/Igβ heterodimer that is responsible for intracellular signal transduction [41]. Interestingly, most cases of FL express IgM; and only a minority expresses IgG, IgA, or no IgH [42]. Immunoglobulins are glycosylated, and this posttranslational modification plays an important structural role in modulating sIg function. In normal B-cells, N-glycosylation is mainly confined to conserved sites in the Ig constant regions, and a few germline-encoded V regions do carry potential N-glycosylation sites. In contrast, the number of potential sIg

glycosylation sites increases dramatically in FL via the process of somatic hypermutation (SHM). Somatic hypermutation (SHM) is part of the antibody affinity maturation process in the GC and involves introducing point mutations in the variable region of rearranged immunoglobulin heavy- and light-chain genes. Importantly, somatic hypermutation is a prominent feature of follicular lymphoma B-cells due to their derivation from GC B-cells.

Zhu et al. investigated the frequency of potential N-glycosylation sites introduced into functional V(H) genes as a consequence of somatic mutation in FL. In a cohort of 70 FL patients, 50 patients had at least one new potential N-glycosylation site in the V-region sequence [43]. These sites occur with a low frequency (8%) in normal cells. Interestingly, there was also a tendency in all studied tumors to lose the naturally occurring N-glycosylation sites that exit with $V_{1\text{-}08}$, $V_{4\text{-}34}$, and V_H5a germline gene sequences. Their finding that motifs are not common in normal somatically mutated B-cells or in nonfunctional VH sequences strongly suggested that the sites are positively selected for in FL. They also suggested that the pattern of N-glycosylation of immunoglobulins in DLBCL biopsies obtained from the lymph node (LN) but not from extranodal sites is perhaps indicative of a role for cells retained in the GC. Furthermore, they argued that the relative lack of motifs in normal memory B-cells, normal plasma cells, MM, and CLL indicates that cells that have exited from the GC do not require N-glycosylation. They posited that there may be a role for carbohydrates in the antigen-binding site as potential site of interaction with lectins in the GC microenvironment, which could activate signaling through the surface immunoglobulin. The signaling by oligosaccharide interactions to tumor microenvironment may free FL cells from dependence on antigen and may contribute to tumor cell persistence or growth.

In a subsequent study, sequencing the tumor-specific immunoglobulin heavy-chain variable region fragment showed that acquired potential N-glycosylation sites (AGS) were found in all 24 (100%) FL cases, but in only 2/23 (9%) cases of B-cell malignancy other than FL [44]. Based on this observation, they argued about considering the presence of AGS as an element capable of defining cases of FL. No subsequent larger cohort has proved this preliminary findings, and no structural studies have been performed to assess whether these sites are actually glycosylated in tumor cells or are merely potential sites for glycosylation.

Further work on this observation demonstrated that oligosaccharides added to Ig in FL are unusually of the high mannose type. Protein mannosylation is a vital type of glycosylation that could be categorized based on the type of glycoprotein linkages to O-mannose glycans and C-mannose glycans [45]. These oligomannose sugars bind to mannose-binding lectin (MBL), a member of the collectin family of proteins that interact with microorganisms and elicit an innate immune response [46]. Using biotinylation and glycosylation analysis of cell surface proteins, mannosylated IgM at the surface of primary lymphoma cells of seven randomly selected cases of FL was identified [47]. Notably, two major C-type lectins with specificity for high-mannose structures are the mannose receptor (MR) and DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing non-integrin) expressed by cells of innate immunity, including macrophages and dendritic cells. This study

highlighted a difference between the effect of C-type lectins binding to FL cells and to normal B-cells. Impressively, C-type lectins mediate intracellular signaling in FL and not in the normal cells.

Two recent studies explained a role of lectin binding to surface Ig in FL as a mechanism for antigen-independent B-cell receptor signaling [48, 49]. They displayed that activating intracellular phosphorylation pathways in FL cells occurs when they encounter DC-SIGN-expressing macrophages as they transit through the tissue or the DC-SIGN on lymphatic endothelium. Interestingly, N-glycans were found to severely impair BCR specificity and affinity to the initial cognate antigen, but activating it by lectins from *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* stimulates FL cells [50].

mTOR

mTOR (mechanistic (formerly "mammalian") target of rapamycin) is a gene belonging to a family of phosphatidylinositol kinase-related kinases that plays a critical role in coordinating cell cycle progression in response to stresses such as DNA damage and nutrient deprivation. The serine/threonine protein kinase *mTOR* forms the catalytic subunit of two distinct protein complexes termed mTORC1 and mTORC2. Importantly, pathways upstream and downstream of *mTORC1* are dysregulated in most human cancers [51]. Many of these pathways have been elucidated, and amino acids were revealed as a regulator of mTORC1 through vacuolar ATPase (V-ATPase) that is required for lysosomal acidification [52]. Two genes, *ATP6V1B2* and *ATP6AP1*, involved in that process were found mutated in 22% and 12% of FL cases, respectively. Green et al. performed whole-exome sequencing of purified tumor B-cells and matched germline DNA from tumor-infiltrating T-cells from 28 FL tumors. Among many discovered novel genes, they identified those two mutated genes. *ATP6V1B2* mutations were found to lie in two hotspots close to the ATP-binding pocket [26].

Another frequently mutated gene involved in *mTOR* pathway is *RRAGC* which encodes a member of the GTR/RAG GTP-binding protein family. It forms a heterodimer with *RRAGA* and *RRAGB*. This complex promotes intracellular localization of the *mTOR* complex [53]. Exome sequencing on 24 FL tumors from five patients showed somatic mutations in *RRAGC* in four of the five cases, and the clustering of mutations resides in the nucleotide-binding domain [54]. Although certain mutations in different contexts were described to decrease S6K, one of the downstream kinases of mTOR, no functional studies were done to investigate these mutations in FL [53]. Another study of exome sequencing of 23 FLs identified two *RRAGC* mutations, and further Sanger sequencing of all *RRAGC* coding exons in 125 FL cases identified a total of 9.4% mutations which are clustering on one protein surface area surrounding the GTP/GDP-binding sites [55]. Interestingly, mutations in *ATP6V1B2* and *ATP6AP1* were described to be preferentially co-occurred with *RRAGC* mutations [54]. Moreover, mutations in *RRAGC*

represent a unique feature for FL in contrast to the mutations in the pathways JAK-STAT, NOTCH, and nuclear factor (NF)-κB which exhibit an overlap between DLBCL and FL.

Others

TNFRSF14 (HVEM)

This gene encodes a member of the TNF (tumor necrosis factor) receptor superfamily that functions in signal transduction pathways that activate inflammatory and inhibitory T-cell immune response. *TNFRSF14* has diverse functions and interacts with multiple ligands, mainly LIGHT, lymphotoxin-α, BTLA, and CD160, a feature that sets it apart from other immune regulatory molecules [56]. Evidently, many sequencing studies have characterized *TNFRSF14* mutations as a frequent event in FL. It was first described as a maker associated with worse prognosis in FL. An expanded cohort consisting of 251 specimens of FL identified 46 cases (18.3%) with nonsynonymous mutations affecting *TNFRSF14* [57]. A subsequent study using QMPSF (quantitative multiplex PCR of short fluorescent fragments) assay of 81 FL tumors showed partial or complete deletion of *TNFRSF14* in 30% of cases, and further sequencing of the eight exons and the exon-intron boundaries identified nonsynonymous mutations in 44% of the cases [58]. Later, whole-exome sequencing of subpopulations of B-cells and tumor-infiltrating T-cells from eight FLs defined mutations in *TNFRSF14* in 25% of cases [27]. Mutations in *TNFRSF14* might contribute to FL development [58], although they probably represent late events during disease evolution [27].

Considering the role of *TNFRSF14* in T-cell biology, a recent study hypothesized that reduced HVEM expression would stimulate allogeneic T-cells inducing graft-vs.-host disease (GVHD) in patients undergoing autologous hematopoietic stem cell transplantation. They found that FL B-cells with *TNFRSF14* aberrations had reduced protein expression and greater alloantigen-presenting capacity than wild-type lymphoma B-cells. The increased immune stimulatory capacity of lymphoma B-cells with *TNFRSF14* aberrations was associated with higher incidence of acute GVHD [59]. Of central concern therefore is to understand how a mutation with such high incidence in FL could be in the favor of the tumor and increase the T-cell effects against the malignant B-cells.

One study that identified a possible mechanism linking GC microenvironment to the pathogenesis of GC lymphomas evaluated the expression of HVEM and BTLA in 198 FL samples. The authors reported that interactions between the HVEM and BTLA receptors are lost in FL [60]. Ligation between HVEM and BTLA provides an inhibitory signal for the T-cells [61]. Consequently, on the same study using a chimeric mouse model of lymphoma, they showed that HVEM acts as a tumor suppressor with increase in follicular T helper cells in HVEM-deficient lymphomas that could be corrected by CAR T-cells [60].

It is noteworthy that *TNFRSF14* mutation/deletion is a characteristic feature of distinct subtypes of FL, diffuse-type FL and pediatric FL. Both of them share t(14;18)-negative nodal FL with a unifying genetic alteration of *TNFRSF14*. Mutations of *MAP2K1*, an essential component of MAP kinase signal pathway, are also frequently detected in pediatric nodal FL in the *TNFRSF14* non-mutated cases indicating distinct roles in lymphomagenesis for both genes [62, 63].

TP53

Mutations of *TP53* are the most commonly observed alteration in cancer, but are infrequent in de novo FL. Paired analysis of FL cases pre- and post-transformation has demonstrated wild-type (WT) *TP53* in the antecedent FL samples, with the emergence of a mutated subclone at the time of transformation [64, 65]. However, later study showed that mutation of *TP53* contributes to histological transformation in only the minority of individuals. Despite this, authors observed that increased expression of MDM2, key regulator of p53, occurred both in the presence of *TP53* mutation/deletion and in cases where WT *TP53* was retained, suggesting that it occurred by a p53-independent mechanism [66]. Up to the present time, many sequencing articles exposed the complete genomic landscape of transformation more clearly and detected a network of genes that are discussed in greater detail below.

Copy Number Alterations (CNAs)

There is a wide spectrum of common recurrent chromosomal abnormalities in FL. These include gains of 2, 5, 7, 6p, 8, 12, 17q, 18, 21, and X and losses on 1p, 6q, and 17p together with many frequent small abnormalities including losses of 1p36.33-p36.31, 6q23.3-q24.1, 9p21.3, and 10q23.1-q25.1 and gains of 2p16.1-p15, 8q24.13-q24.3, and 12q12-q13.13 [67–70]. Given the limited knowledge we have about the targeted genes of these alterations, additional studies are required to discover the complete set of genes involved in the observed CNAs. However, some genes have been highlighted as likely candidates for a subset of these CNAs, namely, *TNFRSF14* in 1p36.33-p36.31 loss, *ARID1A* in 1p36.11-p35.3 loss, *TNFAIP3* in 6q23.3-24.1 loss, *CDKN2A* in 9p21.3 loss, *PTEN* in 10q23 loss, *REL* in 2p15 gain, *MYC* in 8q24 gain, and *MDM2* in 12q15 gain.

Appreciation of the function role of these CNAs could be inferred from the function of these genes in the B-cell development. *ARID1A* is a tumor suppressor gene that promotes the formation of SWI/SNF complexes [71] that control high-order chromatin structure, which fits the theme of chromatin modifier mutations in FL. *TNFAIP3* is a key player in the negative feedback regulation of NF-κB signaling, and *REL* encodes an NF-κB transcription factor [72]. However, most of the published

data point to NF-κB pathway mutation involvement in transformed FL rather than low-grade disease. The *CDKN2A/B* locus encodes two distinct proteins, p16INK4A (p16) and p14ARF (p14) which both function in cell cycle regulation. The p16 protein is a tumor suppressor that modulates pRb-regulated G1-to-S-phase transition, and p14 is also a tumor suppressor that inhibits MDM2-induced p53 degradation [73]. *MDM2* and *MDM4* are genetic modifiers of *TP53* and have p53-independent roles in tumorigenesis [74]. In contrast to TP53, these proteins that control *TP53* pathway now seem to be "druggable" using a variety of strategies. The *PTEN* gene encodes a well-characterized tumor suppressor that inhibits the PI3K/AKT pathway, which feeds into the previously discussed mTOR signaling pathway [75].

Transformed FL

An integral part of the natural history of FL are multiple recurrences that often culminate in histologic transformation. Many abnormalities were detected as a likely mechanism of this transformation, the most notable of which are disruption of the p53 pathway, activation of NF-κB, dysregulation of transcription factors, and evasion of immune surveillance.

An interesting study by Pasqualucci et al. elucidated the genetic landscape of transformation to DLBCL. Sequential FL and tFL biopsies obtained from 12 patients showed that all tFL cases bore unique mutations and CNAs that were not present in the earliest FL clone at diagnosis. Most common was the loss of *CDKN2A/B* that affects both cell cycle regulation and p53-dependent DNA damage responses, thus promoting genomic instability. The second important observation was genetic lesions deregulating *MYC* that might provide many advantages to cancer cells through its role in cell cycle progression, apoptosis, and cellular transformation. In addition, *B2M* and *CD58* mutations were implicated and function in the control of immune recognition by cytotoxic T-lymphocytes and NK cells, implicating escape from immune surveillance as a contributor to the transformation process.

Additionally, a copy number analysis of samples from 225 FLs and 84 tFLs highlighted abnormalities that likely activate the nuclear factor-κB pathway [76]. Similarly, a later study of integrative copy number analysis and gene expression profiles in serial FL biopsies from 44 patients defined enrichment for NF-κB pathway regulators associated with transformation [77]. These two groups highlighted *REL* as candidate for promoting transformation through increased NF-κB signaling. One study using 20 FL/tFL samples described *TP53* mutation, *CDNK2A* loss, and *c-REL* amplification as the recurrent oncogenic events in FL transformation [78]. The other using integrative copy number analysis and gene expression profiles from sequential biopsies diagnosed with FL showed gain of *REL/BCL11A* in the transformed tumors [79]. Finally, whole-exome sequencing of 35 paired FLs and tFLs confirmed the observation of the recurrent mutated genes in NF-κB pathway and p53 pathway.

Clonal Evolution of Follicular Lymphoma

Clonal evolution is intimately linked to the concept of intratumor cellular diversity, and it is always presumed that an elevated rate of mutation in tumor cells promotes that clonal evolution. Study of the patterns of genetic evolution during progression of follicular lymphoma was first performed by analysis of somatic hypermutation. However, it is unclear whether immunoglobulin somatic hypermutation occurs in synchronicity with other somatic mutations and thus whether other somatic events follow identical evolutionary patterns [27]. The importance of discovering the clonal evolution comes with the recent effort of personalizing therapy, which requires developing treatment based on the unique genetics and etiology within each tumor. To prioritize certain mutations as a targeted therapy, it is important to characterize clonal evolution and define the order in which somatic mutations are acquired. Mutations that arise early during the evolution are likely to be represented across all the tumor cells, which makes them better targets. In contrast, mutations that arise late are probably to be restricted to a subclone.

In an early study, the overall frequencies of genomic gains and losses tend to follow a stable pattern through the course of the disease in contrast to the trend for increasing level of somatic hypermutation in initial vs. late FL samples [80]. In an attempt to identify the mutations more precisely, whole-exome sequencing from subpopulations of B-cells and tumor-infiltrating T-cells from eight FL tumors and two relapses provided evidence that *IGH-BCL2* translocations and *CREBBP* mutations are early events, whereas *KMT2D* and *TNFRSF14* mutations probably represent late events during disease evolution [27]. These observations remained true in subsequent studies. The first study to perform whole-genome sequencing of paired indolent and transformed FL identified early driver mutations in chromatin regulator genes (*CREBBP*, *EZH2*, and *KMT2D* (*MLL2*)), whereas mutations in *EBF1* and regulators of NF-κB signaling (*MYD88* and *TNFAIP3*) were gained at transformation [28, 81]. Green et al. presented more evidence of the very early mutations during the disease development by analyzing the phylogenetic relationship of somatic mutations across the coding genomes of 59 sequentially acquired biopsies from 22 patients. They found that *CREBBP* mutations were most significantly enriched within the earliest inferable progenitor. *BCL2-IGH* translocation breakpoints were identified in 19/22 patients, with the same breakpoint maintained throughout the course of disease. Mutations in genes such as *EZH2*, *TP53*, *IRF8*, *TNFAIP3*, *CARD11*, and *TNFRSF14* were not significantly higher at relapse compared with diagnosis (Fisher $P > 0.05$), but they were more frequently detected in only the relapse tumor and not at initial diagnosis. Interestingly, mutations that were specific to relapse tumors occurred significantly more frequently within motifs recognized by either activation-induced cytidine deaminase (consensus WRGY) or apolipoprotein B mRNA editing enzyme catalytic polypeptide [26].

The first study of paired FL in situ (FLIS) and manifest FL (mFL) included ten cases of FLIS, four of these cases were not associated with mFL and six cases

were identified coexisting with mFL. It showed that all paired FLIS and mFL cases were clonally related, based on IGH rearrangement patterns and *BCL2* translocation breakpoint sequences, which provide evidence that FLIS represents a FL precursor lesion. It also identified *EZH2* mutations in FLIS, though the sequencing in this study was restricted to *EZH2* and omitted other recurrently mutated genes in FL [82].

A different aspect of the disease evolution is the genotype and phenotype changes acquired during spread of the tumor from the lymph node (LN) to the bone marrow (BM). Simultaneous mutational analysis of the IgVH genes and the topology of the genealogical trees in paired samples from LN and BM of 21 patients with FL revealed intensive clonal selection of small, centrocyte-like tumor cells in the BM infiltration of FL. It is suggested that the interfollicular compartment of FL, which is also composed of small, centrocyte-like cells, preferentially involves the BM and that the BM provides a microenvironment similar to the germinal centers of LNs where tumor cells retain their biological nature [83].

An interesting case of a donor-recipient pair who both developed grade 2/3A follicular lymphoma 7 years after allogeneic transplantation and donor lymphocyte infusions defined clonal evolution within rare subpopulations during human lymphomagenesis. High-depth sequencing revealed that the malignancy was transmitted via the donor lymphocyte infusion; and this product was found to possess an identical immunoglobulin recombination shared by the two subsequent tumors, as well as a common *BCL2* translocation, *EP300* histone acetyltransferase gene mutation, and 13 other somatic variants. However, the vast majority of mutations identified by exome sequencing of each tumor were not shared. This indicates that transmission was likely via an early follicular lymphoma ancestor that underwent genetic evolution in each individual following transplantation and acquired unique sets of secondary mutations that gave rise to follicular lymphoma over a similar time frame [84].

Conclusion

FL arises via the serial acquisition of genetic alterations, the earliest of which is likely *BCL2-IGH* translocation. Mutation of CMGs is a clear hallmark of FL, and a subset of these events including *CREBBP* mutation likely occurs early during disease genesis. However, genetic alterations are not limited to CMGs, and other events also influence tumor microenvironment interactions and intracellular signaling cascades. It is likely that future personalized therapeutic strategies will be determined based upon combinations of mutations rather than a single event. Further work is therefore required to understand how combinations of genetic mutations contribute to the pathogenesis of this genetically complex and heterogeneous disease.

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Chapter 4
The Microenvironment in Follicular Lymphoma

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Introduction

Several studies show that the survival and growth of follicular lymphoma (FL) cells are strongly dependent on multiple components of the tumor microenvironment [1–3], including T-lymphocytes, follicular dendritic cells (FDCs), macrophages, and other stromal cells [4–7]. Consistent with this, the expansion of FL cells in vitro is extremely challenging in the absence of stroma-like cells [8, 9], CD40 receptor stimulation [10], or specialized germinal center (GC) organoids [11, 12]. Conversely, previous studies have reported a significant expansion of FL cells in vitro in the presence of adherent stromal cells derived from the patient's bone marrow (BM) samples [13], highlighting the notion that stromal cells play an important role in the maintenance of FL and may be responsible for resistance to therapy and subsequent relapse. The close interaction between FL cells and the stroma was also observed in the development of patient-derived xenograft (PDX) models in which the experimental engraftment of FL tumors was facilitated by the inclusion of non-tumoral immune cells [14].

The clinical impact of the tumor microenvironment, supported by the identification of immune gene signatures, associates with survival in patients with FL. Gene expression profiling of non-tumoral immune cells derived from patients with FL has shown two primary immune response (IR) signatures, named IR-1 and IR-2. IR-1 identified genes mainly expressed in T-cells (e.g., CD7, CD8B1, ITK, LEF1, and STAT4) and associated with good prognosis; it is important to note that other T-cell-expressed genes, such CD2, CD4, LAT, TRIM, and SH2D1A, did not associate with survival. IR-2 identified instead,

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genes predominantly expressed in macrophages and dendritic cells (DCs) and associated with poor prognosis [2, 3].

The Role of FDCs in FL

While FDCs are essential for a physiological GC reaction [15], they can also provide co-stimulatory signals to FL cells, promoting adhesion, nursing, growth, and survival of FL cells (Fig. 4.1 and Table 4.1) [15–17]. Early studies demonstrated that cell-cell adhesion between FDCs and FL cells is dependent on the same structures essential to prevent apoptosis of normal GC B-cells [18], including the integrins LFA-1 (CD11a/CD18) and VLA-4 (CD49d/CD29), expressed on FL cells (or normal GC B-lymphocytes), and their receptors ICAM1 (CD54) and VCAM (CD106), expressed on FDCs [18–21]. This close interaction assures the proliferation and support of FL cells, through the release of FDC-generated cytokines and antiapoptotic signals [19, 20], and represents a potential factor of resistance to antitumor drug therapy [22]. Of interest, in addition to expressing high levels of CXCL13 [5], the functional status of the FDCs in physiological conditions is determined by the expression of the complement receptors CD21 and CD35 and the low affinity to IgE receptor (CD23) [23]. While the loss of these markers on FDCs was associated with tumor progression in ex vivo models of FL [5], the reduced expression of CD23, CD35, and CD54 on FDCs did not show a significant impact on the clinical outcome of patients with FL [24]. However, the presence of CD21 on FDCs at time of diagnosis was associated with shorter overall survival, progression-free survival, and time to transformation in clinical studies of FL [25]. FDCs are also an abundant source of the B-cell activator factor (BAFF/BLyS), essential for the development and homeostasis of B-cells [26]. While the role of BAFF in the pathogenesis of FL remains poorly understood, clinical data indicate that increased levels of the BAFF receptor are associated with shorter overall survival in patients with FL [27, 28].

Despite multiple reports showing the negative prognostic impact of FDCs in FL, the intra-tumoral presence of plasmacytoid dendritic cells (CD123+) has been associated with improved survival in patients with FL, showing that understanding of the biology of the different dendritic cell subsets requires further research [29].

The Role of T Follicular Helper (T_{FH}) Cells in FL

In a normal germinal center, T follicular helper cells (T_{FH} cells) are a functional subset of effector T helper cells, with a distinct transcriptional profile, separate from Th1, Th2, and Th17 [30, 31]. T_{FH} cells frequently release CXCL13 and localize to the follicles along with GC B-cells due to the expression of the cognate receptor CXCR5 on both of these cell types [32]. In healthy lymphoid tissues, T_{FH} cells are essential for high-affinity antibody generation [30] and the regulation of multiple

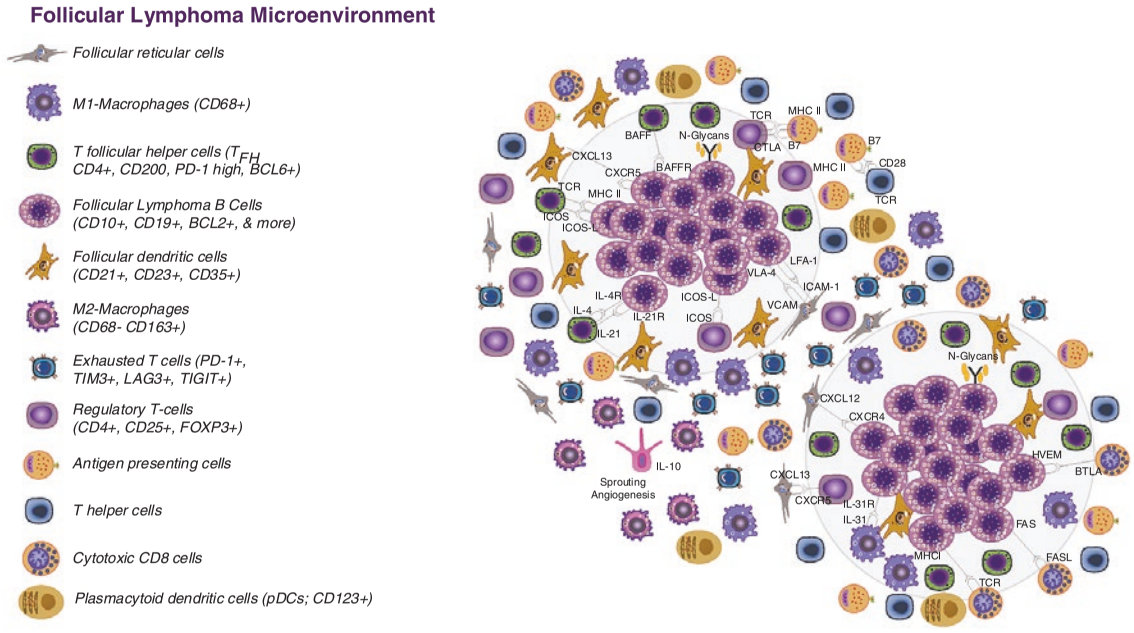

Fig. 4.1 The components of the microenvironment in FL. Follicular dendritic cells (FDCs), follicular T helper (FTH) cells, and follicular reticular cells (FRCs) are essential in the nourishment and maintenance of tumor cells. Secreted chemokines or cytokines such as stroma-derived CXCL12 and CXCL13 or IL-4 and IL-21, whose respective receptors are expressed by the tumor cells, promote growth and survival in FL. Immunoglobulin glycosylation and direct stimulation by professional antigen-presenting cells (FDCs) support the signaling of the B-cell receptor in FL cells. Also, regulatory T-cells (Tregs) are enriched in the FL microenvironment and impair the T-cell response. Tumor B-cells, on the other hand, produce TGF-beta or CCL22 that promotes the conversion and/or recruitment of Tregs. M1-polarized macrophages control malignant growth by supporting a Th1 response, whereas M2-polarized macrophages promote tumor growth by the induction of a Th2 response and promoting angiogenesis. *Abbreviations*: TCR T-cell receptor, VCAM-1 vascular cell adhesion protein 1, ICAM1 intracellular cell adhesion molecule 1, LFA-1 lymphocyte function-associated protein 1, VLA-4 integrin a4b1/very large antigen 4, HVEM herpesvirus entry mediator, BTLA B- and T-lymphocyte attenuator, BAFF; and its receptor BAFFR B-cell activator factor, CTLA cytotoxic T-lymphocyte-associated protein, ICOS; and its ligand ICOSL inducible T-cell co-stimulator

Table 4.1 Role of the main components of the tumor microenvironment in FL

Cell type	Effect on tumor cells	Effect on other stromal cells
FDCs	Promote adhesion, nursing, growth, survival Tumor B-cell migration	Essential for GCR, antiapoptotic, B-cell localization, survival
T_{FH} cells	Contribute to tumor development Overexpress TNF, LTA, IL-4 (JAK/STAT6), or CD40L; high PD-1 expression leads to reduced antitumor response	Regulation of the GCR Supported by the mesenchymal stromal cells
T_{regs}	Intra-follicular Tregs promote tumor survival Inter-follicular Tregs prevent tumor growth Reduce ICOSL in FL B-cells	Decrease the number of follicular DCs
T_{FR} cells	Support of DZ B-cells, chemokine production Promote suppression of humoral immunity and suppress follicular T helper cells	Regulation of GC function and reaction Supported by the mesenchymal stromal cells
T_{EFF} cells	Overexpress TNF, LTA, IL-4 (JAK/STAT6), or CD40L; high PD-1 expression leads to reduced antitumor response	Co-participate with TFR in the regulation of the GC function
F_{RCs}	Structural support and survival of tumor B-cells	Production of reticular fibers, T and DC migration, localization and survival
Macrophages (TAMs)	Provide trophic and survival signals for tumor B-cells Promote tumor progression	Promote angiogenesis

Abbreviations: *FDCs* follicular dendritic cells, *T_{FH} cells* T follicular helper cells, *T_{FR} cells* T follicular regulatory cells, *T_{EFF} cells* T effector cells, *F_{RCs}* fibroblastic reticular cells, *GCR* germinal center reaction, *DCs* dendritic cells, *TAMs* tumor-associated macrophages, *DZ* dark zone

GC functions, along with follicular regulatory T-cells (T_{FR} cells) [33, 34]. Both T_{FH} and T_{FR} cells are supported by mesenchymal stromal cells through an IL-6-dependent mechanism [35] and express CD4, CXCR5, ICOS, PD-1, and BCL-6, while only T_{FR} cells express FOXP3.

When compared to their normal counterpart, T_{FH} cells purified from FL samples show overexpression of TNF, lymphotoxin-alpha (LTA), IL-4, and CD40L, which likely contribute to tumor development [36]. To this regard, PD-1-expressing T_{FH} cells associated with shorter overall survival in patients with FL [37]. Tumor-infiltrating CD4 T-cells, including T_{FH} or non-T_{FH} cells, that express high levels of PD-1 lost responsiveness to cytokine signaling as compared to those not expressing PD-1 or to autologous peripheral T-cell subsets.

Compared to their normal equivalent, T_{FH} cells purified from FL samples also show a reduction in IL-4-, IL-10-, and IL-21-induced phosphorylation of STAT6 and STAT3 [38]. To this regard, recent reports show T_{FH} cells play a prominent role in the pathogenesis of FL via activation of the IL-4/JAK/STAT6 pathway [39], the presence of activating mutations in STAT6 associating with increased levels of IL-4 in the tumor microenvironment [39, 40]. Confirming previous

reports showing high levels of phosphorylated Erk accompanied the increased expression of IL-4 in FL samples, suggests the potential involvement of STAT6 in the biology of FL [41].

Intra-tumoral T_{FH} cells also indirectly promote immune evasion and tumor growth through the induced expression of CCL17 and CCL22 by FL cells, which leads to the recruitment of regulatory T-cells (Tregs) and IL-4-producing T-cells within the tumor microenvironment [42]. In addition, IL-4-overexpressing T_{FH} cells promote increased expression of CXCL12 in the stromal cells of patients with FL, identifying the IL-4/CXCL12 loop as a potential therapeutic target [43].

Finally, T_{FH} cells (along with Th17 cells and natural killer [NK] T-cells) produce high levels of IL-21, whose receptor is mainly expressed on activated and follicle center B-cells [44, 45]. In line with past studies, which have identified the function of IL-21 in FL as mainly immune suppressant [46, 47], recent analyses have shown high levels of IL-21 receptor are associated with decreased survival in patients with FL [48], suggesting that the blockade of this receptor could be an important therapeutic target.

The Role of Stroma-Derived Cytokines in FL

Cytokines produced by stromal cells are essential in promoting migration and homing of lymphocytes into the lymphoid tissue. These include CXCL12/SDF-1, the ligand for the C-X-C chemokine receptor type 4 (CXCR4/CD184), which is not only important in B-lymphopoiesis and sheltering of stem cells in the bone marrow but also plays a critical role in the chemotactic interaction between the lymph node stromal cells and transformed B-cells [49, 50]. Targeting CXCL12 prevents the signaling and migration of FL cells and counteracts the survival cues from the microenvironment in FL cells [51]. In addition, the chemokine CXCL13 binds to the receptor CXCR5 found on mature B-cells and a subset of T helper memory cells and is expressed by FDCs that reside within secondary lymphoid organs [52, 53]. As evidenced by animal studies, the interaction between CXCL13 and CXCR5 is essential for the B-cell organization within the follicle and the development of lymph nodes and Peyer's patches [54, 55]. A report showing important synergistic activity between CXCL13, expressed by T_{FH} cells, and CXCL12, expressed by stromal cells, suggests that these chemokines promote the migration of malignant B-lymphocytes [56]; and such interaction might facilitate the ectopic accumulation of FL cells within determined anatomical structures [57, 58]. Cultured FDCs secreted the monocyte chemoattractant protein-1 (MCP-1), whose activity involves the chemotaxis of several lymphoid cells through the C-C chemokine receptor type 2 (CCR2) [59, 60]. The importance of MCP-1/CCR2 interaction, not only on the chemotaxis of normal B-cells but also in FL cells by potentiating the CXCL12-induced chemotaxis, shows that MCP-1 is an important element for the migration and homing of FL cells [57–59].

TNFα is a cytokine with multiple functions, mainly produced by activated macrophages and CD4 T-cells, with a critical role in inflammation, immunity, and cancer progression [61]. High intra-tumoral levels of TNFα are associated with poor prognosis in patients with FL [62].

Another member of the TNF superfamily, with implications in the pathogenesis of FL, is the herpes virus entry mediator HVEM (TNFRSF14). HVEM is the receptor for the B- and T-lymphocyte-attenuator (BTLA) ligand, frequently mutated and associated with poor prognosis in patients with FL [63–65]. The interaction between HVEM and BTLA is typically lost in FL, leading to the decay of HVEM. This promotes a supportive environment for FL cells, as demonstrated by the robust activation of stromal cells and CD21/CD35-positive FDCs and the surge in the recruitment of T_{FH} cells to the tumor site [66]. Of interest, in vivo restoration of HVEM using CAR T-cells as carriers promoted the expression of BTLA and led to the destruction of tumor B-cells [66].

T-Cell Exhaustion in FL

Intra-tumoral cytotoxic CD8 T-cells are prevalent in tumor samples derived from patients with FL [67], their density correlates with prolonged overall survival [68]. However, these cells can also express the programmed death receptor protein (PD-1), the lymphocyte activation gene 3 (LAG3), and the T-cell immunoglobulin domain and mucin domain protein 3 (TIM3) [69, 70]. Such markers and other inhibitory receptors are associated with T-cell exhaustion [71, 72] and poor clinical outcome in patients with FL [73]. However, extra-follicular PD-1 expression predicted a favorable outcome as compared to low levels of PD-1, and 5-year treatment-free survival increased from 37% to 67% in patients with low intra-follicular CD3 expression [74].

A newly discovered immune checkpoint relevant to the pathogenesis of FL is the T-cell immune receptor with Ig and immune-receptor tyrosine-based inhibitory motif (ITIM) domain (TIGIT). TIGIT is a co-inhibitory receptor, frequently expressed in exhausted effector memory CD8 T-cells [75]. TIGIT and CD226 serve as co-inhibitory and co-stimulatory receptors for the ligands CD112 and CD155, respectively, and are highly expressed by FDCs in the tumor microenvironment [76].

Another mechanism of T-cell exhaustion is the expression of the replicative senescence marker CD57 (B3GAT1). CD57-positive T-cells are unable to proliferate and are unresponsive to FL cells [77–79], associating with a higher risk of transformation [25]. Low CD4+CD57+/CD4+ and low CD4+/CD8+ ratios in FL patients correlated with poor survival, and multivariate analysis demonstrated that CD4+CD57+/CD4+ ratio was the best predictor of overall survival in FL [80].

Interestingly, FL cells can induce these changes, affecting the antitumor activity of tumor-infiltrating T-cells. To this regard, in vitro co-culture of FL cells with healthy T-cells led to the disruption of the cytolytic effector molecule Rab27A, an essential component of the immunological synapse in T-cells [81]. In addition, FL cells can affect the transcription of important genes (PMCH, ETV1, and TNFRSF9) in both CD4 and CD8 tumor-infiltrating lymphocytes, influencing their overall motility and survival and ultimately translating to inferior outcome in patients with FL [82, 83].

Recent RNAseq analysis of tumor-infiltrating lymphocytes derived from patients treated with rituximab-based regimens showed a significant correlation between high levels of CD3 and CD8 and progression-free survival in FL. However, no correlation with other transcripts, including PD-1, ICOS, and FOXP3, was observed [84].

The Role of Macrophages in FL

The detection of high levels of macrophages (CD68+) in patients with FL was associated with poor outcome [85–87]. In particular, a high frequency of intra-follicular macrophages (CD68+ and PD-L1+), combined with extra-follicular regulatory T-cells and intra-follicular CD4+ cells at diagnosis, correlated with a shorter time to transformation in these patients [25]. Also, a high number of CD163+ infiltrating macrophages have been observed in the vicinity of the newly formed vessels in patients with FL [88], associating with further increase in neovascularization, shorter survival, and increased risk of transformation [89].

In a recent report, a consistent decrease in CD4+ cells accompanied by an increase in the absolute number of circulating monocytes in the peripheral blood of patients with FL correlated with poor prognosis [90]. To this regard, elevated serum levels of soluble interleukin-2 receptor (sIL-2R) strongly associated with a high number of CD68+ tissue macrophages in the tumor microenvironment of patients with FL [91]. While decreased absolute lymphocyte count correlated with increased levels in eotaxin, an increased absolute monocyte count also associated with increased levels in MCP-1, other than increased levels in IL-2R.

The recently discovered IL-31/IL-31R interaction showed a potential role in the pathogenesis of FL [92]. IL-31 is a member of the IL-6 cytokine superfamily, produced by activated T helper type 2 (Th2) cells, monocytes, macrophages, dendritic cells, and mast cells [93]. Increased expression of IL-31 induces a shift toward an M2 (pro-tumoral) phenotype of macrophages in FL, with a subsequent negative impact on survival [94]. It is important to note that other studies have found no correlation between the presence of intra-tumoral macrophages or circulating monocytes and outcome in FL, with some studies even showing a potentially positive prognostic significance [86, 87].

The Role of Regulatory T-Cells in FL

Regulatory T-cells interact with tumoral B-cells in patients with FL through ICOS and its ligand, ICOSL, leading to a subsequent decrease in the levels of ICOSL produced by tumoral B-cells [95]. Studies analyzing the role of regulatory T-cells (FOXP3+) and CD4 T-cells in the tumor microenvironment of patients with FL and their influence on clinical outcomes showed conflicting results [78, 85, 96–98].

Farinha et al. have reported that the distribution of FOXP3+ cells, within or around the follicle, is associated with risk of transformation and decreased survival in patients with FL [98]. Regulatory T-cells were able to migrate from the interfollicular space into the GC by downregulating the expression of T-cell area localization chemokine receptor 7 (CCR7) and upregulating the follicular-homing receptor CXCR5. This resulted in a preferential response to the follicle-homing chemokine CXCL13 as compared to the normal homing chemokine CCL19 [99], expressed by DCs and stromal cells in the T-cell-rich zone [100]. Remarkably, as opposed to normal B-cells, tumoral B-cells derived from patients with FL were able to induce a regulatory phenotype of T-cells without TCR stimulation in vitro [101, 102].

Chevalier et al. described the frequency and distribution of dendritic cells (DCs; CD11c) in relation to CD4/FOXP3 regulatory T-cells in patients with newly diagnosed FL and found that tumoral follicles had a decreased number of DCs and increased concentration of regulatory T-cells as compared to hyperplastic follicles. Regulatory T-cells were also increased in peripheral blood at diagnosis and persisted in high numbers after induction of clinical remission in FL patients [103]. High levels of peripheral blood Tregs are associated with inferior clinical outcome suggesting that they likely mediate systemic immune suppression in the patients [103]. Of interest, an increase in intra-follicular DCs correlated with a higher number of intra-follicular regulatory T-cells and was associated with shorter overall survival in patients with FL [104]. It is important to note, however, that as opposed to intra-follicular localization, an accumulation of regulatory T-cells in the interfollicular zone has been associated with a favorable outcome in patients with FL [96, 98, 105, 106], likely secondary to suppression of B-cell proliferation [107].

The Role of Natural Killer (NK) Cells in FL

Limited data is available regarding the role of NK cells in patients with FL. Lapenta et al. showed that dendritic cells loaded with patient-derived apoptotic FL cells secreted high levels of IFN-α, which lead to a robust activation of NK cells with significant antitumoral activity [108]. Further studies are needed to shed light on the function and clinical impact of different subsets of NK cells in patients with FL.

Epigenetics of the FL Microenvironment

Mass cytometry studies demonstrated that a decreased expression of HLA-DR (MHC class II) in FL cells contributes to tumor heterogeneity [109]; this has been associated with recurrent somatic mutations in the acetyltransferase CREBBP, leading to the disruption of the class II transactivator (CIITA) and consequent reduced expression of MHC class II [110–112]. Moreover, the reduced expression of MHC class II led to a decreased number of activated CD4 and CD8 T-cells, contributing to immune evasion mechanisms [110, 111].

Recent genome-wide DNA methylation studies showed an association between progressive intra-tumoral and inter-patient heterogeneity in the cytosine methylation patterns of patients with FL and poor outcomes [113]. A similar relation between aberrant DNA methylation and development of a pro-tumoral microenvironment was confirmed in additional samples derived from 14 patients with FL [114]. Also, a recent study identified wild-type EZH2 and low percentages of CD8+ T-cells and CD163+ macrophages as predictors of early failure of immunotherapy in FL previously treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) [115].

While rarely detected in normal B-cells, high N-glycosylation has been observed in the immunoglobulin (Ig) variable region (VH) genes following active somatic hypermutation in FL cells [116–118]. Indeed, because surface Ig is critical for the survival of FL cells, the tumor-specific expression of mannose-rich N-glycosylation sites in the Ig variable region generates signaling cues between FL cells and its microenvironment [118, 119]. These mannose-rich glycans interact with mannose-specific lectins found in dendritic cells and macrophages, such as the C-type lectin receptor dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN/CD209) [120]. Also, it is important to note that dendritic cells and macrophages from samples of patients with FL overexpress DC-SIGN [118], and the binding of this lectin to FL cells elicits continuous activating signals, promoting tumor survival and proliferation [120, 121].

Future Directions

Future clinical trials will need to take into account the crucial role played by the tumor microenvironment in FL, by targeting relevant immune checkpoint and identifying early biomarkers of the microenvironment activity and response to therapy. Such strategies will be critical in the application of preventive measures in the early stages of FL and in establishing long-lasting responses, by disrupting the cross-talk between cells of the microenvironment and FL cells.

Several new agents able to effectively target the tumor microenvironment are now available for the treatment of patients with FL. These include anti-CD20 monoclonal antibodies, such as obinutuzumab [122, 123], PI3 kinase inhibitors such as copanlisib [124, 125], and the immunomodulatory agent lenalidomide [126]. Other therapeutic options, still under investigation, include modulators of the B-cell receptor, such as Syk inhibitors or BTK inhibitors [127], and the anti-CD79b antibody-drug conjugate polatuzumab vedotin [128, 129].

Finally, the use of chimeric antigen receptor T-cells targeting CD19 approved for the treatment of patients with relapsed or refractory transformed FL [130, 131]. While its safety profile is being optimized, this may represent in the future a promising alternative to the use of allogeneic stem cell transplant for patients with non-transformed FL [132, 133]. More research is needed to decipher the complex functions of the FL microenvironment; this will ultimately lead to the discovery of attractive new therapeutic strategies for the improvement of clinical outcomes in patients with FL.

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Chapter 5
Prognostic Factors in Follicular Lymphoma

Anna Johnston and Judith Trotman

Part 1 Clinical Prognostic Factors

At Diagnosis

Prognostic Indices

Multiple clinical features at diagnosis have been identified as having important prognostic significance in follicular lymphoma (FL). These include the five factors included in the International Prognostic Index (IPI) which was originally developed for aggressive lymphomas [1]. Although the IPI was found to be predictive for progression-free survival (PFS) and overall survival (OS) when applied to patients with advanced-stage FL [2], it was found to identify too few high-risk patients. The need for an index specific to FL led to the international collaborative effort resulting in the Follicular Lymphoma International Prognostic Index (FLIPI) [3]. Clinical characteristics at diagnosis of 4167 FL patients were analyzed to identify factors most strongly predictive of prognosis [3]. The FLIPI, remembered by the anagram "NoLASH," consists of five easily obtainable clinical factors: number of *no*dal areas >4, serum *l*actate dehydrogenase (LDH) > normal, *a*ge > 60, advanced *s*tage, and *h*emoglobin < 120 g/L. The FLIPI was developed prior to the introduction of rituximab-containing therapy but has since been validated in patients treated with R-CHOP [4], a variety of

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Table 5.1 Indices, factors, and risk groups

Index	Factors	Risk groups[a]	Number of factors	5-year overall survival (%)	References
FLIPI	Age ≥60 Stage III or IV Hb <120 g/L Four or more nodal areas LDH > ULN	Low/ intermediate	0–2	89–91	[3, 4, 7]
		High	3–5	59–75	
FLIPI2	Lymph node mass >6 cm Age ≥60 Bone marrow involvement Hb <120 g/L β2-Microglobulin > ULN	Low	0	98	[5]
		Intermediate	1–2	88	
		High	3–5	77	
m7-FLIPI	FLIPI ECOG Genes *EZH2* *ARID1A* *MEFZB* *EP300* *FOXO1* *CREBBP* *CARD11*	Low	See comment	84–90	[4, 7, 9]
		High	See comment	41–65	

[a]These risk groups are for patients treated with immunochemotherapy except for the FLIPI2 which included some watch and wait patients as well as patients receiving treatment without rituximab including local treatments.
Free online calculator is available at http://www.glsg.de/m7-flipi/
Hb hemoglobin, *LDH* lactate dehydrogenase, *ULN* upper limit of normal

rituximab-containing regimens [5] and in a population based cohort in the USA managed with both watchful waiting and rituximab-containing chemotherapy [6]. It allows identification of three groups of patients with distinct prognoses in terms of progression and OS (see Table 5.1). Limitations of the FLIPI are that it was based on a retrospective dataset, it does not define an indication for treatment, and again there are relatively few patients in the high-risk group. Also, some factors now in common use, such as β2-microglobulin (B2M), could not be included due to missing patient data. The International Follicular Lymphoma Prognostic Factor F2 project was developed to address some of these limitations and involved prospective collection of data from 1093 patients with newly diagnosed FL [5]. Using the five variables (B2M > normal, longest diameter of the largest involved node >6 cm, bone marrow involvement, Hb < 120 g/L, and age > 60) identified as being most strongly associated with PFS after treatment, Federico and colleagues developed a prognostic model (FLIPI2) which defined three risk groups, low, intermediate, and high risk, with 3-year PFS of 91%, 69%, and 51%, respectively [5] (see Table 5.1). They also validated the original FLIPI in a cohort treated with rituximab although the FLIPI2 performed better.

In clinical practice, FLIPI is still widely used as a predictor of survival in FL due to the ease with which it can be applied. Importantly, these indices are not generally used to define an indication for treatment for which criteria of high tumor burden (such as the Groupe d'Etude des Lymphomes Folliculaires (GELF) [10] and the British National Lymphoma Investigation (BNLI) [11] criteria) are generally used (see Table 5.1).

Disease Burden Including Baseline Metabolic Tumor Volume

Patients who do not fit established criteria for high tumor burden (such as GELF and BNLI criteria) have a good long-term prognosis, and early treatment of these patients has not demonstrated an advantage in terms of overall survival; hence, watchful waiting is often employed. This was initially based upon data from the pre-rituximab era including a large British study of observation vs. chlorambucil, which showed no improvement in OS with early initiation of chemotherapy [11]. This observation has been confirmed in the rituximab era including an international study, which compared observation to single-agent rituximab followed by rituximab maintenance in low-tumor burden patients [12]. In this study, patients initially managed with watchful waiting or single-agent rituximab followed by rituximab maintenance had 3-year OS in excess of 90% with no significant difference between the two groups [12]. Not surprisingly, rituximab did however improve the time to next treatment in these patients such that 88% of patients did not need additional treatment at 3 years in the rituximab group compared to 46% of patients in the watchful waiting group. A population-based study from Denmark has also shown that these patients have a very good prognosis with the watch and wait population having a similar OS during the first 50 months after diagnosis compared to a matched background population but an increased risk of death after 50 months [13]. Conversely, it is well established that patients with high tumor burden (for which factors such as LDH, B_2M, longest diameter of the largest involved node (LODLIN), and numbers of nodal sites are surrogates) have a comparatively unfavorable prognosis, and the benefit from immunochemotherapy for these patients in terms of PFS and OS has been demonstrated in multiple large randomized studies [14, 15]. Hence, current treatment strategies are based on assessment of tumor burden which has traditionally been done using surrogate clinical parameters. [18F]-Fluorodeoxyglucose (FDG)-positron emission tomography (PET) is a highly sensitive imaging modality that is used to detect malignant disease. It is based on the principle that neoplastic cells take up glucose more rapidly than normal cells [16]. PET in combination with anatomic CT data allows an accurate and reproducible assessment of overall tumor volume. Total metabolic tumor volume (TMTV) is a measure of viable tumor and environmental cells, and pooled analysis of a subset of patients from three large studies has shown that baseline TMTV strongly predicts outcome in high-tumor burden FL in patients receiving R-CHOP without antibody maintenance [17]. TMTV was obtained by summing the metabolic volumes of all local and extranodal lesions. Studies have

shown that 29% of patients with a high TMTV >510 cm^3 had a markedly inferior 5-year progression-free survival of only 33% compared to 65% for patients with TMTV ≤510 cm^3. High TMTV was also associated with inferior 5-year OS (85% vs. 95%). The combination of high TMTV and intermediate to high FLIPI2 allowed definition of three groups with distinct prognoses, ranging from 5-year PFS of 20% with both risk factors to 69% when they are both absent. These findings, and particularly the cutoff for definition of high TMTV, require validation in prospective studies in larger numbers of patients, including those receiving rituximab-bendamustine and importantly patients receiving maintenance. There are also issues related to the optimal software algorithms and threshold SUV used to define disease, as well as reproducibility of findings with different equipment, different treatments, and the interaction of TMTV with other prognostic measures [18].

After Treatment

Response to Initial Treatment

CT-based assessment with measurements of the sum of the products of the diameters of up to six target lesions has been the cornerstone of response assessment for FL for decades. However, with approximately 95% of patients having a response to rituximab-chemotherapy, the discriminatory capacity of the 1999 IWC contrast-enhanced CT-based response assessment [19] consigns most responding patients (with an unconfirmed complete response or partial response) to an uncertain remission in which only close clinical follow-up identifies those with early relapse.

Multiple lines of evidence have recently been presented that demonstrate that early progression after treatment for FL is a strong predictor of inferior OS [20–22]. Approximately 20% of patients will progress within 2 years of initial immunochemotherapy. Analysis of outcomes of almost 600 patients from the US National LymphoCare Study shows that this group has significantly inferior OS (50% at 5 years) compared to the overall cohort (90% at 5 years) [20]. Using event-free survival (defined as time from diagnosis to progression, relapse, retreatment, or death) at 12 and 24 months in a large cohort of patients from the Mayo Clinic, Maurer and colleagues confirmed that patients with early events after immunochemotherapy had especially poor outcomes [21]. They also showed that an early event following rituximab monotherapy or after initiation of treatment following observation predicts poor subsequent survival. In an individual patient-level analysis of multiple randomized trials, Shi et al. found that the 30-month complete remission (CR) rate is a robust surrogate end point for progression-free survival (PFS) in first-line treatment for FL [22].

PET Response

Following the initial hypothesis generating study of PET scans by Trotman and colleagues, performed in the PRIMA (Primary Rituximab and Maintenance) study

[23], two additional European cooperative group prospective studies, the Italian FOLL05 [24] and French PET Folliculaire [8] studies, were reported. Both confirmed the strongly predictive value of PET-CT performed up to 3 months after induction of immunochemotherapy for patients with high-tumor burden disease. A pooled analysis of 246 independently reviewed scans by three expert PET physicians in these three studies was conducted applying the currently recommended cutoff score of ≥4 (defined as FDG uptake in tumor moderately higher than that of the liver) on a five-point scale (5PS; also known as the Deauville criteria) [25]. Forty-one (16.7%) scans were positive with a cutoff of ≥4 (i.e., lymphoma FDG uptake moderately > liver uptake), with substantial reporter concordance. With a median follow-up of 55 months, the HR for PFS and OS of PET+ vs. PET- patients was 3.9 (95% CI 2.5–5.9, $p < .0001$) and 6.7 (95% CI 2.4–18.5, $p = 0.0002$), respectively. For PET+ patients, 4-year PFS was 23.2% (95% CI 11.1–37.9%) vs. 63.4% (95% CI 55.9–70.0%) in those who became PET- ($p < .0001$). Four-year OS was 87.2% (95% CI 71.9–94.5%) vs. 97.1% (95% CI 93.2–98.8%) ($p < .0001$), providing the first large body of evidence of the impact of post-induction PET status on OS (see Fig. 5.1).

Conversely, conventional CT-based response (complete response/unconfirmed complete remission vs. partial response) was weakly predictive of PFS (HR 1.7, $p = 0.02$) but not OS. As an example, Fig. 5.2 demonstrates a series of scans in a patient with newly diagnosed FL with high tumor burden who had a positive scan (score of 4) at the end of induction and who relapsed 6 months later.

More recently, data from the GALLIUM study confirmed the highly predictive power of post-induction PET status after either rituximab- or obinutuzumab-chemotherapy (bendamustine, CHOP, or CVP) for FL [26]. Of 595 patients included in the PET-CT intention-to-treat population, 508 and 519 were included in an OS and PFS landmark analysis, respectively, applying the Lugano 2014 response criteria which incorporate the 5PS. Following induction therapy, 454/595 (76.3%) obtained complete metabolic response (CMR). With median of 43.3 months' follow-up, post-induction PET-CT was highly prognostic for PFS and OS (CMR vs. non-CMR: HR 0.2, 95% CI 0.1–0.3, $p < 0.0001$ and HR 0.2, 95% CI 0.1–0.5, $p < 0.0001$, respectively). Two-and-a half-year PFS from EOI was 87.4% (95% CI 83.8–90.2) for CMR patients vs. 54.9% (95% CI 40.5–67.3) for non-CMR patients; 2.5-year OS was 96.6% (95% CI 94.4–97.9) vs. 84.0% (95% CI 72.9–90.8). With a fivefold increased rate of early progression and death in patients who failed to obtain a complete metabolic response, this data validated the prognostic impact of PET. While highly predictive of outcome, there is still a minority of patients obtaining CMR who progress early, as well as a number failing to obtain CMR who remain in remission during this rather short follow-up. A combined analysis of all four studies will provide a more robust assessment of the predictive power of PET response with longer follow-up, and post-induction PET status is potentially a useful surrogate end point for use in clinical trials. Two large studies are underway (the British-led PETReA study and the Italian FOLL12 trial) which individualize post-induction treatment based on risk according to metabolic response.

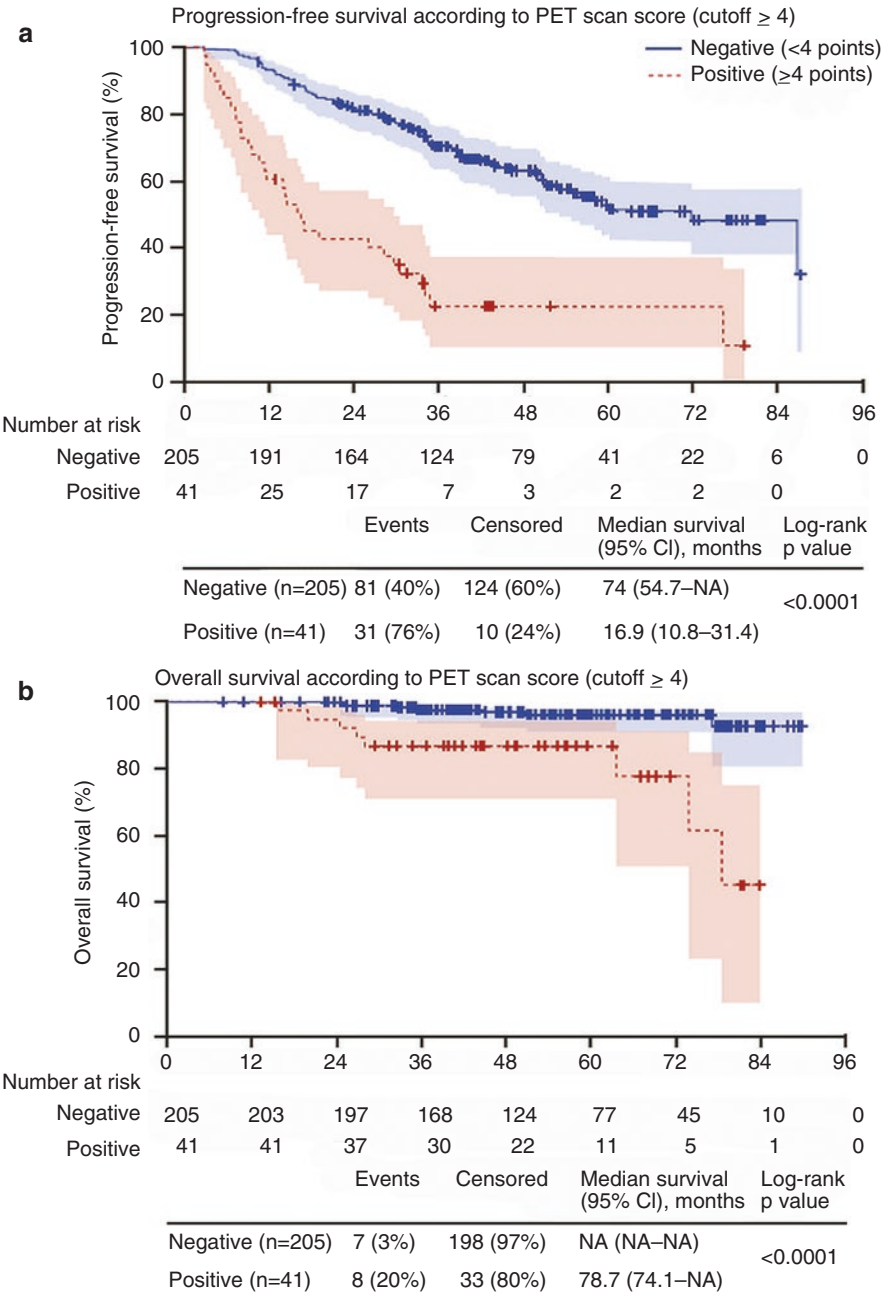

Fig. 5.1 Progression-free (**a**) and overall survival (**b**), according to post-induction PET scan status (cutoff ≥4 on the five-point Deauville scale). Negative scan is <4 and positive is ≥4 [25]. NA not applicable. (Reprinted from Trotman et al. [25], © 2014; with permission from Elsevier)

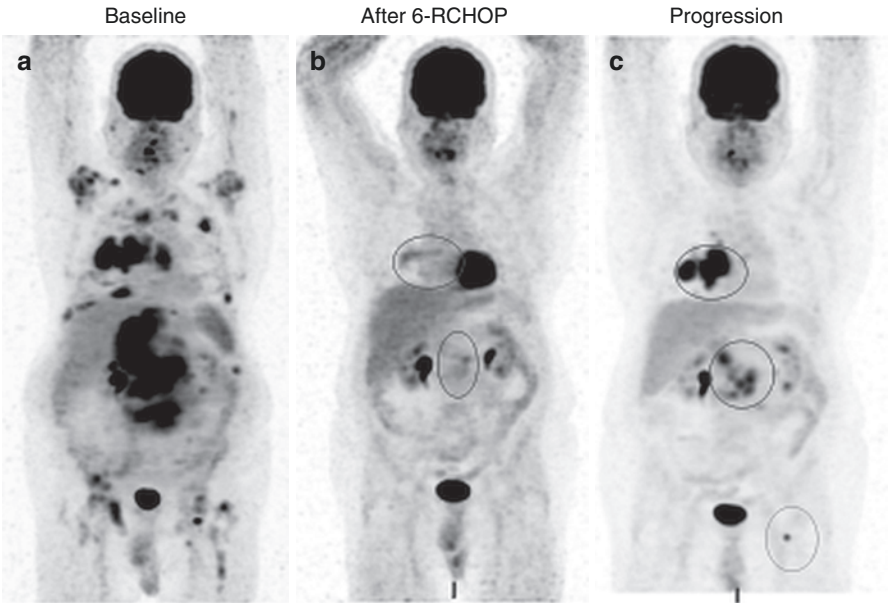

Fig. 5.2 PET images from a 55-year-old man with newly diagnosed FL. (**a**) Baseline PET scan demonstrates extensive disease with a very large intra-abdominal mass, multiple lymph nodes above and below the diaphragm, and sites of bony involvement. (**b**) After induction therapy, there has been substantial regression of disease, but circles highlight residual disease with FDG uptake greater than that of liver (Deauville 4). (**c**) The patient progressed 6 months later. Circles show progression in areas of persistent disease and a new bony lesion in the left femur. (Figure courtesy of Dr Anne-Segolene Cottereau, Department of Nuclear Medicine, Cochin Hospital, Assistance Publique Hôpitaux de Paris, Paris Descartes University, 75014 Paris, France)

Transformation and Prognosis

Transformation to aggressive lymphoma (usually diffuse large B-cell lymphoma) occurs in approximately 30–40% of cases of FL with prolonged follow–up [27]. Histological transformation (HT) has been associated with a very poor prognosis with early series reporting a median survival following HT of 1–2 years [27]. Contemporary data with modern therapy shows a significant improvement in survival following transformation with a median survival of 5 years in the large National LymphoCare Study [28]. Despite recent advances, 5-year survival from diagnosis remained significantly worse for patients who experienced HT at 75% compared with 85% for non-transformed patients [28]. Interestingly, when HT is identified at the time of diagnosis, it did not appear to negatively impact survival [28]. The risk of HT appears to be stable and consistent at approximately 2–3% per year for at least the first 10–15 years following diagnosis [29]. Available evidence suggests that chemoimmunotherapy does not abrogate risk of transformation [28, 29]. Risk factors for transformation include altered performance status, anemia, high lactate dehydrogenase level, B symptoms, histologic grade 3a, and high FLIPI score at diagnosis [30].

Part 2 Biological Prognostic Factors

Histology and Tumor Grade

Tumor cells in FL are malignant counterparts of normal germinal center B-cells. These malignant cells are admixed with nonmalignant cells such as T-cells, follicular dendritic cells, and macrophages that make up the tumor microenvironment [31]. FL is separated into grades 1–3 which are defined by the relative proportion of centrocytes to centroblasts [32]. Grade 3 is further subdivided into 3A and 3B according to the presence of centrocytes (3A) or solid sheets or entire follicles composed of centroblasts (3B). The clinical significance of WHO grades has been controversial, and tumor grading has not been adopted as standard criteria for patient prognostication. Accumulating data however indicate that grade 3B FL is a distinct entity that is clinically different from grade 1, 2, and 3A FL. A study of a population-based cohort of 505 patients with long follow-up [33] demonstrated that the clinical course of patients with grade 1–2 and 3A FL was equally indolent with similar median OS times. In contrast, the clinical course of grade 3B lymphoma more closely resembled that of diffuse large B-cell lymphoma with an inferior median survival time and the survival curve reaching a plateau after 5 years. Treatment with first-line anthracyclines correlated with superior OS in grade 3B disease, whereas grade 1–2 and 3A FL did not seem to benefit from upfront anthracyclines. Grade 3B FL was also less frequently positive for BCL2 and more often positive for TP53. A study of cytogenetic and immunohistochemical profiles of FL has also shown that strictly defined 3B FL is a distinct entity with infrequent BCL2 and BCL6 translocations compared to grade 1–3A FL, infrequent expression of CD10, and increased expression of IRF4/MUM1 [34]. Thus, current treatment guidelines generally recommend that grade 3B FL is treated like diffuse large B-cell lymphoma.

Several unique subtypes of FL also appear to have a distinct clinical course, and this has been recognized in the recently revised WHO classification [32]. Pediatric-type FL (PFL) is now recognized as a definite entity. This is usually a localized disease presenting in the head and neck in young males. Pathological features include high-grade histology, absence of the BCL2/IgH translocation, and absence of BCL2 expression [35]. This variant of FL has an excellent prognosis showing lack of progression or recurrence after local treatment [36]. Duodenal-type FL is another distinct variant presenting with localized disease in the gastrointestinal tract. These patients also have an excellent outcome including cases managed with initial observation [37].

Microenvironment

The tumor cell microenvironment has been found to have a significant impact on the long-term outcome of patients with FL. A seminal study conducted by Dave et al. showed that the prognosis of FL correlates with the molecular features of

nonmalignant immune cells present in the tumor at diagnosis [38]. Using gene expression profiling (GEP) on biopsy specimens of patients with untreated FL, they identified individual genes that were associated with prognosis and then grouped these genes according to patterns of expression into molecular "signatures." Flow cytometry showed that these signatures reflected gene expression by nonmalignant tumor-infiltrating immune cells. The immune response 1 signature was enriched for genes expressed in T-cells and was associated with favorable outcomes. In contrast, the immune response 2 signature which included genes expressed in follicular dendritic cells and macrophages identified a very poor risk group with a ninefold increase in the relative risk of death compared to baseline. Subsequent studies have confirmed the important role of the microenvironment in FL biology, but have yielded conflicting results. Potential explanations include the complex interplay of the cellular immune system and the use of differing treatment regimens across studies, further highlighting the important influence of methodology and lymphoma treatment on interpretation of results.

Although the influence of the tumor microenvironment was initially demonstrated using gene expression profiling, subsequent studies have shown that certain immunohistochemical markers may be used as surrogates [39]. A number of studies have identified an important prognostic role of tumor-associated macrophages [40]; however, it is clear that their role in prognosis is critically reliant on treatment received; in particular, rituximab appears to abrogate the adverse prognostic impact of increased tumor-associated macrophages. Interactions between tumor cells and infiltrating T-cells are the subject of considerable study with follicular helper T-cells and regulatory T-cells demonstrated to be of particular importance. Regulatory T-cells recognize tumor antigens and can suppress antitumor effector cells. They can be identified by positive staining for FOXP3. It has been shown that the pattern of distribution of these cells may be an independent predictor of survival and histological transformation in FL [41]. Follicular helper T-cells have been implicated in the survival of FL B-cells [42] and express programmed death 1 (PD 1) [43]. A recent study however did not show an association of these PD 1-positive follicular helper T-cells with patient outcomes [43]. Given the substantial interest in checkpoint inhibitors in solid tumors and lymphoma, further studies investigating this biology and its effect on prognosis in FL are ongoing.

Molecular Prognostic Markers

The advent of powerful molecular techniques such as next-generation sequencing has allowed genome-wide analysis of FL. This has resulted in improved understanding of the molecular pathogenesis of FL and identification of mutations that can impact on prognosis. It is clear that epigenetic modification is critical to the biology of FL. Genes that regulate histone proteins, and hence modify chromatin, are mutated at a high frequency and appear to be key driver mutations [44–46] (see also Table 5.2). Numerous mutations have also been detected in genes encoding

Table 5.2 Selected molecular alterations in follicular lymphoma and their prognostic significance

Role/function	Gene	Mutation/ alteration	Frequency	Prognostic significance	References
Histone modification	*KMT2D (MLL2)*	Inactivating mutations	60–89%	Core driver mutation, unclear prognostic significance	[44–46]
	CREBBP	Inactivating mutations	32–52%	Driver mutation, associated with inferior PFS, component of the m7-FLIPI	[7, 44, 46–48]
	EZH2	Activating mutations	17–27%	Early mutation in FL, associated with a favorable outcome in patients with high-risk FLIPI, component of the m7-FLIPI	[7, 44, 46, 49]
	EP300	Inactivating mutations	9–19%	Driver mutation, component of the m7-FLIPI	[7, 47]
	ARID1A	Inactivating mutations	6–15%	Impaired DNA repair, correlates with longer failure-free survival (FFS), component of the m7-FLIPI	[7, 44]
Microenvironment	*TNFRSF14*	Inactivating mutations	18–32%	Controversial impact, associated with pediatric-type FL (54% of cases)	[7, 50–52]
Transcription factor	*MEF2B*	Inactivating mutations	8–20%	Driver mutation, component of m7-FLIPI	[44, 45]
B-cell receptor signaling	*CARD11*	Activating mutations	10–15%	Mutations associated with shorter FFS, component of the m7-FLIPI	[7]
Cell cycle regulation	*TP53*	Inactivating mutations	<10%	Significantly associated with inferior overall survival	[7, 53]
Regulation of apoptosis	*BCL2*	Gain-of-function mutations	12–54%	Controversial impact on survival, may be associated with increased risk of transformation	[54, 55]

transcription factors, kinases, and other signaling molecules [56]. Mutations in few individual genes have been associated with prognosis in FL, and some associations are controversial (see Table 5.2).

Whether combining mutational analysis into risk models could help account for the complex interactions between distinct gene mutations is largely unknown [56].

Potentially, clinical parameters could add to such models to improve predictive power. Pastore et al. performed a comprehensive multivariate analysis of recurrent gene mutations and clinical risk factors in patients with symptomatic follicular lymphoma receiving first-line R-CHOP in a German Low-Grade Lymphoma Study Group trial [7]. The authors developed a clinicogenetic risk model (m7-FLIPI) which incorporates seven genes, FLIPI, and the ECOG performance status. The model was validated in a cohort of patients from a British Columbia Cancer Agency treated with R-CVP. The seven genes which emerged as being most strongly predictive in this model (*EZH2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP*, and *CARD11*) are known to be dysregulated in FL and include several genes involved in histone modification. The m7-FLIPI identified a high-risk group (comprising approximately one-quarter of patients) with 5-year failure-free survival (FFS) of 25% (R-CVP) to 38% (R-CHOP) compared to the low-risk group with 5-year FFS of 68% (R-CVP) to 77% (R-CHOP). It significantly outperformed the FLIPI and was able to reclassify a substantial proportion of patients (44–55%) with a high-risk FLIPI to a low-risk group who had outcomes similar to those with low-risk FLIPI. In limited analyses, it also outperformed the FLIPI2 in the identification of high-risk patients. The m7-FLIPI also proved to be predictive of OS with low-risk patients having 5-year OS of 84–90% compared to high-risk patients with 5-year OS of 41–65%. A recent comprehensive genetic study which involved analysis of more than 1700 genes in 105 patients with primarily untreated follicular lymphoma significantly expanded the number of genes known to be recurrently mutated in FL [44]. In a subset of untreated patients with full clinical annotation, these investigators also investigated the utility of the m7-FLIPI. They did not find significant differences in PFS for patients with high- and low-risk scores, but the study was underpowered for this end point [44].

In additional analyses, the m7-FLIPI was also shown to be predictive of early progression within 24 months (POD24) with 76–77% accuracy [57]; however, approximately half of early progressing patients were still classified as low-risk m7-FLIPI. A subsequent model was generated, the POD24-prognostic index (PI), which consisted of only three gene mutations (*EP300*, *FOXO1*, and *EZH2*) along with the FLIPI. Although the POD24-PI had a higher sensitivity for predicting POD24, it came at the expense of lower specificity, suggesting further work is needed to characterize the genetic markers that define high-risk groups. The m7-FLIPI will also require prospective validation in large clinical cohorts, and its ability to prognosticate patients treated with novel therapeutics (which may differentially impact different genetic lesions) is unknown.

A recent French study used gene expression profiling (GEP) of a cohort of patients from the PRIMA study to develop an expression-based predictor of PFS [58]. This predictor is based on expression levels of 23 genes reflecting both B-cell biology and tumor microenvironment. The predictor was further evaluated in three separate independent cohorts using RNA obtained from formalin-fixed paraffin-embedded tissue and was able to predict PFS independent of FLIPI and maintenance therapy. The median PFS of the combined high-risk groups was 3.1 years compared with 10.8 years in the combined low-risk groups [58].

In the future, tools such as the m7-FLIPI and the GEP score may be used to select treatment for patients based on risk of progression at diagnosis, but this concept must be tested in large trials.

Cytogenetics

While cytogenetic abnormalities are identified in most cases of follicular lymphoma, they do not generally contribute significantly to clinical prognostication. The translocation t(14;18)(q32;q21) is present in approximately 80–90% of cases with no evidence of a difference in OS between positive and negative cases [59–61]. The consequence of this translocation is high-level expression of BCL2 protein resulting in resistance to apoptosis [59]. Approximately 5–15% of FL is found to have abnormalities of 3q27 involving the *BCL6* gene, a transcription factor that is essential for normal germinal center development [60–62]. Cytogenetic abnormalities involving 3q27 appear to be associated with grade 3B histology and a more aggressive clinical course [61, 62]. While the majority of lymphomas harboring translocations involving both *BCL2* and *MYC* (cytogenetic "double hit") are high-grade lymphomas with an aggressive clinical course, cases of "double hit" FL are described [63, 64]. Most commonly, this is associated with high-grade transformation [63]. While "double hit" cytogenetics are associated with a worse prognosis in newly diagnosed high-grade lymphoma, the prognostic impact of these translocations on histologic transformation is not well established.

Minimal Residual Disease (MRD) Assessment

The t(14;18) translocation results in the *BCL2/IgH* fusion gene. Levels of this transcript may be used to monitor FL by quantitative PCR in different compartments, particularly the bone marrow and blood. There is a significant body of literature that establishes the prognostic value of minimal residual disease as defined by persistence of *BCL2/IgH* in the setting of autologous transplantation in FL [65, 66]. The prognostic role of MRD after conventional immunochemotherapy has been more contentious. Some studies such as the large Italian FOLL05 trial in the frontline setting have shown that molecular tumor burden correlates with the quality of response to induction treatment as well as duration of remission [67]. There have been others such as the EORTC 20981 study in relapsed FL that did not show an impact of post-induction molecular status on duration of remission [68]. Limitations of MRD monitoring by *BCL2/IgH* transcripts include the fact that a molecular marker can only be detected in approximately half of patients and that the blood and marrow compartments may not be representative of whole-body residual disease.

Integration of MRD Plus PET

There is interest in using integrated prognostic measures to improve outcome prediction in FL. For example, in the FOLL05 study, a subset of 41 patients were analyzed who had both PET and MRD assessment by *BCL2/IgH* PCR at end of induction [69]. This analysis showed that PET and MRD are not strongly correlated with each other and can be used as complementary techniques at the end of treatment. Patients who had a negative PET and were MRD negative had significant improved PFS at 5 years of 75% compared to 35% if either parameter was positive [69]. A similar analysis of the GALLIUM study is underway. The value of such integrated analyses to tailor post-induction maintenance treatment will be tested in FOLL12 trial.

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Part II
Current Therapy for Follicular Lymphoma

Chapter 6
Management of Localized Low-Grade Follicular Lymphoma

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Overview of Localized Disease

Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma. The majority of FL cases are advanced stage at diagnosis, with frequent nodal and bone marrow involvement. Approximately 20% of patients have localized (stage I–II) disease at the time of presentation [1, 2]. Because early-stage FL is uncommon, it is important to exclude the presence of distant disease with a complete staging evaluation, including a bone marrow biopsy and PET-CT scan, before embarking on a course of definitive local therapy.

In patients diagnosed with localized FL, the median age is 60 years. These patients typically have a good performance status and normal LDH. Disease is often limited to one nodal region at a peripheral site, such as the neck or inguinal basin. Extranodal involvement is observed in approximately 25% of cases [3].

There is great variety in the management approaches used for early-stage FL. The National LymphoCare Study was a multicenter, longitudinal, observational study that included patients treated for FL in academic and community practices. It aimed to identify current demographics and patterns of care in the United States. Of the 2728 subjects enrolled, 474 patients had stage I disease at diagnosis. Management of these patients with stage I disease consisted of radiation therapy (RT) in 23%, rituximab alone in 13%, rituximab with chemotherapy in 30%, and observation in 29%. Among stage I patients not receiving RT as

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initial therapy, 21% received RT within 90 days of completing initial treatment, suggesting a planned combined modality approach [2]. Thus, various treatments are used frequently in this setting. Furthermore, all of these approaches have been associated with favorable outcomes [4]. As no clinical distinction is reliably made between grade 1 and 2 diseases and as many centers treat grade 3A or grade 3B disease as DLBCL, the goal of this chapter is to discuss the management strategies for early-stage grade 1–2 FL.

Radiation Therapy

Radiation therapy (RT) is a recommended approach for stage I or contiguous stage II, low-grade (grade 1–2) FL and has been the historical standard of care. Multiple institutions have reported their experience treating localized FL using definitive RT [5–9], as summarized in Table 6.1. These series demonstrated local control rates of >90% within the irradiated area. However, relapse with systemic disease outside of the radiation field was common, with 10-year relapse-free and overall survival rates of approximately 50% and 60%, respectively. A plateau in the disease-free survival curve was observed beyond 10–15 years, suggesting that a proportion of patients were cured with RT alone. An important limitation of these older series is that at least some patients were treated before metabolic imaging was used for staging. Therefore, patients may have had undiagnosed advanced-stage disease. Furthermore, the RT fields were more extensive and salvage options more limited, so it may be difficult to apply the data to today's experience and prognosis discussions.

To address these concerns regarding older series, the International Lymphoma Radiation Oncology Group (ILROG) reported the outcomes of curative RT for localized FL in patients treated in the modern era, all of whom were staged using PET-CT scans prior to RT. In this cohort, the 5-year freedom from progression (FFP) was 70.2% and overall survival was 95.8%. The 5-year FFP was 74.3% for the subset of patients with stage I disease [10]. Thus, outcomes after RT in these PET-staged patients were better than in some earlier series, suggesting that the curative potential of RT for truly localized FL may have been underestimated previously.

Radiation Dose

The accepted radiation dose for the treatment of FL was established by two randomized dose de-escalation studies. First, Lowry et al. randomized patients with indolent NHL, primarily grade 1–2 FL, to receive the historical, standard dose of 40–45 Gy or the experimental, reduced dose of 24 Gy. No difference in disease response, local progression, disease-free survival, or overall survival rates was observed between the

Table 6.1 Reports of definitive treatment of stage I/II follicular lymphoma with radiation therapy

Institution	Number of patients	Years of treatment	Median follow-up (years)	Freedom from relapse	Overall survival	Local control	Radiation dose	Radiation field	Comments
Princess Margaret Hospital [5]	190	1967–1978	10.6	53%, 12 years	58%, 12 years	87%, 12 years	20–48 Gy	IFRT	No difference in local control for doses between 20 and 40 Gy, when controlling for other prognostic factors
Stanford University [6]	177	1961–1994	7.7	44%, 10 years	64%, 10 years		35–50 Gy	TLI, STLI, EFRT, IFRT	Relapse was rare beyond 10 years after RT
University of Florida [7]	72	1965–1995	8.5	59%, 10 years	46%, 10 years	Four in-field recurrences	20–50 Gy	TLI, EFRT, IFRT	RT dose was not a significant prognostic factor on multivariate analysis, suggesting that low dose was adequate
Harvard University [8]	106	1972–2000	12	46%, 10 years	75%, 10 years	Seven in-field recurrences	Median 36.7 Gy	EFRT, IFRT	The leading cause of death was lymphoma. Second malignancy rates did not differ from the expected number, based on SEER data.
British Columbia Cancer Agency [9]	237	1986–2006	7.3	49%, 10 years	66%, 10 years	3 isolated in-field recurrences; 11 in-field and distant recurrences	20–40 Gy	EFRT, INRT ≤5 cm	No difference in patterns of failure or survival outcomes in patients treated with EFRT or INRT ≤5 cm. Relapse beyond 10 years after RT was rare
International Lymphoma Radiation Oncology Group [10]	310	2000–2016	4.2	70.2%, 5 years	95.8%, 5 years	Six in-field recurrences	Median 30 Gy (range 24–36 Gy)		All patients were staged by PET-CT prior to RT

arms. Lower toxicity rates were observed in the 24 Gy arm [11]. Since no loss of efficacy was associated with the reduced dose compared with the previous standard dose, 24 Gy in 12 fractions became the accepted dose for definitive RT.

The reported efficacy of even lower radiation doses prompted the FORT trial. This non-inferiority study randomized patients to receive a total dose of 24 Gy in 12 fractions or 4 Gy in 2 fractions. In the patients treated with just 4 Gy, the overall response rate (ORR) was 81% (48% complete response [CR] and 32% partial response [PR]). The CR rate was higher in patients treated with 24 Gy. However, given the high ORR, ease of administration, and minimal toxicity associated with 4 Gy, the authors concluded that this very low dose is a useful alternative to 24 Gy in instances when local control is less of a priority [12].

Additionally, 4 Gy should be considered if there is concern that 24 Gy may be associated with excess toxicity. As one example, in the treatment of FL of the orbit, 4 Gy in two fractions is associated with high response rates and minimal toxic effects [13, 14]. Conversely, moderate doses to the orbit, in the range of 24 Gy, have been associated with late toxicity in a substantial proportion of patients [15]. Therefore, a reasonable approach for FL of the orbit is to treat with 4 Gy initially and to escalate the dose only if needed for refractory disease. This strategy may be used in other settings, as well, based on the risk of toxicity from 24 Gy and the importance of establishing local control.

Radiation Volume

Historically, radiation field design has included total lymphoid, extended field, involved field, and involved site techniques. No randomized studies have compared larger and smaller fields. However, retrospective data published by Campbell et al. show no significant difference in the patterns of failure or survival outcomes in patients treated with large, regional fields or smaller fields encompassing the involved node(s) with margins of no more than 5 cm. The authors conclude that the field size may be reduced to include only the involved nodes with a margin of $\leq$5 cm, without loss of efficacy [9]. Based on such data, the ILROG guidelines recommend a “generous” involved site approach for the definitive treatment of FL [16], as shown in Fig. 6.1.

Systemic and Combined Modality Therapy

Lymphoma remains a common cause of death, and distant relapse is the dominant pattern of failure after initial treatment with RT for limited-stage FL. The use of

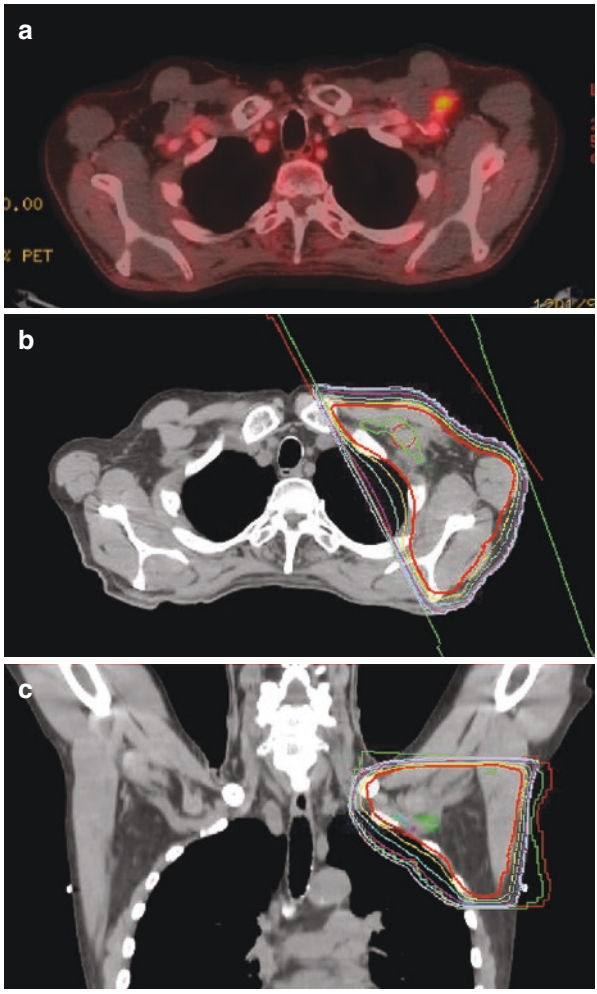

Fig. 6.1 A 67-year-old male presented with a left axillary mass. An excisional biopsy revealed grade 2 follicular lymphoma. A PET-CT showed a 3.7 cm left axillary lymph node with an SUV of 4, with no other evidence of disease (**a**). A bone marrow biopsy was negative for lymphomatous involvement. The patient was treated with definitive radiation therapy to a total dose of 24 Gy (axial slice in (**b**) and coronal slice in (**c**); red isodose line = 24 Gy)

systemic therapy, including cytotoxic chemotherapy, immunochemotherapy, and anti-CD20 antibody monotherapy, in combination with RT or alone, has been investigated by multiple groups attempting to address these issues. Thus far, while improvements in PFS have been seen with some regimens/combinations, no reproducible benefit in OS has been demonstrated, as summarized in Table 6.2. Thus, the use of systemic therapy in limited-stage FL is not recommended routinely outside a trial or in select cases with high burden disease.

Table 6.2 Reports of chemotherapy or combined systemic and radiation therapy for limited-stage follicular lymphoma

Institution	Number of patients treated with systemic therapy	Treatment regimen (s)	Years of treatment	Median follow-up (years)	Freedom from relapse	Overall survival	Comments
Prospective							
MD Anderson [17]	85	30–40 Gy IFRT + cytotoxic regimen without Rituxan	1984–1992	10	72% at 10 years	80% at 10 years	Myelodysplasia and second cancer rates were worrisome
MSKCC [18]	44	RT (regional median 40 Gy) +/− CHOPx6	1980–1988	7	83% vs. 47% $p < 0.03$	88% vs. 66% NS	
British National Lymphoma Investigation [19]	148	RT (25–35 Gy) +/− oral chlorambucil	1974–1981	18	33% vs. 42% at 10 years NS	52% vs. 42% at 10 years NS	
Retrospective							
Ruella et al. [20]	43	Rituximab ×4 cycles → RT	1999–2011	8.6	51% at 10 years	84% at 10 years	NS difference in PFS or OS vs. RT alone on MVA
LymphoCare [4]	206	CT and RT or rituximab alone	2004–2007	4.75	Not reported	Not reported	MVA showed PFS benefit but not OS benefit for immunochemotherapy +/− RT vs. RT alone or observation

Janikova et al. [21]	28	Rituximab or rituximab-RT	1999–2012	<5	85.7–91.7% at 3 years	85.7–100% at 3 years	Small retrospective; limited follow-up in Rituxan arms in particular
Michallet, et al. [22]	87	Rituximab, rituximab + CT, +/− RT	1967–2011	7	At 7.5 years, 60% for rituximab + CT vs. 19–26% for other therapies	66–100% at 7.5 years; NS difference between cohorts	Poor outcomes for RT alone and observation cohorts attributed to imbalance in referral pattern; limits interpretation
Mondello et al. [23]	72	Rituximab +/− IFRT	1995–2012	8	Median 5 years rituximab, 6 years rituximab + RT	Not specified	Improved PFS vs. RT but NS difference in OS
Oslo University [24]	139	CT with or without RT	1980–2005	15	At 10 years, ~25% for CT, ~45% for observation, ~55–60% for RT or CT + RT	At 10 years, ~50% for CT or observation, ~70% for RT or CT + RT	Follow-up shorter for systemic therapy-treated patients but not specified; few rituximab patients. NS difference on MVA between treatment groups
Sancho et al. [25]	73	CT with or without RT	1989–2012	6.8	At 10 years, 61% for CT + RT and 39% for CT	At 10 years, 81% for CT + RT and 72% for CT	NS difference in outcomes on MVA between treatment groups; ~1/2 patients received rituximab

IFRT involved field radiation therapy, *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisone, *RT* radiation therapy, *CT* chemotherapy, *NS* not significant, *PFS* progression-free survival, *OS* overall survival

Historical Trials of Adjuvant Chemotherapy with Radiotherapy

Early investigations of adding systemic therapy to RT in FL occurred in an era before "rigorous" staging techniques or modern classification of indolent NHL, which complicates their comparison to current series. Overall, they demonstrated that systemic therapy conferred no OS benefit and added toxicities not considered justifiable for treatment of asymptomatic, low-burden disease.

- Seymour et al. [17] at MD Anderson Cancer Center conducted a prospective study of risk-adapted chemotherapy (cyclophosphamide, vincristine, prednisone, bleomycin +/− doxorubicin depending on risk features) with 30–40 Gy IFRT in indolent NHL including 85 patients with limited-stage FL from 1984 to 1992. With a median follow-up of 10 years, 10-year PFS was 72% and OS 80% in FL patients. However, two cases of myelodysplasia and ten second malignancies (four within RT field) and the acute toxicities of therapy tempered enthusiasm for this regimen, without direct comparative proof of its benefit in comparison to RT alone.
- Yahalom et al. [18] at Memorial Sloan Kettering Cancer Center conducted a randomized prospective trial of regional RT followed by six cycles of CHOP chemotherapy in 44 patients with stage I intermediate-low-grade NHL between 1980 and 1988. At a median follow-up of 7 years, there was an improvement with combined modality therapy for PFS (83% vs. 47%) but no significant difference in OS (88% vs. 66%, $p = 0.2$). Of note, these patients' treatment predated the use of advanced imaging techniques and the current classification of NHL.
- The British National Lymphoma Investigation group conducted a randomized trial [19] of low-grade limited-stage NHL from 1974 to 1981 in 148 patients, who received either RT alone or RT and oral chlorambucil. At a median 18-year follow-up, no PFS or OS difference was seen.

Retrospective Comparisons of Radiation Alone to Systemic Therapy with or Without Radiation

More contemporary retrospective studies have evaluated varying combinations of the anti-CD20 antibody with systemic therapy regimens and/or RT. These have found at most a suggestion of benefit for PFS but not OS with the addition of systemic therapy to RT:

- Ruella et al. [20] evaluated patients with limited-stage grade 1–3A FL who underwent either RT alone ($n = 51$) or RT followed by four cycles of rituximab anti-CD20 therapy ($n = 43$). At a median follow-up of 10.9 years, they found improved 10-year PFS in the combined therapy vs. RT alone group on univariate

analysis, but not on bivariate analysis adjusting for stage. No difference in OS was seen.

- The LymphoCare [4] observational study included a subset report of stage I patients who were staged with CT or PET and bone marrow biopsy. This analysis included a comparison of 206 patients treated with systemic therapy and RT to those managed with observation, anti-CD20 therapy, immunochemotherapy, or RT alone. A PFS benefit over RT alone or observation was seen on multivariable analysis for those receiving either immunochemotherapy or combination systemic therapy with RT. Again, no OS difference was identified. No difference was seen in PFS for these combinations vs. anti-CD20 monotherapy.
- Janikova et al. [21] reported a small retrospective series with short follow-up (5 years or less for all subgroups) in which 93 patients with stage I–II grade 1–3A FL were treated with RT alone ($n = 65$), rituximab alone ($n = 14$), or rituximab and RT ($n = 14$). The 3-year PFS was worse for RT alone vs. rituximab/RT or rituximab alone (57.4% vs. 85.7% vs. 91.7%, respectively). However, no multivariable analysis was performed, and time periods and follow-up were substantially different between the RT and combination/rituximab arms. OS was not significantly different.
- Michallet et al. [22] reported a series of 145 early-stage FL patients undergoing RT, rituximab, chemotherapy, chemotherapy and rituximab, chemotherapy and RT, or observation. Improved 7.5-year PFS was seen in the immunochemotherapy arm (60%) compared to all other arms (19–26%). However, the exceptionally poor performance of these other arms, especially RT, compared to multiple other series was noted and ascribed by the authors to referral of patients to their center at the time of relapse. Notwithstanding this issue, which clouds our assessment of the study comparison, OS was not significantly different between arms.
- Mondello et al. [23] reported on 108 early-stage FL patients treated with RT ($n = 36$), rituximab ($n = 38$), or combination rituximab and RT ($n = 34$) with 8 years of follow-up. Despite the higher incidence of adverse features in the rituximab-containing groups, they observed improved PFS for rituximab alone or in combination with RT, compared to RT alone (median PFS of 5–6 years vs. 2.3 years). While OS trended toward improvement in the rituximab arms ($p = 0.059$), it did not reach significance.
- The Oslo University Hospital series [24] included 404 early-stage FL patients who underwent RT, observation, chemotherapy, or chemotherapy and RT. Most patients were treated before the introduction of rituximab. On multivariate analysis, no differences in PFS or OS were seen according to initial management.
- Sancho et al. [25] reported on 130 patients with limited-stage FL managed with RT ($n = 46$), RT and chemotherapy ($n = 30$), chemotherapy alone ($n = 43$), or observation ($n = 11$). No OS benefit was seen, but in those treated with combined RT and chemotherapy, multivariable analysis indicated significantly improved PFS (HR 0.3, $p = 0.024$).

Ongoing Trials

- MD Anderson Cancer Center is conducting a trial of RT with rituximab followed by maintenance rituximab (NCT0143628).
- The German Low-Grade Lymphoma Study Group (GLSG) is conducting the MIR (Mabthera® and Involved field Radiation) phase II trial of induction rituximab followed by restaging and concurrent rituximab with RT (NCT00509184).

Observation

Despite the data for potentially curative treatment with RT, some have argued against its use in limited-stage disease, due to the long, indolent natural history of FL [26], the frequent relapses outside radiation fields, and the lack of improvement in OS. Instead, they have argued for observation in the setting of low-volume disease. A desire to avoid or delay the toxicity of immediate treatment has thus extended to the limited-stage population. This practice pattern is evident from the significant rate of observation, in lieu of immediate treatment, in large registry studies, with over 400 limited-stage FL patients each, conducted in the United States (28.7%) [2] and Norway (~15%) [24]. While published series of selected patients undergoing observation have demonstrated no difference in OS compared to immediate therapy (excepting a SEER analysis comparing RT- to non-RT-treated patients, which did not delineate observed vs. systemic therapy-treated patients [27]), only limited data are available regarding observation in the setting of limited-stage disease. Nonetheless, observation may be the preferred option in patients with significant co-morbidities, noncontiguous stage II disease, or fully resected stage I disease.

Observation Outcomes

Data for observation in limited-stage low-grade FL stems primarily from retrospective studies with varying time periods, staging methods, definitions of observation, and reasons for treatment initiation. A particularly rigorous investigation from Stanford evaluated 43 patients with a median follow-up of 86 months, who underwent uniform staging with bone marrow biopsy and computed tomography. Of note, this study was conducted in the pre-PET era. These patients did not receive any therapy for at least 3 months after diagnosis. They achieved an impressive median overall survival of 19 years and 10-year freedom-from-treatment rate of 56%. These outcomes were superior to those from a series of patients treated with RT [6] from the same institution (median overall survival of 13.8 years). However, for the 37% of patients who required treatment, overall survival was 8.3 years. Furthermore, four patients experienced transformation, even in this highly selected population.

Soubeyran et al. [28] reported on 26 patients at their institution who were followed with observation after full excision of their disease ("stage I0"). They reported a 50% crude rate of freedom from relapse after a median follow-up of 6.3 years. The predominant pattern of relapse was at distant sites.

In another single institutional retrospective series, Michallet et al. [22] observed 36 patients (definition not given in manuscript) with unclear median follow-up (likely short given the overall cohort was 7-year median follow-up and most observation patients were treated in more recent era). At 7.5 years, the progression-free survival rate was 26% and overall survival was 72%.

Further data has been made available from registry studies or investigations of observation in all stages of disease, though not all have specified outcomes for the limited-stage cohort [29, 30]. The LymphoCare prospective observational registry of FL patients managed from 2004 to 2007 at community practices primarily reported outcomes for stage I disease that was "rigorously" staged by bone marrow biopsy and either CT or PET. Thirty-five patients were managed with observation, defined as having received no therapy for 3 months after diagnosis [4]. This cohort did not have actuarial outcomes specified; however, OS was reported to be not significantly different for patients who were observed or given immediate therapy, with a median follow-up time of 57 months. Lastly, a recent series from the Oslo University Hospital [24] compiled outcomes of 63 patients undergoing observation. With a median follow-up of 15 years, the crude rate of progression was 46%, and no difference in OS was observed for patients who were observed compared to those who received immediate therapy.

Selection for Observation

As observation is used for differing reasons, selection criteria in published work have varied. Common reasons include the following:

- Fully excised stage I FL.
  - *Rationale*: Lower benefit to local therapy in the absence of gross disease.
  - *Data*: Soubeyran et al. [28] reported a series of 26 patients achieving 50% crude relapse-free survival at 6.3 years of follow-up. In the Oslo University Hospital series [24], those patients undergoing observation after full excision demonstrated superior PFS compared to those with asymptomatic residual disease ($p = 0.03$).
  - *Comment*: In rigorously staged patients with no residual disease after excisional biopsy, observation may be considered after counseling patients that a substantial progression risk may remain. Given the low morbidity of modern doses of radiation for FL, performing a more radical surgery to allow observation is not supported.

- Concern over RT toxicity due to large field requirement and/or location of disease.
  - *Rationale*: Multifocal areas of noncontiguous FL may require large fields to encompass all sites of disease, resulting in more treatment-related toxicity. Furthermore, noncontiguous stage II disease may portend a higher risk of occult distant involvement outside of the RT field. Additionally, observation might be recommended if disease is in close proximity to radiosensitive normal tissues, causing concern for RT-induced toxicity. Together, a lower therapeutic ratio from local RT may justify observation.
  - *Data:* In the Stanford series [31], the rationale for observation in 33% of cases was large abdominal field/salivary gland involvement. Most patients (74%) had stage II disease. In the Oslo series [24], observation was recommended in 43% of cases based on stage II presentation with nonadjacent nodal involvement.
  - *Comment*: As noted in the previous RT section, randomized controlled trials of RT for indolent B-cell NHL have recently established a relatively low dose of 24 Gy as the standard dose, 4 Gy as an alternative dose with lower control but minimal toxicity, and smaller fields for treatment. Thus, while select cases of discontinuous widespread or bulky stage II disease may merit consideration of management as "advanced" disease with observation [32], an attractive alternative is treatment with RT to just 4 Gy. This very low dose results in high response rates with minimal risk of toxicity.
- Patient comorbidities.
  - *Data:* No specific data.
  - *Comment:* As with other malignancies, an understanding of the life expectancy of a sick or elderly patient relative to the natural history of limited-stage FL should guide management. In the setting of a limited life expectancy, the use of either observation or very low-dose (4 Gy in two fractions) RT may be appropriate.

Comparison to Treatment

Comparison of outcomes after observation vs. immediate treatment is challenging. First, observation cohorts are subject to selection bias. Furthermore, most data for observation remains retrospective, with follow-up shorter than the ~10 years typically needed before the PFS curves plateau after RT. With that said, currently published series of observation for limited-stage low-grade FL have not demonstrated significant differences in OS compared to immediate therapy with varying approaches, including RT, combined modality systemic therapy, and radiation and anti-CD20 therapy combinations. These comparative data are summarized below:

- The Oslo University Hospital [24] analyzed 404 patients with early-stage FL managed with either observation (15%; fully excised stage I or discontinuous stage II), RT alone (48%), systemic therapy (16%; including cytotoxic regimens, immunochemotherapy, or anti-CD20 antibody alone), or combined chemotherapy and RT (16%). On univariate analysis, RT-treated patients demonstrated improved OS compared to systemic therapy-treated patients and a trend toward improved OS compared to observation patients ($p = 0.054$). However, multivariable analysis demonstrated no difference in PFS or OS between cohorts.
- Michallet et al. [22] evaluated 145 patients with limited-stage FL treated or referred upon relapse to their hospital from 1967 to 2011 with a median 7-year follow-up. Treatment was observation ($n = 36$), RT ($n = 21$), rituximab alone ($n = 7$), chemotherapy/RT ($n = 18$), or immunochemotherapy ($n = 36$). Monotherapy with rituximab was associated with a poor complete response rate. Only immunochemotherapy was associated significantly with improved PFS but no difference in OS. The authors conclude that observation is reasonable, but, when treatment is required, immunochemotherapy may be preferred. However, this conclusion is challenged by an exceptionally poor performance in 7.5-year PFS of their observation (26%), RT (19%), chemotherapy/RT (26%), and chemotherapy (23%) cohorts. These PFS rates are significantly inferior to outcomes reported for each approach in multiple other series. The authors suggest that these outcomes were poor, because some patients were referred to their center at the time of relapse.
- The LymphoCare [4] observational registry evaluated a cohort of 206 patients with stage I FL who underwent "rigorous" staging, including a bone marrow biopsy and PET or CT. The subgroup of 35 patients who were observed experienced no significant difference in OS compared to those treated initially with immunochemotherapy, anti-CD20 therapy alone, RT alone, or combined chemotherapy and RT. However, PFS was superior for those receiving either combined RT and systemic therapy or immunochemotherapy.

Conclusion

A variety of management approaches are used for early-stage, low-grade FL. The standard of care for stage I and contiguous stage II disease remains involved site RT. Typically, a dose of 24 Gy is used for definitive therapy; however, a total dose of just 4 Gy is reasonable in some cases. Incorporation of systemic therapy into management may result in improved PFS, but no improvement in OS has been shown. This strategy is an area of active study. Lastly, observation may be an appropriate strategy in select patients. A long natural history and evolving treatment approaches complicate the study of FL. Multi-institutional collaboration, with standardized pretreatment evaluations, management strategies, and follow-up schedules, is recommended to provide further insight into the optimal treatment of early-stage grade 1–2 FL.

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Chapter 7
Current Management and Novel Approaches to the Management of Follicular Lymphoma

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Frontline management of follicular lymphoma (FL) requires a careful consideration of a number of clinical features including the patient's age and fitness, burden of the disease, prognostic biomarkers, and the patient's wishes. Strategies for patients with newly diagnosed and untreated disease can range from watchful waiting to an aggressive, anthracycline-based induction therapy with consideration of post-induction consolidation and maintenance. Fortunately, most patients with newly diagnosed FL will experience prolonged survival despite the propensity for relapse, and as a result, therapy decisions must also take into account the possibility of long-term toxicities and the need for future treatments. In this chapter, we review the indications for treatment of FL, currently available therapies, and the role of maintenance treatment.

Identification and Management of Patients with Low Tumor Burden

The primary goal of the initial evaluation for a patient with untreated FL is to determine whether or not treatment is actually required at the time of diagnosis. Although counterintuitive to many patients who are facing a new diagnosis of cancer, the

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deferral of initial therapy is often the most appropriate approach and can delay the toxicity and risks of therapy for several years. Horning and Rosenberg were among the first to describe outcomes for patients with low-grade non-Hodgkin lymphoma who were initially observed, reporting a median time to initiation of therapy of 3 years and a median overall survival of 11 years in a series of 83 patients with low-grade NHL at Stanford who were initially observed [1]. Subsequent studies, including randomized controlled trials, have failed to identify a survival benefit for patients with low-grade NHL who initiate therapy at the time of diagnosis [2–4]. However, these studies were all conducted prior to the use of rituximab, and the comparator arms included oral alkylating agents, interferon-based immunotherapies, or combination of cytotoxic regimens. As a result, their impact on treatment decisions in the current era is somewhat limited.

Despite the more than 20 years since its publication, the study by Brice et al. comparing watchful waiting to either oral alkylator (prednimustine) or interferon-alpha supplied the treatment-initiation criteria that are most frequently utilized in the modern era (i.e., GELF criteria; Table 7.1). Although they were utilized to identify "low tumor burden" in a different era, these criteria are frequently utilized in current clinical practice.

In the modern (i.e., rituximab) era, additional assessments of early therapy for patients with low tumor burden have been completed and suggest excellent outcomes regardless of approach. The most frequently explored therapy for untreated patients is rituximab, which is currently utilized alone and in various combinations in the management of patients with untreated and relapsed FL as well as in a maintenance approach after initial therapy. Ardeshna et al. conducted a randomized phase 3 study of watchful waiting, 4 weekly doses of rituximab, or 4 weekly doses of rituximab followed by maintenance rituximab (1 dose every 2 months for 2 years) in 379 patients with untreated FL with a low tumor burden [5]. The criteria to identify low tumor burden in this study closely approximated the GELF criteria. Similar to prior studies, there was no improvement in overall survival (OS) between the rituximab-treated and the watch and wait groups. However, patients assigned to watch and wait had a significantly shorter time to next treatment (median of 31 months vs median not reached in the rituximab maintenance group). Additionally,

Table 7.1 GELF criteria for initiation of therapy in follicular lymphoma

Any nodal or extranodal mass > 7 cm in diameter
Involvement of at least 3 nodal sites, each >3 cm in diameter
Presence of any systemic and/or B symptoms
Splenic enlargement with inferior margin below the umbilical line
Compression syndrome (ureteral, orbital, gastrointestinal)
Pleural or peritoneal fluid collection
Leukemic phase (> 5×10^9/L circulating cells)
Cytopenias (neutrophil count <1.0×10^9/L or platelet count <100×10^9/L)

patients receiving rituximab maintenance reported an improved quality of life with decreased anxiety/depression and improvement in the Mental Adjustment to Cancer score at 7 months post study entry when compared to patients assigned to watch and wait. These findings would suggest that further treatment can be delayed by initiating therapy early in the course of patients with FL, but the study was limited by the fact that retreatment (or initial treatment) with single-agent rituximab was considered a subsequent therapy. However, rituximab as a single agent utilized as induction followed by maintenance was associated with good quality of life and limited comorbidities and is a reasonable option for patients with low tumor burden. However, the true impact of this intervention on the long-term outcome of the disease is likely modest as nearly all patients with low tumor burden FL will have prolonged survival and early intervention does not appear to result in any appreciable difference.

While Ardeshna et al. attempted to explore the role of induction alone vs induction + maintenance for patients with low tumor burden, the study was amended due to low accrual to ultimately be a comparison of watch and wait vs induction + maintenance. However, in ECOG 4402 (RESORT), Kahl et al. evaluated the role of rituximab maintenance vs rituximab re-treatment in patients with FL and low tumor burden [6]. In this study, 408 patients received 4 weekly doses of rituximab and responding patients ($n = 299$) were randomly assigned to maintenance (one dose of rituximab every 13 weeks until progression) or retreatment (observation and retreatment with 4 doses at the time of progression). The primary endpoint for this study was time to treatment failure, defined as: no response to rituximab retreatment, time to progression of <26 weeks, initiation of an alternative therapy, or inability to complete planned therapy. No differences between arms were identified with regards to the primary endpoint of TTF (50% vs 53% at 5 years for retreatment vs maintenance, respectively). However, secondary analyses suggested that patients in the retreatment arm were likely to require cytotoxic therapy sooner and to have an inferior response duration when compared to patients receiving maintenance. Subsequent analyses of quality of life have identified no significant benefit to either approach [7].

Based on the available studies, observation of patients who are asymptomatic and have a low tumor burden remains a reasonable option for newly diagnosed patients with follicular lymphoma. We continue to utilize the GELF criteria in the absence of a clear alternative approach to identifying patients with high tumor burden requiring therapy. However, in patients for whom watchful waiting is not felt to be appropriate due to patient preference or patient-specific clinical findings, induction with 4 weekly doses of rituximab without maintenance is appropriate therapy for the majority of patients and most patients will not require cytotoxic therapy for many years (80% free of cytotoxic therapy at 5 years) and will spare the costs associated with maintenance rituximab in this cohort of patients likely to experience prolonged OS.

Additional assessments of tumor burden have been utilized to justify initiation of therapy, including the FL international prognostic index (FLIPI) [8]. Although this

index was initially developed in the pre-rituximab era to identify patients at risk for early death from FL, its use of several features associated with tumor burden, including extent of nodal disease, disease stage, and lactate dehydrogenase level, has resulted in its use as an indication for initiation of therapy in several recent studies including E2408 and CALGB50904, both of which investigated the role of intensified induction therapy in patients with high-risk FL [9, 10]. Although there is frequent overlap between patients who have high tumor burden by GELF criteria and those who have intermediate or high FLIPI, these are not mutually exclusive, and it is reasonable to consider both measures when deciding on the appropriate timing of therapy. Biologically based approaches to risk stratification of asymptomatic patients remains challenging, and while recurrent mutations and gene expression signatures have been utilized to determine underlying disease risk, these are not typically utilized in practice and to date have not superseded clinical assessment when determining tumor burden and the need for immediate therapy for newly diagnosed patients [11].

Management of Patients with Advanced-Stage FL

Although there are a number of potential options for patients with limited stage and/or low tumor burden at the time of diagnosis, most patients with FL will at some point in their treatment have advanced-stage disease that requires systemic therapy. At the present time, none of the currently available therapies are considered curative, so it is essential that any discussion of therapy includes considerations of short- and long-term toxicities in addition to a review of efficacy and expected remission durations.

Historically, > 50% of all patients with FL with newly diagnosed FL have been treated with rituximab in combination with chemotherapy [12]. However, identifying the optimal chemotherapy backbone and the best CD20 antibody has been challenging with a number of studies conducted comparing potential approaches. Most patients currently receive either CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), CVP (cyclophosphamide, vincristine, and prednisone), or bendamustine, nearly always combined with a CD20 monoclonal antibody such as rituximab. Additional studies have been conducted to evaluate the role of targeted or other novel therapies in the frontline setting, both alone and in combination with traditional regimens.

R-CHOP has long been the gold standard regimen for many NHL subtypes, but the importance of anthracycline use in the upfront setting for indolent NHL has not been as well established. The FOLL05 study conducted in Italy compared R-CVP to R-CHOP and to a fludarabine-based therapy (R-FM). In this study, R-CVP was inferior to both R-CHOP and R-FM, with a lower response rate and an inferior 3-year PFS (52% vs 68% (R-CHOP) vs 63% (R-FM), $p = 0.011$) [13]. This study was recently updated after a median follow-up of 7 years and demonstrates no evi-

dence of an impact on OS with either regimen, although patients treated with R-CVP remained at higher risk for relapse and need for subsequent treatment [14].

Both R-CVP and R-CHOP have been compared to R-Bendamustine (B-R) in a randomized study. The BRIGHT study was a noninferiority randomized study comparing R-CVP or R-CHOP (investigator choice) with B-R, with the primary endpoint of this study being the CR rate [15]. In this study, the CR rate for B-R was 30% vs 25% for R-CHOP/R-CVP, meeting the noninferiority endpoint, while the overall response rate was >90% for both arms. This study was recently updated with 5-year follow-up, and while the PFS appears to be improved for patients who received B-R, there was no difference in OS.

The second large randomized study comparing B-R to R-CHOP was the STiL study conducted in Europe [16]. This study randomized 514 patients (279 patients with FL) to either B-R or R-CHOP for 6 cycles. Although the study included patients with a variety of NHL subtypes, results were presented based on disease histology, and within the FL cohort, there was a significant improvement in PFS for B-R compared to R-CHOP (median not reached vs 41 months). Subsequent follow-up for this study has continued to demonstrate a benefit in time to next treatment and PFS although there is no significant difference in OS between these arms [17].

These studies have consistently identified toxicity concerns for both approaches that require consideration when selecting therapy. Myelosuppression is significant with both approaches. The rate of grade 3–4 neutropenia with B-R ranges from 29–44% between the two studies compared to 69–87% for R-CHOP. However, grade 3–4 lymphopenia is increased with B-R (62–74% vs 33–43%). As a result, patients receiving both regimens are at risk for while on treatment. Grade 3–4 thrombocytopenia occurs in up to 12% of patients receiving these regimens with no clear difference between B-R and R-CHOP. Fortunately, nonhematologic toxicity ≥ grade 3 is unusual with both regimens, although R-CHOP results in alopecia in nearly all patients and carries an increased risk of neuropathy and cardiomyopathy while B-R is associated with cutaneous reactions more frequently than R-CHOP. Despite the reported toxicities while on treatment, both R-CHOP and B-R can successfully be administered to patients of nearly all ages with close clinical monitoring.

In addition to the investigation of the appropriate chemotherapy backbone for untreated patients with FL, the ideal CD20 monoclonal antibody remains a topic of study. Rituximab has been included in the management of patients with FL for several years after it was demonstrated to improve outcomes for patients receiving CVP and CHOP in two separate studies [18, 19]. However, newer antibodies including ofatumumab and obinutuzumab have been assessed in this setting as well [10, 20–22].

Ofatumumab is a fully human monoclonal antibody to CD20 which has been evaluated in a number of B-cell malignancies. Its clinical use in FL has been limited, but it has been safely combined with bendamustine and CHOP in the front-line setting where the overall response rate is at least 90% with both regimens [21, 22]. These studies were both phase 2 studies, and there are no randomized studies comparing ofatumumab vs rituximab in the front-line setting in FL. Ofatumumab is

currently only approved in the United States for chronic lymphocytic leukemia and its role in the management of FL is likely very limited. Ofatumumab was recently utilized in the upfront setting in CALGB 50904, where it was combined with bendamustine ± bortezomib [10]. The study was not designed to identify a benefit vs rituximab, and while the ofatumumab was well-tolerated without a significant safety concern, the efficacy did not appear to be significantly improved over a similarly designed study with rituximab presented simultaneously (E2408) [9].

On the other hand, obinutuzmab is a glyoengineered type II CD20 monoclonal antibody and has demonstrated some impressive activity in FL, both in the frontline setting and at relapse [20]. The recently published GALLIUM study randomized patients with untreated FL to receive either obinutuzmab + chemotherapy or rituximab + chemotherapy [20]. Chemotherapy was predetermined at each site and could be either bendamustine, CVP, or CHOP. Over 1200 patients were enrolled and received between 6 and 8 cycles of therapy. The primary endpoint of the study, PFS, compared outcomes between patients receiving rituximab or obinutuzumab regardless of chemotherapy regimen, and patients receiving obinutuzumab had improved 3-year PFS (80% vs 73%, $p = 0.001$). There was no significant improvement in OS, and concerns have been raised regarding the toxicities encountered in this study, especially with regards to myelosuppression and infection. At the present time, obinutuzumab + chemotherapy is considered an option for patients with FL, is currently FDA approved for that indication, and is listed in the NCCN Guidelines. However, its use has not been universal due to persistent toxicity concerns as well as a perceived incremental benefit over rituximab in the GALLIUM study that many feel is not reflective of a true clinical improvement.

Incorporation of Novel Therapies in the Upfront Management of Advanced-Stage FL

While most patients in the United States continue to receive chemotherapy-based induction regimens when therapy is indicated, a number of newer therapies have been investigated, with and without chemotherapy. Lenalidomide is an oral immunomodulator which is currently FDA approved for relapsed mantle cell lymphoma in addition to indications in multiple myeloma and myelodysplastic syndrome. It has been combined with rituximab in untreated FL in two phase 2 studies with slightly different rituximab treatment schedules (weekly during cycle 1, then 4 additional doses vs day 1 of each cycle) [23, 24]. Patients received treatment for up to 1 year in both studies, and lenalidomide was dosed at 20–25 mg on days 1–21 of a 28-day cycle. The overall response rate was 95%, and the CR rate was quite high, ranging from 72% to 87%. The duration of response was also high, with a 5-year PFS of >70% in both studies. Grades 3–4 hematologic toxicities were relatively common, and nonhematologic toxicities were limited but included rash, infection, and fatigue among others. In general, the rate of grades 3–4 nonhematologic

toxicity is low. However, roughly ¼ of patients required a dose reduction at some point in their treatment. As a result of these promising findings, R-lenalidomide has recently been compared with R-chemotherapy in the phase 3 RELEVANCE study, with formal reporting of the results still pending.

The Bruton's tyrosine kinase inhibitor, ibrutinib, is currently approved for management of mantle cell lymphoma, Waldenstrom's macroglobulinemia, marginal zone lymphoma, and chronic lymphocytic leukemia. However, its efficacy in FL has been more modest, with a response rate of only 37.5% and median PFS of 14 months in a phase 2 study in patients with relapsed/refractory FL [25]. In combination with rituximab, however, patients with untreated FL had an overall response rate of 82%, and the median PFS was not reached with limited duration of follow-up [26]. Further follow-up will be needed to determine whether the ibrutinib is truly providing benefit in this setting as opposed to the rituximab as the response to single-agent rituximab is well described in untreated FL. There are randomized studies currently enrolling which will aim to answer this question.

The triplet of rituximab, ibrutinib, and lenalidomide has also been evaluated in a phase 1 study through the Alliance cooperative group (A051103) [27]. This study included lenalidomide for up to 18 cycles and ibrutinib until progression. There were no dose-limiting toxicities identified, but the incidence of rash was high (36% of patients with grade 3 rash). The overall response rate was high at 95%, and the 12-month PFS was 80%. However, the frequency of rash and other toxicities as well as lack of clear improvement on other combinations including R-lenalidomide made this combination less appealing, and the authors recommended not to pursue this triplet further.

Similarly, the PI3K inhibitor, idelalisib, is currently approved as monotherapy for relapsed follicular lymphoma but has not been safely combined with other agents, especially in the front-line setting [28]. In combination with lenalidomide and rituximab (A051202), the incidence of grade 3–4 rash was 50%, and several patients experienced hepatotoxicity and sepsis-like syndromes, prompting close of the study after only 8 patients were enrolled, and additional studies have confirmed the unacceptable toxicity of this combination [29, 30]. In addition, the combination studies idelalisib with bendamustine and rituximab in the front-line setting has been associated with significant infectious toxicities including pneumocystic jiroveci pneumonia and cytomegalovirus reactivation. Additional PI3K inhibitors are currently being evaluated for indolent NHL and their risks of infectious and immune-mediated toxicities continue to be assessed [31].

The proteasome inhibitor bortezomib has also been evaluated in untreated FL, and in combination with rituximab, the ORR is 76%, with a CR rate of 44% [32]. Based on these promising findings, subsequent trials have evaluated the role of bortezomib in combination with chemotherapy, including a phase 2 study of bortezomib combined with R-CHOP [33]. In this phase 2 study that included 20 patients with FL, the ORR was 100%, and the 4-year PFS was 83%. To limit neuropathy, the vincristine dose was capped at 1.5 mg, and bortezomib was administered at a dose of 1.6 mg/m^2 on days 1 and 8 of each cycle. Neuropathy of any grade was common,

but only 2 patients (out of 29 treated patients across NHL histologies) experienced grade 3 neuropathy.

Bortezomib has recently been evaluated in two randomized studies using a CD20-antibody + bendamustine backbone (E2408 and CALGB50904) [9, 10]. Despite promising efficacy in early studies, including with chemotherapy, neither of these studies demonstrated an improvement in PFS outcome with inclusion of bortezomib. While secondary analyses may ultimately find a subset of patients who may benefit from its use, we would generally not recommend combination of bortezomib with bendamustine-based induction therapies in FL.

Summary of Induction Approaches

We have summarized the currently available data for the most commonly utilized (and recently studied) approaches to induction therapy for advanced-stage FL in Table 7.2. While we await further description of outcomes from studies that are

Table 7.2 Currently available and investigated induction therapies for advanced-stage FL

Regimen	Source	ORR/CR rate	PFS	OS
Rituximab	E4402/Kahl [6]	71%/12%	–	5 years: 94%
R-CVP	Marcus [35] FOLL05/Federico [13]	81%/41% 88%/67%	38mo (DOR) 3 yr: 52%	4 yr: 83% –
R-CHOP	FOLL05/Federico [13] StIL/Rummel [16]	93%/73% 91%/30%[a]	3 yr: 68% Med: 41mo	– 10 yr: 66%
R-B	StiL/Rummel BRIGHT/Flinn [15] E2408/Evens [9]	93%/40%[a] 97%/31%[a] 90%/58%	Med: NR 5 yr: 66% 3 yr: 74–76%	10 yr: 71% 5 yr: 82% 3 yr: 84–87%
R-Lenalidomide	Fowler [24] CALGB50803/Martin [23]	98%/87% 95%/72%	3 yr: 79% 5 yr: 86%	3 yr: 94% 5 yr: 100%
G-CHOP	GALLIUM/Marcus [20, 36]		3 yr: 80.6%	
G-Bendamustine	GALLIUM/Marcus [20, 36]		3 yr: 84.1%	
O-Bendamustine	CALGB50904/Blum [10]	92%/59%	4 yr: 53%	4 yr: 87%
R-Ibrutinib	Fowler [37] Arm 1: R-Ibrutinib Arm 2: Ibr lead-in	85%/35% 75%/35%	1 yr: 87% 1 yr: 77%	1 yr: 98% 1 yr: 100%
B-V-R	E2408/ Evens [9]	91%/74%	3 yr: 81%	3 yr: 90%
B-V-O	CALGB50904/Blum [10]	84%/57%	4 yr: 67%	4 yr: 84%
V-RCHOP	Cohen [33]	100%/75%	4 yr: 83%[a]	4 yr: 93%[a]

Note: The authors recommend a review of individual studies when considering the impact of these data as studies often have differing inclusion/exclusion criteria and tumor burden requirements for study entry

Abbreviations: *ORR* overall response rate, *CR* complete response, *PFS* progression-free survival, *OS* overall survival, *R* Rituximab, *CVP* cyclophosphamide, vincristine, prednisone, *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisone, *B* bendamustine, *G* obinutuzumab, *O* ofatumumab, *V* bortezomib

[a]Denotes study that included additional indolent NHL subtypes

evaluating novel therapies in combination and separate from chemotherapy, most patients are safely and effectively managed with chemotherapy-based induction regimens. While the prospect of a non-chemotherapy-based induction regimen is intriguing, one must be mindful of the financial burden of long-term oral therapy as well as the impact on quality of life of long-term therapy, especially when "mild" toxicities persist for many months [34].

Post-induction Outcomes

Fortunately, most patients with FL will respond to initial therapy, regardless of the chosen regimen, and survival is prolonged for most patients with FL. In the FLASH study which combined patient-level outcomes from 13 randomized studies, the median PFS after induction therapy for FL was >7 years, and achievement of CR at 30 months post initiation of induction therapy was considered a suitable surrogate for PFS [38]. However, a subset of patients (19–25%) will experience a relapse of disease within 2 years of diagnosis and will have inferior outcomes. In an analysis from the National LymphoCare Study, only 50% of patients treated with R-CHOP with early progression were alive at 5 years compared to 90% of patients who did not experience an early relapse. These findings were validated in the FLASH cohort, where the 5-year OS for early progressors is 62% vs 87.5% in patients who do not experience early progression [39].

Management of patients with early relapse is challenging and is the subject of many ongoing investigations, including S1608, which will randomize patients with early relapsing FL to three arms: (a) obinutuzumab + umbralisib; (b) obinutuzumab + lenalidomide; (c) obinutuzumab + CHOP. In addition, the role of stem cell transplantation has been assessed for patients with early relapse, and autologous stem cell transplant appears to improve outcomes compared to patients who do not receive autologous transplant from a NLCS/CIBMTR study recently published [40]. The role of allogeneic transplantation in this setting is also being evaluated. Additional approaches to the management of relapsed/refractory FL are discussed in more detail in Chap. 12.

Post-induction Therapies Designed to Improve Outcomes

Rituximab Maintenance

Despite the likelihood of prolonged survival for most patients with advanced-stage FL, attempts to prolong the initial duration of response are ongoing given the fact that nearly all patients with FL will ultimately relapse. The most frequently utilized approach to post-induction therapy is rituximab monotherapy, which has been utilized in a variety of schedules and settings. To date, none of the published studies

have identified an OS advantage with maintenance rituximab although there does appear to be a consistent benefit in PFS.

Some of the initial evaluations of maintenance rituximab included various schedules. Hainsworth et al. published the results of a phase 2 study which utilized rituximab monotherapy administered for 4 consecutive weeks every 6 months for up to 2 years (for total courses) [41]. In this series of 60 patients, the ORR at the end of treatment was 73%, and the median PFS was 34 months. This maintenance schedule was also utilized in E1496, which randomized patients receiving CVP to rituximab maintenance (given for 4 weekly doses every 6 months for 2 years) or observation [42]. This study was updated recently with prolonged follow-up, and the maintenance arm outperformed the observation arm (median PFS 4.8 years vs 1.3 years) but there is no significant difference in OS [43]. Ghielmini et al. conducted a randomized study of rituximab maintenance where all patients received 4 weekly doses and were then randomized to observation vs 4 additional doses of rituximab spaced 2 months apart [44]. The group of untreated patients receiving maintenance had improved PFS compared to the observation group (36 vs 19 months).

The largest study to date to evaluate the role of maintenance rituximab in FL was the PRIMA study which assessed rituximab administered as a single dose every 3 months for up to 2 years after completion of one of three induction therapies (R-CHOP, R-CVP, or R-fludarabine, cyclophosphamide, mitoxantrone) [45]. The study included roughly 1200 patients, and the 3-year PFS was 75% for the maintenance group and 58% in the observation group (< 0.0001). There were also significant improvements in time to next anti-lymphoma therapy and time to next chemotherapy, although there were no significant differences in OS. The study was recently updated with longer-term follow-up, and the maintenance arm continues to outperform the observation arm, with a 10-year PFS of 51% vs 35% [46]. However, the OS continues to be identical (80%) in both arms.

Despite the evidence of benefit with rituximab maintenance after CVP, R-CVP, and R-CHOP, its use in the modern era when bendamustine is more frequently utilized is less clear. There have been no randomized studies designed to evaluate the role of maintenance rituximab after receipt of B-R. The German low-grade lymphoma study group recently presented the MAINTAIN trial which randomized patients receiving B-R to 2 vs 4 years of rituximab maintenance, at a schedule of 1 dose every 2 months for 2 years [47]. While there appeared to be a trend toward a PFS benefit with 4 years of maintenance vs 2 years, this was not statistically significant (HR 0.63, 95% CI: 0.36–1.11). The authors also compared the 2-year arm to the previously published StiL NHL1 study that compared B-R to R-CHOP without maintenance [16], and there was a significant improvement in PFS for patients receiving maintenance (HR 0.68, 95%CI: 0.47–0.87), although it is important to recognize the limitations of a cross-study comparison.

A large North American retrospective study evaluating this question was also recently presented by Hill and colleagues and suggested that patients achieving a CR after bendamustine-based induction did not benefit from maintenance rituximab while patients achieving PR did appear to experience a benefit in PFS (but not OS) [48]. A secondary analysis of the BRIGHT study also evaluated the role of rituximab

maintenance for patients receiving B-R after completion of study therapy at the discretion of the investigator [49]. The schedule/duration of rituximab maintenance was not prescribed by the study and varied by investigator. In this study, there did appear to be an improvement in PFS for patients receiving rituximab maintenance vs those that were observed. Although there appeared to potentially be a benefit in OS as well, the study requires cautious interpretation due to the many limitations inherent in this type of analysis.

In general, maintenance rituximab can be considered to improve PFS, especially in patients who are not receiving bendamustine as part of their induction therapy. Patients who do receive maintenance rituximab should do so with the understanding that there is likely no long-term OS benefit. In addition, toxicities associated with therapy are often limited but can include serious infections. Unfortunately, there is not a widely adapted schedule, although most physicians currently utilize a once every 2- or 3-month schedule. The RESORT trial (E4402) described a potentially clinically significant decrease in rituximab blood concentrations for patients receiving maintenance every 3 months, suggesting that a schedule of maintenance every 2 months may be more appropriate, although these schedules have not been compared in a randomized study [50].

Incorporation of Additional Agents as Maintenance in FL

While rituximab (or other CD20 antibodies) have been most frequently utilized in FL patients achieving first remission, other agents, including lenalidomide, have been assessed. In E2408, one arm included R-lenalidomide maintenance, and other studies have included a prolonged course of lenalidomide [9]. To date, none of these studies have suggested a role for additional agents as maintenance for patients achieving a first remission, although the definition of what constitutes "induction" vs "consolidation" or "maintenance" is becoming more challenging in the era of chronic, oral therapies. At this time, maintenance therapy with agents other than rituximab or obinutuzumab should only be undertaken in the context of a clinical trial.

Long-Term Toxicities Associated with Therapy

Most patients will recover from treatment without significant toxicities, and short-term toxicities including nausea/vomiting, alopecia, and acute myelosuppression are frequently manageable with current supportive care measures. Given the length of survival expected for most of the patients, however, careful monitoring of latent toxicities is important. Up to 6% of patients in the National Lymphocare study experienced deaths related to treatment [51]. Commonly encountered issues include conditions related to bone marrow toxicity (myelodysplastic syndrome and acute myelogenous leukemia), secondary malignancies, and occasionally long-term organ toxicity.

Given the latency of many of the toxicities identified, long-term follow-up from prior clinical trials is often the best method of identifying long-term toxicities. The FOLL05 study compared R-CVP vs R-CHOP vs R-FM in untreated FL and was recently updated. Out of 504 patients randomized, 41 secondary malignancies were identified, impacting almost 10% of patients at 8 years, and including 21 patients who never experienced a relapse of their FL [14]. In the StiL NHL1 study comparing B-R to R-CHOP, 75 secondary malignancies (out of 447 patients) have been encountered with long-term follow-up, including 14 hematologic malignancies [17]. In addition to the potential risk of secondary malignancies related to chemotherapy exposure, the risk of malignancies is increased in patients who complete a series of radiographic exams. In one study examining the impact of serial CTs on an individual's cancer risk, the risk of death from lung cancer, for example, for a 20-year-old female completing 10 full-body CT scans, is estimated at 0.47% [52]. Although this represents a small absolute increase in mortality risk related to scans, it is critical that physicians consider the potential long-term ramifications of serial CT scans for patients with FL who are in complete remission, especially as there may not be any long-term lymphoma-related benefit [53]. The identification of late secondary malignancies is always concerning although it is also important to recognize that patients who develop one cancer are more likely to develop a second cancer and that FL is typically a disease of elderly patients, who are also predisposed to the development of cancer. Given the typically prolonged life expectancy for patients with FL, we recommend that they continue to receive all recommended age-appropriate cancer screenings, including dermatologic skin exams, prostate exam, mammogram, and colonoscopy, as indicated and in discussion with their primary care physicians.

Additional long-term toxicities may be related to the chemotherapy received and should be considered when selecting a treatment. For example, the long-term incidence of anthracycline-induced cardiomyopathy is low, but this can be a devastating result of treatment considering the length of life expected in this disease. Although much of the literature in this arena is based on breast cancer outcomes, it is clear that anthracyclines can be associated with increased risk of heart failure in patients with NHL and the current NCCN survivorship guidelines recommend close monitoring for cardiac symptoms, consideration of an echocardiogram after 1 year post-treatment, and management of modifiable risk factors during and after treatment.

Conclusions

Evaluation and management of advanced-stage FL requires consideration of the patients' comorbidities and wishes regarding therapy given the fact that most newly diagnosed patients can expect a prolonged life span, regardless of the type of therapy chosen. In many cases, no therapy is required and patients can be observed for several years without any significant impact on their OS. When therapy is indicated, a number of regimens are currently available and should be chosen in collaboration

with the patient based on expected short- and long-term toxicities and expected remission duration. While there are a number of currently available prognostic markers, none are as informative as the progression of disease within 24 months, which unfortunately is not known at the time of diagnosis and initial therapy selection. Future studies are needed to identify which patients are at highest risk for early progression and require alternative approaches to prevent premature death from FL. For the remaining patients, life expectancy may approach that of the general population and management of treatment-related toxicities will allow these patients to enjoy a good quality of life throughout their disease course.

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Chapter 8
Transformed Follicular Lymphoma

Michael J. Leukam and Sonali M. Smith

Introduction

Follicular lymphoma (FL) is the prototype of indolent lymphomas and typically has a prolonged clinical course with waxing and waning disease burden requiring intermittent therapy. However, this indolent phenotype can undergo biologic transformation to a more aggressive histology, mandating a distinct clinical approach with urgent intervention. The earliest description of transformed follicular lymphoma (TFL) was in 1942 by Gall and Mallory when they described an aggressive lymphoma arising 8 years after an initial diagnosis of FL [1]. Since then, the definition of TFL includes any follicular lymphoma that acquires a more aggressive clinical picture, and upon re-biopsy, will show diffuse large B-cell lymphoma, high-grade B-cell lymphoma, Hodgkin lymphoma, lymphoblastic lymphoma or gray zone lymphoma, with a prognosis equal to or worse than the aggressive subtype it most resembles [2–7]. The management of TFL is not uniform, and there is a lack of dedicated prospective trials for this subset of lymphomas. Nevertheless, outcomes in the modern era appear to be improving, and more targeted therapies are on the horizon.

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Pathogenesis of Transformed Follicular Lymphoma

The eventual emergence of a transformed follicular lymphoma has its seeds in the premalignant phase, likely from a common progenitor cell. The mutational gene expression profile of a transformed lymphoma retains genomic markers of a normal B cell, a purported common progenitor (premalignant) B cell, and a follicular lymphoma, in addition to the aggressive acquired phenotype.

The specific oncogenes driving histologically transformed follicular lymphomas have roots in the normal function of germinal center B cells. Noncancerous B cells in the light zone of the germinal center first undergo somatic hypermutation but not cell division [8]; they express low levels of the growth-promoting proto-oncogene *BCL6* [9]. After somatic hypermutation and class switch recombination, B cells in the light zone undergo selection based on antigen presentation by follicular helper T cells and dendritic cells. B cells with weak B-cell receptor affinity are targeted for apoptosis, while those selected by the helper cells initiate cell division in part through upregulation of *MYC* and *NF-κB* [10, 11]. These selected cells migrate to the dark zone, where they undergo clonal expansion and proliferation [8]. Normal B cells in the dark zone express high levels of BCL6, which suppresses DNA damage-sensing and checkpoint proteins (such as TP53, CDKN1A, and ATR), anti-apoptotic proteins such as BCL2, and expression of growth-promoting oncogenes such as *MYC* [9, 12–15]. The journey to a malignant B cell is marked by abnormal expression of some of these normally suppressed genes in a cell already primed for brisk proliferation.

Early Mutations Attributable to Progenitor Cells

The mutational pattern of purported common progenitor cells within an individual patient has been investigated through identifying mutations common to all lymphomatous clones, including both indolent and transformed clones. The earliest genomic event, present in 80–90% of patients, is a translocation between chromosome 14 and 18 resulting in the juxtaposition of the anti-apoptotic oncogene *BCL2* and the heavy-chain enhancer region (*IgH*), or a different rearrangement resulting in deregulated increased BCL2 expression [16–18]. *BCL2* rearrangements likely occur at an early stage in B-cell development in the bone marrow during VDJ recombination. Instability at the *IgH* site on chromosome 14 is associated with defective Rag1-mediated VDJ recombination, and breaks at chromosome 18 near the *BCL2* locus are believed to be due to the inherent fragility of CpG island sites [19, 20]. Those patients without a *BCL2-IgH* rearrangement fall into two subgroups: those that demonstrate an elevated BCL2 protein level without the *BCL2-IgH* rearrangement [18, 21] and those with elevated BCL6 levels, which can be associated with *BCL6*

rearrangements [22] or trisomy 3 [23]. Overexpression of a cell survival factor seems to be necessary but not sufficient for the development of follicular lymphoma – an identical *BCL2-IgH* re-arrangement to that seen in follicular lymphoma can be found in up to 50% or more of healthy blood donors [24].

Though common progenitor clones gain anti-apoptotic properties promoting cell survival in the bone marrow, they can continue to differentiate similarly to normal germinal center B cells. Additional common mutations may explain the transition to a follicular lymphoma. Mutations in genes coding for chromatin remodeling factors such as *CREBBP*, *KMT2D* (also known as *MLL2*), and *EZH2* are additional events seen across both nontransformed and histologically transformed disease within patients [25–28]. *CREBP* mutations are among the earliest attributable mutations to the development of follicular lymphomas in hierarchical mutational analysis and are associated with a decreased antigen presentation phenotype which may aid in the avoidance of immune surveillance [29]. *EZH2* and *KMT2D* mutations are both associated with promotion of the germinal center phenotype and decreased expression of tumor suppressor genes [27, 28, 30]. Mutations in some cell signaling pathways are also seen in paired samples of follicular lymphoma and histologically transformed lymphoma in the same patient, indicating the mutation typically precedes histologic transformation. For example, mutations in *RRAGC* are uniquely enriched in follicular lymphoma patients and are seen in paired transformed tumors as well [31].

Early mutations in the purported common progenitor population are associated with the later phenotype of histologically transformed lymphoma. Founder populations containing *BCL2* rearrangements, when transformed, tend to fall into the germinal center B-cell (GCB) phenotype, while the absence of a *BCL2* rearrangement predicts for an activated B-cell (ABC) phenotype [32] (Table 8.1).

Table 8.1 Mutations seen in both FL and TFL (possibly originating in a common progenitor cell)

Mutation	Effect	Frequency	References
BCL2 re-arrangement, *t(14;18)*	Increased BCL2 expression, cell survival	80–90%	[16–18]
BCL6 re-arrangement	Increased BCL6 expression, cell survival	~30% of t(14;18) negative FL	[21, 22]
Trisomy 3	Multiple effects, increased BCL6	40% of t(14;18) negative FL	[23]
KMT2D loss of function	Chromatin remodeling, epigenetic dysregulation	35–80%	[27, 28, 30]
CREBBP loss of function	Chromatin remodeling, epigenetic dysregulation	50–60%	[25, 26, 29, 33]
EZH2 loss of function	Chromatin remodeling, epigenetic dysregulation	20–25%	[25, 26, 34]
RRAGC loss of function	Increased mTorc signaling	18–25%	[31]

Patterns of Clonal Evolution

To investigate the patterns of clonal evolution in transformed follicular lymphoma, a series of elegant experiments have been carried out which take advantage of the tendency of follicular lymphoma cells to undergo somatic hypermutation and class switch recombination of the variable regions of the immunoglobulin heavy chain in a similar fashion to normal germinal-center-derived B cells. After creating a taxonomy of mutations in the immunoglobulin heavy chain, investigators have not found evidence of linear, stepwise evolution in follicular lymphoma clones, but rather, found a pattern of branched, divergent complex evolution that suggests multiple lineages arising from a progenitor population [35–39]. Additional studies investigating class switch recombination, copy number alterations, and uniparental disomy confirm the pattern of branching clonal evolution of both follicular lymphoma clones and histologically transformed follicular lymphoma from a common trunk consistent with a progenitor population [38, 40, 41]. More recently, next-generation sequencing techniques combined with phylogenetic analyses of both coding and noncoding elements have been applied to the question of clonal evolution in histologic transformation. When comparing paired sequential follicular lymphoma and histologically transformed tumors, one study found that all cases progressed via branched divergent evolution [25] while another study found 80% of cases progressed via branched evolution while 20% had elements of a linear sequential model [26]. Thus, there is building support for a common progenitor cell that gives rise to both the indolent and transformed component in follicular lymphoma.

Genomic Changes Associated with Transformation

A loss of programmed cell death is a hallmark of both FL and TFL. An interesting mutational pattern has been found in the *FAS* gene, a cell surface death receptor that is a member of the tumor necrosis factor superfamily. In the nonmalignant germinal center, signaling via FAS receptor triggers apoptosis in B cells that fail selection due to weak affinity or self-reactivity [42]. Approximately one-third of the TFL cases examined harbored a *FAS* mutation [26]. The mutation was also present in the FL precursor of all paired samples with a *FAS* mutant TFL, but was absent in all unselected FL cases. This suggests that *FAS* mutation might be a predictor of transformation, though further investigation would be required to establish test characteristics.

While epigenetic and pro-survival factors dominate early development of FL, transformation is marked by oncogenic activation and loss of tumor suppressors. The well-described tumor suppressor gene *TP53* encodes a transcription factor with a broad range of antiproliferative downstream effects including control of cell cycle

arrest, apoptosis, senescence, DNA repair, and metabolism [43]. Mutations in *TP53*, loss of chromosome arm 17p (which contains *TP53*), and upregulation of the *TP53* repressor *MDM2* are specifically enriched in histologically transformed lymphomas [44–47]. *CDKN2A* is a tumor suppressor gene that acts, in part, to suppress the function of TP53. Mutations in *CDKN2A* are rare at the time of diagnosis of FL, but when present, portend a poor response to therapy and a poor overall survival [48]. Bi-allelic loss of function of *CDKN2A* is relatively common in TFL and, through its effects on the DNA-repair pathway through *TP53* and the loss of regulation of the cell cycle, may represent an important step in the development of mutations and proliferative ability in TFL [26].

Somatic hypermutation is the mechanism by which proliferating B cells diversify the B-cell receptor prior to affinity selection. This process involves a highly increased rate of mutation in the B-cell receptor locus mediated by activation-induced cytosine deaminase (AID) [49]. De novo DLBCL often contains mutations in the 5′ untranslated or coding regions of multiple genes consistent with aberrant AID activity. These phenomena, deemed "aberrant somatic hypermutation," are not found in nonmalignant germinal center B cells or in nontransformed FL [50], but can be found at the time of transformation in serial samples of TFL cases [26, 51]. Many of the targeted genes in aberrant somatic hypermutation have a known oncogenic function (e.g., *PAX-5* and *MYC*), which suggests a possible pathogenic role for this process in transformation (Table 8.2; Fig. 8.1).

Table 8.2 Frequency of selected mutations in transformed FL

Mutation	Effect of mutation	Frequency in TFL	Frequency in FL	References
TP53 loss of function	Loss of DNA repair, loss of cell cycle control	30%	15%	[26, 45, 46, 52]
MDM2 overexpression	Suppression of TP53	80%	0%	[44]
MYC	Cell growth, altered metabolism, genomic instability	25% translocations, 33% amplifications	0%	[26, 52]
CDKN2A	Increased proliferation	46%	0–8%	[26, 48]
FAS	Increased cell survival	33%	Unknown	[26]
B2M	Alterations in interactions with surrounding T-cells	25%	10%	[52]
CD58	Alterations in interactions with surrounding T-cells	5%	0%	[26]
Aberrant somatic hypermutation	Deletions and insertions in 5′ region of several genes	55–87%	Unknown	[26, 51]

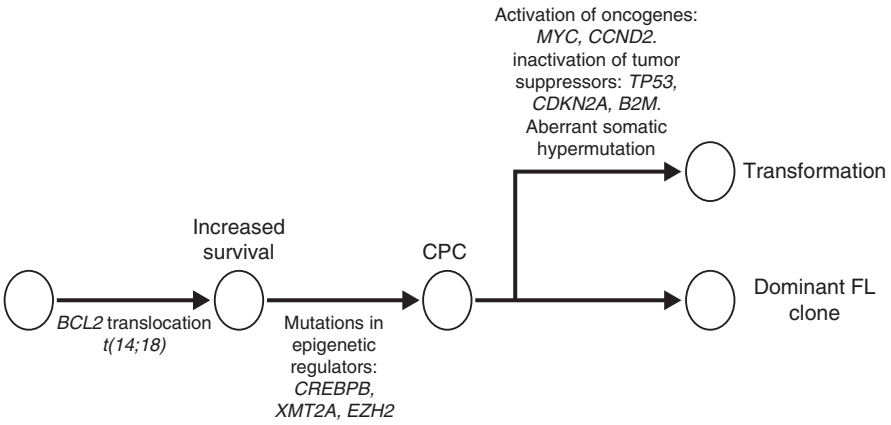

Fig. 8.1 Simplified summary of genomic evolution of transformed follicular lymphoma

Role of the Tumor Microenvironment in Transformation

Gene expression profiling studies have confirmed the critical role of the FL microenvironment in terms of prognosis. The rich immunologic environment of a lymph node, including follicle dendritic cells, T cells, and other B cells contributes to the risk of transformation as well [53]. The role of the surrounding microenvironment in the development of FL and subsequent histologic transformation is an active area of research. The development of FL is associated with a decrease in clonal diversity in tumor-associated CD8+ T cells [54] and a decrease in nonmalignant germinal center B cells [55]. In a large gene expression profile study of follicular lymphoma patients, the strongest predictors of early mortality were alterations in gene expression in nonmalignant T-cell populations [56], and the T-cell populations in "poor-risk" FL with early transformation have a different phenotype than in "good-risk" FL unlikely to undergo early transformation, namely, a T-helper1 phenotype [57, 58]. A shorter time to transformation is seen in FL patients who are found to have higher levels of immune checkpoint protein expression such as PD1 on infiltrating T cells [59]. Finally, mutations in FL cells which further affect interactions with surrounding T cells such as beta-2 microglobulin [52] and CD58 [26], and a fall in the absolute number of infiltrating T cells [60, 61], are specifically associated with histologic transformation. These findings together suggest a role for alterations in interactions with the microenvironment in pathogenesis and immune evasion in histologic transformation. A model for transformation in which a supporting activated immune network drives rapid mutation in FL cells, leading to genomic instability and accumulation of transformative mutations, is supported by gene expression and cytokine profiling studies [61]. Further studies of the role of the microenvironment in development of TFL may allow better prognostication of high-risk patients requiring escalated therapy or early consolidation and may

further elucidate the mechanisms and appropriate application of immunomodulatory or immune checkpoint inhibitor therapies.

Definition and Diagnosis of Transformed Follicular Lymphoma

Patients with follicular lymphoma have an approximate 2–3% risk of transformation to aggressive lymphoma, although overall rates of transformation may be decreasing following the introduction of monoclonal antibodies [6, 62–65]. Of note, there may be a population with FL who will never transform, as several studies have identified a possible plateau in the transformation rate after approximately 15 years [66, 67].

Histologic Definition

The definition of TFL includes any follicular lymphoma that acquires a more aggressive clinical picture, and upon re-biopsy, will show diffuse large B-cell lymphoma, high-grade B-cell lymphoma, Hodgkin lymphoma, lymphoblastic lymphoma or gray zone lymphoma, with a prognosis equal to or worse than the aggressive subtype it most resembles [2–7]. Not all clinically suspected cases of TFL will be found to have true histologic transformation on biopsy, and not all confirmed cases of TFL on biopsy will demonstrate clinical signs of transformation, complicating inclusion criteria for clinical trials [68].

Histologic grade 3B follicular lymphoma bears particular mention as a challenging disease which straddles the narrow divide between high-grade follicular lymphoma and DLBCL, containing features of both. Increasing histologic grades of FL are defined by an increasing number of centroblasts per high power field. If there are more than 15% centroblasts per high power field, the disease is classified as grade 3B, and a diffuse, follicular-obliterating architecture of solid sheet of centroblasts is observed that is similar, yet distinct from DLBCL [69]. The lack of characteristic surface markers such as CD10, the relative paucity of mutations are typically found in FL such as IGH re-arrangement, and an increased frequency of mutations not frequently seen in FL such as *BCL6* translocations or rearrangements suggests that grade 3B FL is not an evolutionary step in the transformation of lower grade FL but rather a distinct entity [70–73]. The mutational landscape of grade 3B FL is heterogeneous, frequently having more in common with germinal center B-cell lymphoma than grade 1–2 FL, despite the histologic similarity with FL [74]. Clinically, areas of DLBCL are frequently found in close examination of grade 3B FL biopsies, which would reclassify the disease as TFL [75]. Those remaining cases have an outcome similar to DLBCL and are usually treated clinically as transformed [76, 77]. Further research is necessary to clarify the driving pathways in grade 3B FL and DLBCL to elucidate potential pharmacologic targets.

Clinical Diagnosis

Due to the clonal heterogeneity of FL and the possibility for isolated initial areas of transformation, confirming a histologic diagnosis of transformation is not always straightforward. When following patients with known FL over time, transformation may be first suggested by a change in overall clinical status and emergence of symptoms. Clinical features suggestive of potential transformation include declining performance status, new "B" symptoms, rapid growth of nodal disease, increased extranodal sites of disease, newly disseminated disease, development of cytopenias, elevated serum lactate dehydrogenase, elevated serum calcium, and increase in International Prognostic Index score [6, 66, 78, 79].

If transformation is clinically suspected, biopsy confirmation should be pursued both for diagnostic and prognostic analysis. Identifying the best site for biopsy can be difficult, particularly since the transformation may not be present in all involved disease sites. A useful tool in identifying the ideal site for biopsy is functional imaging with FDG-PET, where the area with the highest uptake is most likely to reveal the aggressive component. In one study of biopsy-proven TFL, the corresponding standardized uptake value (SUV) on the concurrent PET scan ranged from 3 to 38 with a median of 12 [80]. The specificity for transformation of elevated SUV on PET increases with higher SUV; a cutoff of 10 is approximately 80% specific for transformation [81], whereas a cutoff of 13 confers a specificity of approximately 90% [82] and a cutoff of 14 is associated with specificity of approximately 95% [83]. An SUV of 17 or above demonstrates a positive predictive value of 100% in one study [83]. Biopsy remains the gold standard for diagnosis and provides additional clinical information which may alter treatment such as the development of "double-hit lymphoma" (see below); therefore, a highly suspicious PET scan should be followed by biopsy targeted toward the areas with highest SUV.

However, not all patients will have a diagnostic specimen despite a strong clinical suspicion of transformation, and not all patients will have accessible sites for biopsy. Because of the extensive clonal heterogeneity and sampling error, a biopsy may still miss the critical site of transformation due to sampling error. In the event of concerning clinical signs/symptoms and suspicious PET, a negative biopsy cannot be taken as evidence for a lack of transformation. Additional biopsy or treatment for TFL based on clinical and radiographic findings alone may need to be pursued in those cases.

Treatment of Transformed Follicular Lymphoma

The treatment of TFL depends on the clinical context, with three frequently encountered scenarios: simultaneous diagnosis with FL, subsequent transformation after a known diagnosis of FL that is treatment-naïve, and subsequent transformation following prior therapy for established FL. Historically, patients with TFL had a very

poor prognosis, with five-year overall survival rates under 25% [66, 84]. Therefore, TFL was treated aggressively, including incorporation of up-front autologous or allogeneic hematopoietic stem cell transplant (SCT). In the pre-rituximab era, use of early autologous SCT (ASCT) consolidation improved survival rates in patients with transformed follicular lymphoma to approximately 50% [85]. In the modern era of chemoimmunotherapy era (marked by the addition of the anti-CD20 antibody rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone, known as "R-CHOP"), outcomes have substantially improved, and the ability to achieve complete remission and long-term survival akin to de novo DLBCL is possible.

Chemoimmunotherapy

The treatment of TFL in all cases (simultaneous diagnosis of transformed and indolent components, treatment naïve transformation of known indolent lymphoma, and transformation of previously treated indolent lymphoma) starts with chemoimmunotherapy, specifically R-CHOP. Rates of complete remission and overall survival are similar after treatment with standard chemoimmunotherapy to matched patients with DLBCL; however, the group with transformed disease have a higher rate of relapse of indolent lymphoma [78]. While the aggressive, histologically transformed component of TFL requires urgent intervention and can potentially be successfully treated, the underlying follicular lymphoma is not considered curable and is subject to late relapse even after successful therapy.

Outcomes after initial treatment for patients with both DLBCL and transformed indolent lymphomas have improved in the rituximab era of combined chemoimmunotherapy [65]. The current recommendation for most patients who present with transformed disease at the time of first diagnosis or who have been followed with observation only is standard chemoimmunotherapy such as rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Retrospective studies suggest a long-term survival rate of approximately 60% for minimally pretreated after up-front treatment with standard chemoimmunotherapy [65, 85, 86]. There is some limited evidence from a noncontrolled study that supports consolidative autologous or allogeneic SCT after chemotherapy, based on superior short-term outcomes compared to historical controls [87]. However, more recent studies have found nonsignificant differences in survival comparing TFL to de novo DLBCL treated with standard chemoimmunotherapy [88]. Given the reasonable rate of long-term survival (similar to that of de novo DLBCL) with or without consolidation therapy, and the inability to cure the indolent lymphoma even with aggressive consolidation therapy, patients who achieve a complete remission after initial therapy are not routinely recommended for consolidative autologous SCT (ASCT). There are no currently validated methods to identify patients at higher risk of relapse with minimally treated histologically transformed disease who might benefit from ASCT, though such a tool would be of great usefulness.

Special Case: Patients with Prior Anthracycline Exposure

The choice of chemotherapy should take into account prior exposure to anthracyclines. Patients receiving multiple lines of therapy for incurable, recurrent diseases such as FL are at risk for reaching cardiotoxic lifetime doses of anthracycline chemotherapy. Regimens such as R-ICE (rituximab, ifosfamide, carboplatin, and etoposide) [89], GDP or DHAP, followed by high-dose chemotherapy and transplant should be considered for patients with prior anthracycline exposure. For those who have not been previously exposed to anthracyclines or who can tolerate additional exposure, the combination of rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (R-EPOCH) demonstrated good activity in a phase II study as a salvage agent for CD20+ relapsed and refractory lymphomas (including transformed follicular lymphomas) with a 28% rate of complete response and median event-free survival of approximately 12 months [90]. In that trial, 19 of the 50 patients (38%) proceeded to high-dose chemotherapy and ASCT [90]. In sum, in patients with newly diagnosed follicular lymphoma previously treated with chemotherapy, the current evidence supports treatment with salvage chemotherapy followed by consolidative ASCT if the disease demonstrates chemosensitivity. The choice of salvage therapy should take into account prior therapy to minimize toxicity.

Special Case: "Double Hit Lymphoma"

Approximately 25% of new diagnoses of TFL are "double-hit lymphoma (DHL)" or "high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements" which is now recognized as a separate entity from DLBCL in the 2016 World Health Organization classification system [69]. The term "double hit" refers to the dual presence of *MYC*-associated proliferation plus *BCL2*-associated anti-apoptotic effects related to chromosomal rearrangements and leading to a very aggressive and chemoresistant phenotype. DHL that arises from FL has a similarly poor prognosis to de novo diagnoses when treated with standard-dose chemoimmunotherapy regimens such as R-CHOP [91–93]. While prospective data supporting the use of intensified therapy in this population is lacking, retrospective studies suggest superior outcomes when intensified chemotherapeutic regimens such as either dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), R-hyperCVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and cytarabine), or R-CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate alternating with ifosfamide, etoposide, and cytarabine) are used in place of R-CHOP [94]. In patients with DHL who achieve complete remission after completion of initial chemotherapy, there has been no demonstrated benefit in retrospective studies of consolidative SCT [93, 94].

Role of Consolidative Stem Cell Transplant

Patients exposed to prior lines of chemotherapy for indolent lymphoma prior to transformation have a poorer response to standard therapy after transformation [95], particularly those patients previously exposed to rituximab [89]. The best outcomes in this patient group have been achieved with salvage chemotherapy as a bridge to consolidative high-dose chemotherapy and ASCT, so long as there is chemosensitive disease and a reasonable performance status. This approach is supported by a subset analysis from a large National Cancer Institute of Canada Clinical Trials Group study which found similar outcomes of both patients with relapsed/refractory DLBCL and transformed indolent lymphomas (including follicular lymphoma) after either gemcitabine, dexamethasone, and cisplatin (GDP) or dexamethasone, cytarabine, and cisplatin (DHAP) and then high-dose chemotherapy with ASCT. In both the transformed indolent group and DLBCL group, the 4-year survival was approximately 40% [96]. Another study comparing outcomes after high-dose chemotherapy and ASCT in heavily pretreated DLBCL and transformed indolent lymphoma patients found approximately 60% 3-year survival in both groups without a significant difference in outcomes based on immunohistochemically defined cell of origin [97].

One limitation to ASCT is reduced eligibility due to disease refractoriness, comorbidities, or advanced age/frailty. In a series of 105 consecutive referrals for ASCT for transformed indolent lymphomas, only 48% actually proceeded with ASCT; those patients not offered transplant were primarily due to progressive disease [98]. In those cases of TFL where initial salvage therapy does not show a chemosensitive response (progressive or refractory disease), currently available options include clinical trials, chimeric antigen receptor T cells (CAR-T), allogeneic hematopoietic stem cell transplant (SCT), or radioimmunotherapy; clinical trial participation should be a high priority. Given the histologic and genomic similarity of histologically transformed lymphoma and DLBCL, and the lack of dedicated trials for relapsed/refractory TFL, support for these various treatment strategies is extrapolated from the DLBCL literature and those trials that included TFL in the population of aggressive lymphoma patients.

A major limitation of allogeneic SCT for the treatment of follicular lymphomas including relapsed/refractory cases is the significant associated nonrelapse mortality. In several comparisons of myeloablative allogeneic SCT to ASCT for all patients with transformed follicular lymphomas, there was no improvement in overall survival, but significantly more toxicity in the allogeneic transplant group [85, 99, 100]. While reduced-intensity allogeneic SCT has shown efficacy in multiply relapsed transformed follicular lymphoma (47% 4-year survival), there remains considerable toxicity (32% 4-year non-relapse mortality) [101]. Therefore, until further measures are developed to limit toxicity of allogeneic SCT, this therapy should be reserved for patients who experience early relapse after ASCT or who are otherwise ineligible for ASCT.

Biologic and Targeted Agents

Targeted therapies and immunotherapies are currently in use and under investigation for relapsed and refractory aggressive B-cell lymphomas including TFL, including radioimmunotherapy, immunomodulatory agents, and immune checkpoint inhibitors. Few of the single-agent therapeutic options for refractory or relapsed disease have been compared to each other. Given the limited data to guide therapy for relapsed/refractory TFL, referral for evaluation for a clinical trial is always preferred.

Radioimmunotherapy

Yttrium-90 (Y90) ibritumomab tiuxetan, an anti-CD20 antibody conjugated to a beta-emitting radioactive source, was FDA-approved in 2002 for treatment of specific lymphomas including transformed follicular lymphoma. Patients with disease resistant to standard chemoimmunotherapy may be considered for second-line radioimmunotherapy. Multiple studies have compared Y90 ibritumomab tiuxetan to rituximab monotherapy, finding superior outcomes with the radioimmunotherapy, including some patients with long-term responses (time to progression greater than 3 years in 24% of patients) [102–104]. A study of Y90 ibritumomab tiuxetan monotherapy in patients with relapsed or refractory aggressive B-cell lymphomas and who were ineligible for ASCT or allogeneic SCT (primarily due to age) found an impressive ORR of 65–67%, but a poorer median survival in patients previously treated with rituximab (21.4 months vs 4.6 months) [105]. A retrospective registry study of 215 patients treated with radioimmunotherapy included 39 patients treated with Y90 ibritumomab tiuxetan as monotherapy for relapsed/refractory aggressive large B-cell lymphoma, and found a two-year overall survival rate of 50%, which compares favorably to historical controls (the authors cite, as comparison, the 40% three-year overall survival rate in the CORAL study of salvage chemotherapy and ASCT [89]). Trials comparing radioimmunotherapy to other salvage therapy options including ASCT or allogeneic SCT are lacking, and uptake of radioimmunotherapy in general has been hampered by concerns for acute and delayed hematologic toxicity including risk of secondary hematologic malignancies as well as difficulties with reimbursement and in finding partners in radiation oncology to administer the medication [106]. However, secondary hematologic malignancies have actually been rare in clinical practice [107]. Further refinement of treatment protocols and comparative studies clarifying the place of radioimmunotherapy in the sequence of therapies for relapsed and refractory disease may lead to a larger role in the future.

Therapies Targeting the Microenvironment

Lenalidomide is an immunomodulatory agent which is approved for the use of several hematologic malignancies including multiple myeloma, and has been

investigated specifically in relapsed/refractory TFL. A phase II trial of lenalidomide monotherapy in patients with relapsed or refractory aggressive non-Hodgkin lymphoma included a subset analysis of 23 patients with TFL, finding an ORR in 57% of those patients, and a median PFS of 7.7 months [108]. This compares favorably to other studies of single-agent salvage therapy in this population. For example, in a phase II trial of bendamustine in relapsed/refractory FL (including 20% transformed cases), there was a median duration of response of 2.3 months in the TFL subgroup. Lenalidomide has also been studied in combination with rituximab in relapsed/refractory aggressive B-cell lymphoma. In a phase II trial of lenalidomide with rituximab ("R-squared" regimen), 9 out of 45 total patients with significant pretreatment burden had transformed follicular lymphoma; an ORR of 33%, median PFS of 3.7 months, and median OS of 10.7 months were observed [109]. In that trial, the patients who responded proceeded with SCT, which improved the median response duration to 30.9 months [109].

Based on the known immune dysregulation of the microenvironment in transformation to and maintenance of aggressive lymphoma, including overexpression of immune checkpoint protein programmed death 1 (PD1) [59, 110], immunotherapies such as checkpoint inhibitors have been investigated as a promising future therapy for both indolent and transformed lymphoma. A phase II trial of an immunotherapy agent (pidilizumab) in patients who had residual disease after ASCT included 13/66 patients with transformed indolent lymphoma, demonstrating a 16-month PFS of 72% and an ORR of 51% among patients with measurable disease [111]. Another anti-PD1 antibody (nivolumab) has been tested in various hematologic malignancies including both DLBCL and FL. A phase II study of nivolumab in patients with relapsed/refractory lymphoid malignancies found a rate of partial or complete response in 38% of patients with DLBCL and 40% of patients with FL [112]. Further studies are ongoing in both aggressive and indolent lymphomas.

Emerging and Future Approaches

While there are few studies focusing solely on transformed follicular lymphoma or other transformed indolent lymphomas, many trials for FL or DLBCL also include cases of TFL, and potential effectiveness in TFL can be extrapolated to some extent from the response of FL and DLBCL in pioneering trials of cell therapy and targeted agents. While some of the treatments discussed in this section have gained FDA approval, none are yet in widespread use.

Cellular Immunotherapy

A recently developed cellular therapy using bioengineered T cells targeting CD-19 antigens present on lymphoma cells (CAR-T therapy) has shown promise in relapsed/refractory lymphomas including transformed FL. Patients undergoing this

therapy undergo T-cell harvesting, followed by insertion of a chimeric T-cell receptor targeting CD19 with additional activating factors. After expansion of the engineered cohort, the patient receives T-cell depleting chemotherapy followed by infusion of the engineered cells. A phase I trial of axicabtagene ciloleucel (axi-cel) anti-CD19 CAR-T product included patients with TFL, and demonstrated a remarkable rate of durable remission in otherwise refractory patients (40% CR rate at 18 months). Based on remarkable results in phase I trials (see Table 8.3), the FDA granted approval to axi-cel for the indications including the treatment of

Table 8.3 Selected clinical trial results in TFL

Drug studied	Target	Type of trial	Population studied	Outcomes	References
Axicabtagene ciloleucel (CAR-T)	CD19	Phase I	TFL 16%	ORR: 82% CR: 54% 18-month OS: 52% 18-month CR: 40%	[114]
CTL019 (CAR-T)	CD19	Phase I	Included relapsed/ refractory FL and DLBCL	CR (DLBCL): 43% CR (FL): 71%	[116]
Ibrutinib	BTK	Phase I	FL 29% DLBCL 13%	ORR: 60% CR: 16%	[120]
Ibrutinib	BTK	Phase II	Relapsed/ refractory FL (all patients)	ORR: 37.5%, poorer response with CARD11 mutations	[121]
Copanlisib	PI3K	Phase II	Relapsed/ refractory indolent and aggressive lymphomas	ORR: 27.1% in aggressive lymphoma. Increased activity in PI3K overexpressors.	[125]
Fostamatinib	Syk	Phase I/II	Indolent and aggressive lymphoma; FL 31% DLBCL 34% TFL 9%	ORR (FL): 10% ORR (DLBCL): 24%, 1 out of 6 TFL had response	[129]
Entospletinib	Syk	Phase II	Indolent lymphoma, FL 59%	ORR: 13%	[130]
Venetoclax	BCL2	Phase I	Non-Hodgkin lymphoma; DLBCL 32% FL 27%	ORR (FL): 38% PFS (FL): 11 months ORR (DLBCL): 18% PFS (DLBCL): 1 month	[131]
Venetoclax + rituximab or bendamustine	BCL2	Phase II	FL	Early results: ORR 33% with venetoclax + rituximab	[132]

Table 8.3 (continued)

Drug studied	Target	Type of trial	Population studied	Outcomes	References
Vorinostat	HDAC	Phase I	Indolent lymphoma including FL (4 patients)	2 unconfirmed CR (one lasted 18 months), 1 PR.	[138]
Vorinostat	HDAC	Phase II	Included 17 FL patients	ORR (FL): 47% PFS (FL): 15.6 months	[139]
Vorinostat	HDAC	Phase II	Included 39 patients with R/R FL	ORR (FL): 49% PFS (FL): 20 months	[140]
Vorinostat + rituximab	HDAC	Phase II	Indolent NHL including FL	ORR: 46% ORR (treatment naïve): 61%	[141]
Abexinostat	HDAC	Phase I/II	14 patients with FL	ORR (FL): 64.3% PFS (FL): 20.5 months	[142]
Abexinostat	HDAC	Phase II	Included DLBCL and FL	ORR (FL): 56% Response duration (FL): 10.2 months ORR (DLBCL): 31% Response duration (DLBCL): 1.9 months	[143]
Mocetinostat	HDAC	Phase II	Included DLBCL and FL	ORR (FL): 11.5% PFS (FL responders): 11.8 months ORR (DLBCL): 18.9% PFS (DLBCL responders): 26.3 months	[144]
Vorinostat + azacitidine + high-dose chemotherapy	HDAC, hypo-methylator	Phase I	R/R DLBCL	15-month EFS: 65% 15-month OS: 77%	[135]
Decitabine + R-CHOP	Hypo-methylator	Phase I	DLBCL	CR rate: 91.7%	[137]

transformed follicular lymphoma after two prior lines of systemic therapy [113–115]. A second (not yet approved) anti-CD19 CAR-T construct (CTL019) was tested in patients with relapsed/refractory FL or DLBCL, finding a similarly impressive initial remission rate (Table 8.3) [116]. Wide deployment of CAR-T therapy for lymphoma is currently limited by cost, need for specialized centers to administer the therapy, and significant toxicities such as cytokine-release syndrome and neurotoxicity [115]. Further studies will be required to assess the durability of response, the need for further consolidative therapy, and to optimize both the CAR-T product and response protocols to minimize toxicity.

Therapy Targeting Cell Signaling Pathways

Consistently, mutations causing aberrant B-cell survival (such as BCL2 and BCL6) and epigenetic dysregulation (KMT2D, EZH2, CREBBP, and others) are seen early in the development of FL. Therapies targeting intracellular and intercellular signaling pathways relating to mutations underlying both indolent and transformed FL may be useful both in the treatment of TFL and in the prevention of relapse of the indolent component.

Targeting Signaling Pathways

B-cell receptor signaling is necessary for B-cell survival during selection in the light zone of the follicle. A "failure to die" through overexpression of survival factors such as BCL2 is a canonical early mutation in the development of FL, but is by itself insufficient for lymphomagenesis. A potential second-growth signal has been identified in activation of the B-cell receptor (BCR) pathway. A significant proportion of FL cell lines have found to have self-antigen recognition leading to constitutive activation of BCR signaling [117], and mutations in the BCR pathway are found in approximately 45% of cases of FL [118]. Several individual elements in the BCR pathway have been investigated in follicular and aggressive lymphomas that may prove useful in certain cases of TFL.

Bruton's tyrosine kinase (BTK) is tightly associated with the BCR, and is necessary for activation of downstream signaling that results from activation of the BCR [119]. Ibrutinib (PCI-32765) is a selective, irreversible small-molecule inhibitor of BTK which has been studied in several B-cell malignancies [120]. Ibrutinib has been studied in multiple B-cell malignancies including DLBCL and FL with response rates ranging from approximately 40–60% with some complete responses (see Table 8.3) [120, 121]. Interestingly, single-agent activity of ibrutinib in refractory cases of the activated B-cell (ABC) subtype of DLBCL (which represents the minority of TFL) is particularly potent compared to the GCB subtype [122]. To date, ibrutinib has not yet been studied specifically in TFL, though by extrapolation from efficacy in FL and DLBCL, it may have a future role in selected cases.

The phosphatidylinositol 3-kinase (PI3K) family of intermediate signaling molecules are involved in several cellular processes downstream of B-cell receptor activation that are implicated in tumorigenesis, including survival and growth [123]. While the PI3K-delta inhibitor idelalisib has shown promising activity in indolent lymphomas including FL, it has not yet been shown to have a significant effect as monotherapy for aggressive lymphoma [124]. Copanlisib, another PI3K inhibitor with both delta and alpha inhibition, has been shown to have activity in aggressive lymphoma (see Table 8.3). Increased antitumor activity was seen in those patients whose tumors had increased expression of PI3K, suggesting a role for personalized therapy based on pretreatment expression patterns [125].

Spleen tyrosine kinase (Syk) signaling in FL and DLBCL increases cell survival as well as activation of the cell cycle [126, 127]. Syk activity has also been linked to

tumor cell invasion and angiogenesis in follicular lymphoma [128]. Several trials of Syk inhibitors have found a modest response rate in indolent lymphomas, but a somewhat better rate in aggressive lymphoma (see Table 8.3) [129, 130]. One out of the 6 TFL patients included in a trial of the Syk inhibitor fotstamatinib had a response to therapy. Future studies may be focused on the use of Syk inhibitor in combination with other therapies for aggressive lymphoma given the limited response rate in trials of monotherapy [129].

Pro-survival Pathways

Given the importance of BCL2 and other pro-survival factor overexpression in lymphomagenesis of FL, there is biologic plausibility to targeting these factors in treatment of the disease. Venetoclax, an oral selective BCL2 inhibitor, has been more effective as monotherapy in nontransformed FL than in aggressive lymphoma (see Table 8.3) [131]. Venetoclax has also been studied in combination with therapies such as rituximab and bendamustine in FL [132]. Studies of venetoclax monotherapy or in combination with chemoimmunotherapy in aggressive lymphomas are ongoing. Due to the high frequency of *BCL2* mutations in TFL, owing to the *IgH* re-arrangement in the founding progenitor cell population, BCL2 inhibition may be uniquely potent in TFL compared to other aggressive lymphomas, though this hypothesis has yet to be supported by trial data.

Epigenetic Modifying Agents

Preclinical studies have identified histone deacetylation and DNA hypermethylation as mechanisms of chemoresistance in relapsed/refractory aggressive lymphomas [133]. Because of the prevalence of early mutations in epigenetic regulatory genes in FL and persistence to TFL, epigenetic modification therapy may potentially be useful in treating aggressive and indolent forms of FL and is an active area of investigation. Multiple histone deacetylase inhibitors (HDAC inhibitors) have been studied in non-Hodgkin lymphoma, particularly in indolent lymphomas, with some evidence of single-agent activity, and certain HDAC inhibitors have been FDA approved for use in cutaneous and peripheral T-cell lymphomas. Specifically, vorinostat, abexinostat, and mocetinostat, all oral HDAC inhibitors, have been studied in several phase I and II trials in both FL and DLBCL (see Table 8.3). The ORR in trials of HDAC inhibitor monotherapies for both FL and DLBCL have been limited, though some of those responders have had prolonged responses. A significant improvement in ORR is seen in combination with rituximab (see Table 8.3), particularly in treatment-naïve disease. Given the potential promise of HDAC inhibition in treatment of both indolent and aggressive lymphomas, especially in combination with established therapies, these agents may have a particular role in the treatment of transformed lymphomas that contain characteristics of both indolent and aggressive lymphoma.

One limitation to HDAC inhibition is that when histone deacetylation is inhibited in cell lines, the activity of DNA methyltransferases can be increased. Methylation of CpG residues in the promoter region of a gene leads to significantly decreased expression, or epigenetic "silencing." Increased methyltransferase activity was observed following exposure to an HDAC inhibitor in a study of vorinostat plus high-dose chemotherapy for ASCT conditioning [134]. A follow-up study added a hypomethylating agent to the vorinostat plus high-dose chemotherapy regimen and found that double epigenetic modulation of high-dose chemotherapy engendered a surprisingly high event-free and overall survival in refractory aggressive lymphoma compared to historical data (see Table 8.3) [135].

Even in the absence of HDAC inhibitor exposure, non-Hodgkin lymphoma cells have increased DNA methylation compared to nonmalignant cells. One study of the PTPL1 tumor suppressor gene found that it was methylated and suppressed in approximately 60% of DLBCL samples and only 6% in control reactive lymph nodes [136]. In that same study, PTPL1 expression was restored in cultured cell lines upon exposure to the hypomethylating agent 5-azacitidine, suggesting a potential therapeutic role. In another study, an elegant series of in vitro studies demonstrated direct growth inhibition of DLBCL cell lines upon exposure to the hypomethylating agent decitabine as well as potentiation of the lethal effect of doxorubicin [137]. These findings were confirmed in cell lines derived directly from newly diagnosed DLBCL cases and several genes with differential methylation and therefore expression were identified. Finally, the investigators launched a phase I trial of decitabine pretreatment prior to R-CHOP in patients with newly diagnosed DLBCL, finding a complete response in 11/12 patients. Ten of those 11 responders remained in a complete remission at 13-month follow-up. While these studies were done in patients with DLBCL, the prevalence of mutations in epigenetic regulatory genes in FL and TFL suggests that these treatments may be particularly important in the treatment or even prevention of TFL. Further studies of hypomethylating agents in non-Hodgkin lymphoma are ongoing (Table 8.3).

Combination Therapy

Given the limited benefits seen within trials of monotherapy with targeted agents, researchers have studied combinations of targeted therapies with different mechanisms of action in non-Hodgkin lymphoma. In one recent relevant preclinical study, FL and activated B-cell subtype DLBCL cell lines with resistance to the B-cell signaling inhibitor ibrutinib were found to be particularly sensitive to BCL2 inhibition with venetoclax, and a synergistic effect was observed in cell lines with exposure to both venetoclax and ibrutinib [145]. A phase I trial of idelalisib (PI3K inhibitor), lenalidomide (immunomodulatory agent affecting the microenvironment), and rituximab (anti-CD20 antibody) in patients with follicular lymphoma demonstrated excessive toxicity including unexpected toxicities not anticipated from the use of the agents in single-agent trials [146]. This demonstrates a risk of combination targeted therapies: the "on target, off tumor" effects of inhibiting

multiple critical cell signaling pathways may lead to synergistic toxicities that are severe and difficult to predict. Delivery systems that are specific to tumor cells or development of more specific targeted therapies that preferentially target malignant cells may be necessary before wide adoption of combinations of targeted therapies is feasible.

Another potential strategy is the use of drugs with more than one mechanism in a single agent. For example, a phase I trial of CUDC-907 monotherapy – which is both a histone deacetylase (HDAC) inhibitor and a PI3K inhibitor – in relapsed/refractory multiple myeloma and lymphoma found three responders in the five patients enrolled with transformed follicular lymphoma [147]. The safety profile in this trial was acceptable with dose reductions due to adverse events in 14% and treatment discontinuation in 16%.

Conclusion

Histologic transformation of indolent FL remains a diagnostic and therapeutic challenge despite an increasing understanding of the biology of transformation and advancements in therapy. It is now known that TFL emerges from one of many FL clones, most likely from a common progenitor cell that does not represent the dominant FL clone in many cases. Genomic changes associated with transformation differ from those implicated in FL lymphomagenesis and include classic tumor suppressor and growth-promoting genes. Aberrant somatic hypermutation and failure of apoptosis are thought to drive much of the increased mutational burden over time in FL, and the constant rate of transformation of 2–3% per year supports a model of steady accumulations of mutations until a critical threshold for histologic transformation to an aggressive lymphoma is met.

Diagnosis of TFL requires careful monitoring for clinical changes that might signal a change in the underlying disease, with confirmatory testing consisting of PET imaging and biopsy. While biopsy is the "gold standard" diagnostic test, treatment must be initiated at times if clinical suspicion is high enough and a biopsy is not technically or practically obtainable.

The frontline treatment of TFL is chemoimmunotherapy, usually with R-CHOP. Anthracycline-sparing regimens such as R-ICE should be considered in patients with significant prior anthracycline exposure. Intensified regimens such as DA-EPOCH-R should be considered for patients with higher risk features such as concomitant *MYC* and *BCL2* rearrangements. Historically, most TFL patients who responded to chemotherapy were offered ASCT, though currently this is limited to patients who have chemosensitive disease and were exposed to one or more lines of cytotoxic chemotherapy for FL, and for patients with relapsed or refractory TFL which is sensitive to salvage chemotherapy. Patients who relapse early after ASCT could be considered for allogeneic SCT, though transplant-related mortality and morbidity limits use of this therapy. Refractory disease or multiply-relapsed disease should lead to a referral for a clinical trial, or consideration of emerging therapies

such as CAR-T cellular immunotherapy or radioimmunotherapy. Areas of active research in TFL therapy include small molecule inhibitors and antibodies targeting B-cell receptor signaling or anti-apoptotic pathways, therapies aimed at modifying the microenvironment, and epigenetic modifying agents.

Despite progress, there is significant room for improvement in the diagnosis and treatment of TFL. Future research could help better elucidate the mechanisms of transformation and allow for treatment aimed at prevention of transformation, or discover a treatment for TFL that also prevents later relapse of indolent disease. Interpreting trial data for TFL can be difficult given the low numbers at any individual center and exclusion of TFL from many trials of aggressive lymphomas. Cooperative trials will be necessary to answer many of the open questions about optimal treatment in TFL.

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Chapter 9
Cellular Therapy for Follicular Lymphoma

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Introduction

Follicular lymphoma is an exquisitely chemosensitive disorder, with a high response rate, but upon treatment with conventional chemotherapy also a very high recurrence rate. A dogma, emerging in the early days of combination chemotherapy, and hard to dispel is that follicular lymphoma is an incurable disorder. Dose intensification with autologous stem cell rescue was one of the earliest methods available to overcome inherent resistance of residual lymphoma cells and has proven remarkably effective. Allogeneic transplantation avoids some of the problems associated with autologous transplantation such as the issue of bone marrow involvement and also exploits GVL effects. Both procedures therefore convincingly prove that follicular lymphoma is curable.

But both autologous and allogeneic transplantation have limitations and toxicities, and – mainly due to the increased availability of novel therapies – their use has become more limited. Here, we review the data on both procedures and discuss their current indications and outcomes. We also briefly introduce the concept of CAR-T cell therapy which has revolutionized the treatment of aggressive B-cell lymphomas and which also has great potential in follicular and transformed lymphoma.

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Autologous Transplantation Initial Experience

In 1988, Frei et al. showed in preclinical models a linear correlation between increasing doses of chemotherapy and tumor cell apoptosis, providing the rationale for high-dose chemotherapy followed by autologous stem cell transplant as a therapeutic strategy in patients with relapsed refractory malignancies [1]. Groups at Dana Farber in Boston [2] and at St. Bart's in London [3] were the first to systematically investigate autologous transplantation for follicular lymphoma. They used a TBI containing conditioning regimen and reported that patients transplanted in second or third remission obtained durable remissions in approximately 50% of cases. These data have been repeatedly updated and most remissions have been durable. With a length of follow-up of a minimum of 12 years, 10-year progression-free survival was 48%, and 10-year overall survival was 54% [4]. Of interest, longer survival was observed for patients receiving autologous stem cell transplant at the time of second remission as compared to subsequent time-points, supporting its use at early stage of treatment.

But these initial studies also found a high rate of therapy-related MDS. The risk for MDS persisted for up to 10 years after transplant and its cumulative rate was approximately 10% [5]. Subsequent studies have confirmed these findings but also have identified the major risk factors for t-MDS. It is associated with more advanced patient age, more previous relapses (and therefore more prior exposure to chemotherapy), and also the use of TBI and or high-dose etoposide in conditioning regimens [5]. In subsequent studies, TBI has been mostly avoided for this very reason and instead BEAM chemotherapy (BCNU –Etoposide-Cytarabine-Melphalan) has been used. Provocative studies have shown that in a substantial fraction, the marrow of patients destined to develop MDS harbored chromosomal abnormalities even before transplant [6, 7]. This suggests that chemotherapy exposure prior to transplant contributes to a substantial degree to the occurrence of t-MDS after transplant.

A retrospective analysis of 693 patients with relapsed refractory follicular lymphoma from the EBMT registry, treated with autologous stem cell transplant at time of first or second remission, between 1979 and 1995 (before rituximab was available), the majority after conditioning with TBI, confirmed the curative potential of this therapeutic strategy in patients with chemotherapy-sensitive disease [8]. Three hundred and seventy-five patients (54%) relapsed at a median of 1.5 years post autologous transplant (range 1 month–13.5 years). Ten-year and 15-year progression-free survival were 31% and 27%, respectively, and factors associated with shorter survival were low quality of response before transplant, and use of conditioning regimens other than TBI. Ten-year and 15-year overall survival were 52% and 47%, respectively, and factors associated with shorter survival were primary refractory disease, age > 45, and TBI-based conditioning. Non-relapse mortality was higher for those receiving TBI. These apparently contradicting results may be due to the fact that while experiencing a longer progression-free survival, patients receiving conditioning with TBI had a higher incidence of second cancers (13.5% vs 3.5%),

particularly therapy-related MDS and AML (8.5% vs 1.7%), as compared to patients who received non-TBI-based conditioning.

A subsequent retrospective analysis was performed by the German group, and included 241 patients with relapsed refractory follicular lymphoma, treated with autologous stem cell transplant between 1990 and 2002 [9]. TBI was used as conditioning, while BEAM or Bu/Cy was reserved to patients who had been previously irradiated. After a median follow-up of 8 years, 10-year progression-free survival was 49% and 10-year overall survival was 75%. Of interest, a plateau in progression-free survival was observed at year 6, suggesting a subgroup of patients were cured.

The impact of the introduction of rituximab and the use of more effective non-TBI-based conditioning has been assessed in more recently published studies. The Canadian group performed a retrospective analysis of 100 patients with relapsed refractory follicular lymphoma treated with autologous stem cell transplant at the time of first or second remission and for primary refractory disease, between 1993 and 2008 [10]. Patients were younger than 70 years, had a good performance status, and about half received a non-TBI-based conditioning. After a median follow-up of 5 years, 5-year progression-free survival was 56% (estimated 54% at 10 years), and 5-year overall survival was 70% (estimated 63% at 10 years). Use of rituximab within 6 months from transplant was associated with longer progression-free survival. Three out of 4 patients who later developed therapy-related MDS or AML had received TBI as conditioning regimen.

The British group has conducted a retrospective analysis of 70 patients, aged <70, with relapsed follicular lymphoma treated with autologous stem cell transplant preceded by BEAM conditioning [11]. 66% of patients had received rituximab as part of their treatment before transplant, and 84% were transplanted at the time of first or second remission. After a median follow-up of 7 years (maximum of 20 years), 7-year progression-free survival was 60%, and OS was 76%. Patients transplanted in first or second remission fared better than those transplanted in subsequent remissions, showing a plateau in progression-free survival at year 6. In addition, 3 out of 3 patients who developed therapy-related malignancies had received transplant beyond first or second remission, corroborating the idea that the number of chemotherapy regimens used before transplant may increase the risk to develop second cancers.

Recently, the Spanish GELTAMO group reported the long-term outcomes of 655 patients with follicular lymphoma who had undergone autologous transplantation between 1989 and 2007 [12]. Only 16% received TBI containing regimens.

Approximately a third had transplant in first remission, the remainder with more advanced disease. With a median follow-up of 12 years from autologous transplant median PFS and overall survival were 9.7 years and 21.3 years respectively. The best predictor of outcome was disease status at transplant (Fig. 9.1). Patients who underwent HDT/autologous transplant in CR1 had projected 12-year PFS and OS of 74% (95% CI, 66–80%) and 78.5% (95% CI, 72–79%), respectively. For patients with more advanced disease, prior exposure to rituximab was a favorable feature. For patients who underwent transplantation in CR ≥2 or PR ≥2 who had received rituximab before autologous transplant (n = 90), 9-year PFS and OS were 61%

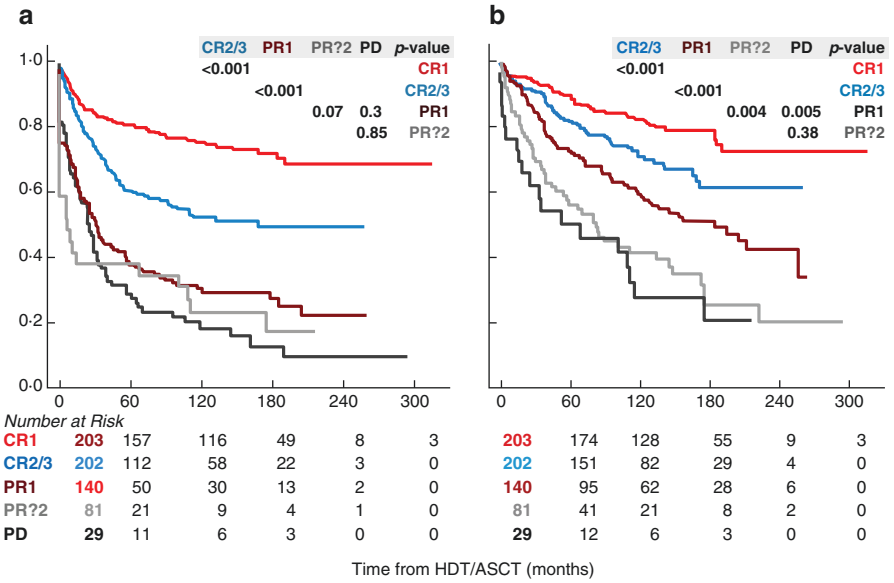

Fig. 9.1 Autologous transplant in 655 patients with follicular lymphoma reported by GELTAMO. Outcomes in patients according to disease status at transplantation. Shown are Kaplan–Meier curves of PFS (**a**) and OS (**b**) from the time of HDT/autologous transplant according to disease status at the time of HDT/autologous transplant. PD, refractory disease. The overall patient population according to the decade of transplantation: 1989–1999 versus 2000–2007. Shown are Kaplan–Meier curves of PFS (**a**) and OS (**b**) from the time of HDT/autologous transplant. (Reprinted from Jimenez-Ubieto et al. [12], (C) 2017, with permission from Elsevier)

(95% CI, 51–73%) and 75% (95% CI, 65–80%), respectively, with no relapses occurring beyond 5.1 years after autologous transplant. The cumulative incidence of second malignancies was 6.7% at 5 years and 12.8% at 10 years. For t-AML/MDS the figures were 2.5% and 6.8%, respectively.

Collectively, these analyses – as summarized in Table 9.1 – establish a number of findings.

- Virtually no relapses are observed 6 years after autologous stem cell transplant. Therefore, follicular lymphoma can be cured by autologous transplantation.
- Several studies showed that prior treatment with rituximab improves long-term survival and progression-free survival after autologous transplantation [11, 12].
- TBI-based conditioning is associated with a higher risk to develop therapy-related malignancies, particularly MDS and AML [5, 8, 13, 14], not observed with the use of BEAM. But t-MDS is also associated with older age [13, 14] and with more extensive pretreatment [5, 13] which may induce premalignant lesions prior to transplant and cell collection.
- Second remission, as compared to first or subsequent remissions, may be the optimal time to pursue autologous stem cell transplant, balancing potential benefits and risks [4, 8, 11]. Some have recently argued – again – for its use

Table 9.1 Autologous transplant performed for relapsed follicular lymphoma

Study	No.	Median follow-up (years)	Conditioning	EFS/ PFS (%)	Overall survival (%)	TRM (%)	Relapse rate (%)	Comments
Schouten et al. (2003) [15]	89	5.75	TBI based	55	71	10	43	Only prospective trial comparing autologous transplant and chemotherapy only
Montoto et al. (2007) [8]	693	10.3	TBI based	31	52	9 (5-year)	54	13.5% secondary malignancies in TBI based vs 3.5% in non-TBI conditioning
Rohatiner et al. (2007) [4]	121	13.5	TBI based	48	54	22	49.5	15 secondary AML/MDS, 4 other secondary malignancies
Kornacker et al. (2009) [9]	241	8	TBI based	49	75	6.2	47	Five secondary neoplasms; only 3/103 relapses occurred after 6 years
Peters et al. (2011) [10]	100	5.4	Variable, 40% TBI	56	70	7	40	Improved EFS if rituximab administered within 6 months of autologous transplant
Kothari et al. (2014) [11]	70	6.8	BEAM	60	76	12	NR	All secondary malignancies occurred in patients transplanted in later than second remission
Jimenez-Ubieto (2017) [12]	655	12	Variable 17% TBI	49	62	10	42	Improved EFS if pre-transplant rituximab, female, disease status and short interval diagnosis to autologous transplant. 6/8% t-AML and MDS

EFS event-free survival, *PFS* progression-free survival, *TRM* transplant-related mortality, *TBI* total body irradiation, *BEAM* BCNU/etoposide/Ara-C/melphalan

as consolidation of first remission [12]. This will be discussed in the next section.

To date only one phase 3 randomized study, the C.U.P. (Conventional chemotherapy, Unpurged graft, Purged graft) trial, conducted by the EBMT group, has investigated the safety and efficacy of autologous stem cell transplant in patients with relapsed refractory follicular lymphoma [15]. The study included 89 patients, aged <65, accrued during the pre-rituximab era, randomized to 3 treatment arms: CHOP-like only, CHOP-like followed by purged TBI, and CHOP-like followed by unpurged TBI. In light of poor accrual, the study was terminated early, and the 2 TBI arms were combined in a single group for the final statistical analysis.

Two-year progression-free survival and 2-year overall survival were significantly longer for patients randomized to the TBI-containing arm as compared to the chemotherapy only arm (55% vs 26%, and 71%v vs 46%, respectively).

Efforts to further improve on the efficacy of autologous transplant have mostly relied on the intensification of the conditioning regimen but have largely failed. Radio-immunotherapy using tositumomab (Bexxar ®) or ibritumomab (Zevalin®) in combination with BEAM has been extensively tested in recurrent B-cell lymphoma, including follicular lymphoma, and were not superior to Rituximab BEAM [16]. Bento et al. recently reported an EBMT registry study comparing R-BEAM with Z-BEAM in follicular lymphoma and did not find any differences in outcome [17].

Randomized Studies: Newly Diagnosed

The majority of studies of autologous stem cell transplant as a consolidation strategy at the time of first remission have been performed in the pre-rituximab era and have used either high-dose or CHOP-like chemotherapy only as conditioning regimen.

Two-hundred and forty patients with relapsed advanced-stage follicular lymphoma were enrolled in the German Low Grade Lymphoma Study Group (GLSG) and randomized at the time of first remission to either autologous stem cell transplant or maintenance with interferon alpha [18]. At a median follow-up of 4 years, 2-year progression-free survival was significantly higher for the patients enrolled in the transplant arm (79% vs 53%). However, patients treated with transplant were also more likely to develop therapy-related malignancies (4% vs 0%). A similar patient population was included in the GELA (Groupe d'Etude des Lymphomes de l'Adulte) study and compared CHOP-like chemotherapy combined with interferon-alpha to CHOP/TBI-based conditioning followed by autologous stem cell transplant as a consolidation strategy at the time of first remission [19]. After a median follow-up of 7 years, no differences in 7-year progression-free survival (28% vs 38%, $p = 0.11$) or 7-year overall survival (71% vs 76%, $p = 0.53$) were observed between the 2 groups. Another French study, the GOELAMS trial, also compared CHOP-like chemotherapy combined with interferon-alpha to TBI-based conditioning fol-

lowed by autologous stem cell transplant as a consolidation strategy at the time of first remission [20]. Patients randomized to transplant had a significantly longer 5-year progression-free survival (60% vs 48%, $p = 0.05$), confirmed on a recent follow-up, showing a longer 9-year progression-free survival (64% vs 39%, $p = 0.004$) [21]. However, patients randomized to transplant also had a higher risk to develop second cancers (19% risk at 5 years), including 6 cases of MDS/AML and 4 cases of solid tumors, leading to death in 7 patients, as compared to none in the non-transplant arm.

Only one randomized phase 3 study, the Italian GITMO trial, comparing chemotherapy to a non-TBI-based conditioning regimen followed by autologous transplant at the time of first remission has been conducted in the rituximab era [22]. One-hundred and thirty-six patients, aged <60, with relapsed advanced stage follicular lymphoma were included in the study. Patients randomized to transplant had a significantly longer 4-year progression-free survival (61% vs 28%), but no differences in overall survival were observed between the 2 arms, likely as a consequence of a higher incidence of therapy-related malignancies in the transplant arm (7% vs 2%).

The lack of clinical benefit on overall survival with autologous transplant as consolidation for first remission has also been confirmed in a meta-analysis of 701 relapsed patients from 3 different randomized phase 3 studies [23]. As a consequence, the EBMT Lymphoma working party has agreed on supporting the use of autologous transplant after relapse, but not as consolidation of first remission [24]. Most recently however, the Spanish group found – with a median follow up of 12 years (interquartile range 8–15 years) – a projected 12 year PFS of 74% for patients transplanted in first remission. They argue that previous studies lacked sufficient follow-up, that autologous transplant remains a superior treatment and that it should be considered for patients in first remission [12].

Purging and Maintenance

Based on concern raised by early reports of potential contamination of autologous grafts with residual tumor cells, graft purging, by means of ex vivo or in vivo use of either monoclonal antibodies of chemotherapy, has become a significant focus of research in the field of autologous stem cell transplantation in patients with lymphoma [2, 25]. This is particularly relevant in patients affected by follicular and mantle cell lymphoma, in whom bone marrow involvement is a frequent finding [26, 27].

In a retrospective analysis conducted by the CIBMTR (Center of the International Bone Marrow Transplant Registry), patients receiving ex vivo purged autologous stem cell transplant had a significantly longer progression-free survival ($p = 0.003$) and overall survival ($p = 0.004$) than patients receiving an unpurged graft [28]. Similar findings were confirmed in a subset analysis of patients affected by follicular lymphoma [29].

Given the technical difficulty associated with the use of ex vivo purging, subsequent studies have focused on the investigation of in vivo purging via use of monoclonal antibodies, based on encouraging preclinical data reported with the in vivo use of rituximab and high-dose cytarabine in patients with follicular and mantle cell lymphoma [30, 31]. The latter technique was investigated by an Italian group in a prospective phase 2 study, including 64 patients with relapsed refractory follicular lymphoma [32]. Purging was confirmed through PCR for bcl-2 in all evaluable patients, comparing favorably to historical controls with chemotherapy only, and associating with longer duration of remission after transplant.

The use of in vivo purging with rituximab was evaluated by the EBMT Lymphoma working party in a phase 3 randomized trial, including 280 patients [33]. After a median follow-up of 8 years, no differences in 10-year PFS were observed for patients randomized to the purged arm as compared to the unpurged arm (48% vs 42%, $p = 0.18$). The same study also evaluated the use of rituximab maintenance – a strategy widely used after frontline treatment for follicular lymphoma. Patients were randomized to four post autologous transplant doses of rituximab each given 2 months apart vs no maintenance. Ten-year PFS at 54% was significantly superior for those receiving maintenance vs 37% for those receiving no maintenance ($p = 0.01$). Neither purging nor maintenance affected overall survival. Since maintenance rituximab is relatively straightforward and non-toxic, we usually recommend its use after autologous transplant for follicular lymphoma. In an effort to increase the efficacy of maintenance therapy, others have combined rituximab with interferon and in phase 2 studies have reported excellent long-term survival and MRD negativity [34].

Current Role of Autologous Transplantation, Survival, QOL, Toxicities, and Cost

The introduction of rituximab was a watershed event in the treatment of lymphoma leading to dramatically improved survival [35]. Since then several new drugs and classes have been added to the armamentarium including newer monoclonals, imids, BTK inhibitors, PI3 kinase inhibitors, bendamustine, and most recently bcl2 inhibitors [36]. There are a plethora of treatment options for patients with recurrent disease. What role does autologous transplantation still have in this landscape?

A recent report focused on patients with early treatment failure, i.e., failure within 2 years after initial therapy [37]. Such patients constitute approximately 20% of all patients with follicular lymphoma and are increasingly recognized as those with the worst prognosis [38, 39]. By using two registries, the national lymphocare study and the CIBMTR registry, they were able to assess the effect of autologous transplant on long-term survival in patients with early treatment failure. All patients received rituximab-based chemotherapy as frontline treatment; 174 non-auto HCT

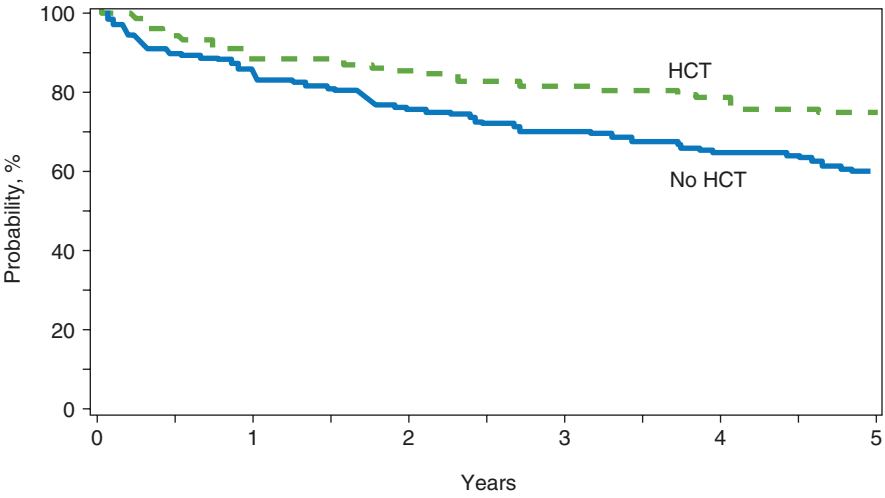

Fig. 9.2 Overall survival of patients relapsing within 2 years after initial chemotherapy and receiving transplant within 1 year of failure vs other treatments at relapse. Five-year overall survival for HCT cohort vs non-HCT cohort 73% vs 60% (***p*** = 0.02). (Data from Casulo et al. 2017 [37]. Used from Casulo et al., ©2017, with permission from Elsevier)

patients and 175 auto HCT patients were identified. There was no difference in 5-year OS between the 2 groups (60% versus 67%, respectively; $p = 0.16$). A planned subgroup analysis showed that patients with ETF receiving auto HCT soon after treatment failure (≤1 year of ETF; $n = 123$) had higher 5-year OS than those without auto HCT (73% versus 60%, $p = 0.05$) (Fig. 9.2). On multivariate analysis, early use of auto HCT was associated with significantly reduced mortality (hazard ratio, 0.63; 95% confidence interval, 0.42–0.94; $p = 0.02$). These data are reminiscent of the historical data which consistently show that more benefit is derived from autologous transplantation when used earlier in the course of the disease.

Similar results were reported by Jurinovic et al. [40]. They analyzed a group of younger patients with follicular lymphoma who relapse after initial chemotherapy with CHOP, RCHOP, or MCP given as frontline therapy in prospective trials. One hundred and sixty-five such patients were identified of whom 113 had early relapse (i.e., POD24 or relapse within 24 months of initial diagnosis). Among the 113 POD24 patients, 52 underwent cytoreduction followed by SCT as part of second-line therapy, and 46 did not undergo autologous transplant despite successful cytoreduction. Autologous transplant for POD24 patients was associated with significantly higher 5-year second-line PFS (adjusted HR 0.36, 95% CI [0.22;0.59], $p < 0.0001$) and OS rates (adjusted HR 0.52, 95% CI [0.29;0.93], $p = 0.027$) (Fig. 9.3). Multivariate analysis demonstrated that autologous transplant had a stronger impact on favorable treatment outcome compared to the addition of rituximab.

Finally, a retrospective analysis from the Provincial Alberta Cancer Registry and Alberta Lymphoma Database in Canada identified 517 patients with follicular

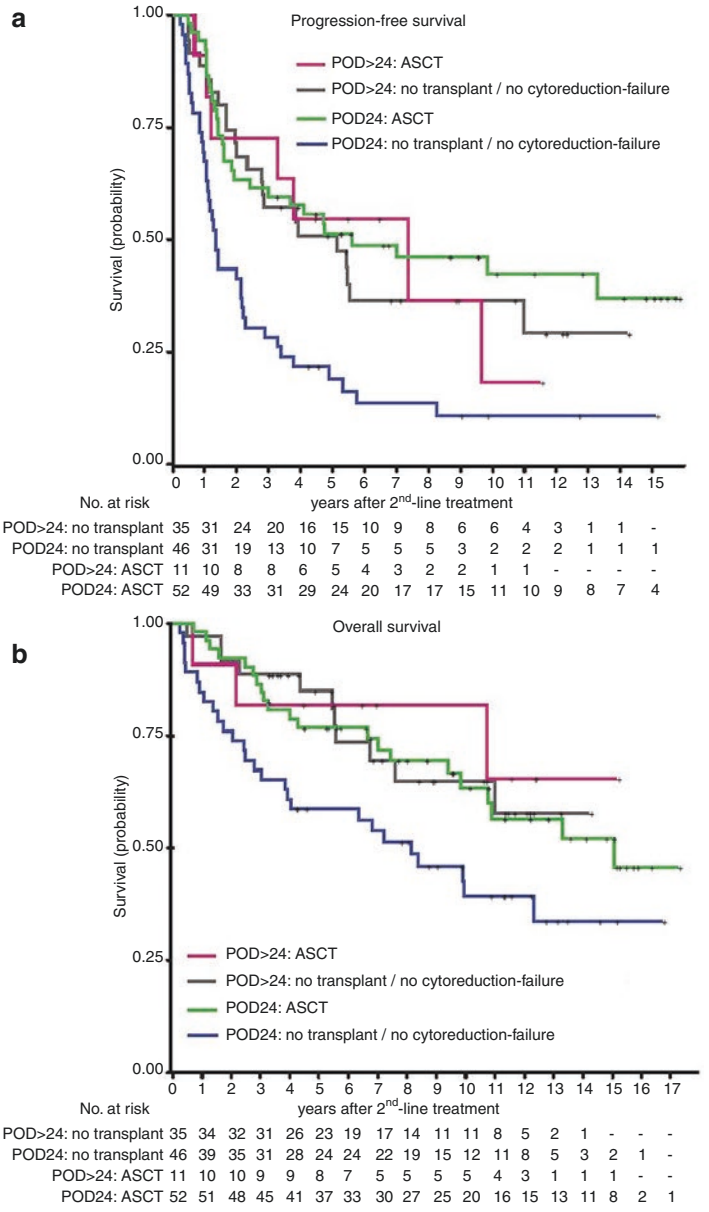

Fig. 9.3 Progression-free survival (**a**) and overall survival (**b**) of patients relapsing after initial chemotherapy and responding to salvage chemotherapy. Green: Early Relapse (POD 24) cytoreduction followed by autologous transplant. Blue: Early Relapse (POD 24) cytoreduction – no autologous transplant. Pink: Late Relapse (POD >24) cytoreduction followed by autologous transplant. Black: Late Relapse (POD >24) cytoreduction – no autologous transplant. There is a significant PFS and OS advantage for those with early relapse who undergo autologous transplant compared to those who don't. There is no advantage for autologous transplant for those with later relapses. (Data from German Lymphoma Study Group (Jurinovic et al., 2018) [40])

lymphoma under the age of 70, of whom 84 had a relapse within 24 months from completing chemoimmunotherapy [41]. Five-year survival was superior for 50 patients who received autologous transplantation compared to 34 non-transplanted patients within the early relapse group (85.4% vs 57.9%, $p = 0.001$),

These data provide strong evidence for a benefit of autologous transplantation in patients with unfavorable features, i.e., early treatment failure after appropriate initial therapy (Vignette 9.1). For those with more prolonged initial remissions, we are not aware that any study showed a survival benefit for autologous transplant. Still, we consider it an attractive treatment option for younger patients with recurrent follicular lymphoma [42]. Indeed it offers the prospect of cure in contrast to the novel drugs, none of which are considered curative and which require prolonged or indefinite administration, with major problems of compliance, cost, and an incompletely established toxicity profile. In several cases, post approval observations have uncovered serious unexpected toxicities of so-called targeted treatments [43, 44]. Autologous transplant on the other hand has gone through an evolution in conditioning regimens and patient selection that have led to gradual improvement in outcome and reduction in toxicities. Lastly, the toxicity of intensive therapy may be preferable to the cumulative toxicity of prolonged exposure to drugs. Retrospective studies demonstrate that definitive therapy with autologous transplant results in better quality of life compared with non-curative approaches [45].

Allogeneic Transplantation

Historically, the use of allogeneic stem cell transplantation in patients with relapsed refractory low-grade lymphoma has been reserved to cases failing all other standard therapeutic options [46]. But few patients have relapsed after allogeneic transplantation, showing its curative potential even in patients unable to achieve remission. The efficacy of allogeneic stem cell transplant in patients with low-grade lymphoma is mainly due to its graft versus lymphoma effect [47, 48], along with the absence of graft contamination [28].

Other than patient qualification, the issues which have surrounded the integration of allo-SCT into treatment strategies include the timing of transplant, intensity of conditioning regimens (myeloablative-MAC or reduced intensity-RIC), and efforts to reduce transplant related mortality (TRM) with methods such as graft manipulation. The data on the largest studies are summarized in Table 9.2 and briefly discussed here.

One-hundred and thirteen patients from 50 different centers, treated with sibling allogeneic stem cell transplant, mostly preceded by TBI-based regimen, were included in an initial observational study conducted by the CIMBTR [49]. Three-year PFS and 3-year OS were both 49%. Factors associated with longer survival were young age, good performance status, achievement of remission before transplant, and use of a TBI-based conditioning regimen. CIBMTR subsequently analyzed 904 patients with relapsed refractory follicular lymphoma, 176 treated with sibling allogeneic transplant (all receiving myeloablative conditioning, the majority

Table 9.2 Largest studies of allo transplant for relapsed follicular lymphoma

Study	No.	Median follow-up (years)	Conditioning	EFS/PFS (%)	Overall survival (%)	TRM (%)	Relapse rate (%)	Comments
Van Besien (2003) [29]	176	3	MAC	61	51	30	21	TRM of allogeneic transplant declined over time.
Hari et al. (2008) [52] {van Besien, 2003 #22490}	208	3	MAC (88) and RIC (120	55% (RIC) vs 67% MAC	62% RIC vs 71% MAC	23 (RIC and MAC)	17% (RIC) vs 8% (MAC)	Day 100 grade 2–4 aGVHD, 44% RIC vs 36% (MAC); 3-year cGVHD, 62% RIC vs 36% (MAC)
Khouri et al. (2008, 2012) [53, 54]	47	8.9	FCR	72	78	15 (5-year)	6	Grade 2–3 aGVHD, 13%; 3-year cGHVD, 58%
Thomson et al. (2010) [59]{Khouri, 2012 #26810;Khouri, 2008 #23605}	82	3.5	FMC	76	76	15	26	Grade 2–3 aGVHD, 13%; cGVHD, 30%
Delgado et al. (2011) [60]	164	4	(RIC) ± ATG/ alemtuzumab	52, T-cell depletion; 67, conventional	74, T-cell depletion; 74, conventional	18, T-cell depletion; 17, conventional	28, T-cell depletion; 14, conventional	Grade 2–4 aGVHD, 15% (T-cell depletion), 23% (conventional) cGVHD, 25% (T-cell depletion), 46% (conventional)
Sureda et al. (2018) [57]	1567	4.5	RIC (1165) and MAC(355)	52	61	29	19	Grade 2–4 aGVHD, 20%; 1-year cGHVD, 45%

aGVHD acute graft-versus-host disease, *ATG* antithymocyte globulin, *cGVHD* chronic graft-versus-host disease, *EFS* event-free survival, *FCR* fludarabine/cyclophosphamide/rituximab, *FMC* fludarabine/melphalan/cyclophosphamide, *PFS* progression-free survival, *RIC* reduced-intensity conditioning, *TRM* transplant-related mortality

TBI-based) and 728 with autologous transplant [29]. Despite more advanced disease and worse performance status, patients treated with allogeneic stem cell transplant had a lower recurrence rate than patients treated with autologous transplant. However, no differences in progression-free or overall survival were observed, mostly as a consequence of a higher transplant-related mortality in the allogeneic arm (30%). Similar results were observed in the EBMT registry study, with a 4-year transplant-related mortality of 38% in the allogeneic group [50].

As a consequence, subsequent studies were aimed at decreased transplant-related mortality, while preserving the efficacy of allogeneic stem cell transplant. To this regard, lower transplant-related mortality had been reported in small-size retrospective studies with the use of reduced intensity conditioning as opposed to myeloablative regimens [51]. To confirm these findings, the CIBMTR retrospectively analyzed the outcome of 208 patients with relapsed refractory follicular lymphoma treated with either regimen between 1997 and 2002 [52]. The use of a reduced intensity regimen was associated with a higher rate of relapse and acute and chronic GVHD at 3 years. However, it was also associated with a significantly lower transplant-related mortality, translating in similar progression-free and overall survival as compared to the use of myeloablative regimens.

These data supported the worldwide use of reduced intensity conditioning over myeloablative regimens in the following years. To this regard, the MD Anderson group retrospectively analyzed the long-term outcome of 47 young patients with relapsed follicular lymphoma, receiving reduced intensity conditioning followed by sibling allogeneic stem cell transplant, mostly matched, at time of remission [53]. The conditioning regimen included fludarabine (at the dose of 30 mg/m^2/daily) and cyclophosphamide (at the dose of 750 mg/m^2/daily) for 3 days, while rituximab (at the dose of 375 mg/m^2 on day −13 and 1000 mg/m^2 on day −6, +1, and + 8) was used to prevent GVHD. All patients achieved complete remission, 11% experienced grade ≥ 2 acute GVHD and 36% grade ≥ 2 chronic GVHD. Transplant-related mortality at 1 year was 10%, 5-year progression-free survival was 83%, and 5-year overall survival 85%. In a more updated follow-up, 9-year PFS was 72% and 9-year OS was 78%, with a plateau in survival at year 6 [54]. It is important to note, however, that patients included in this study were mostly young, with a good performance status, and sensitive to chemotherapy, likely explaining their excellent outcome. In addition, the use of reduced intensity conditioning remained associated with a high risk of chronic GVHD, a common cause of morbidity in patients receiving allogeneic transplant [55, 56]. An updated registry study was recently reported jointly by CIBMTR and EBMT [57]. Sureda et al. reported on 1567 patients who underwent allo-transplant for follicular lymphoma from HLA-identical donors (73% matched sibling and 27% HLA-identical unrelated donor) between 2001 and 2011. The median follow-up was 55 months. The 5-year probabilities of OS and PFS were 61% and 52%, respectively. The 5-year cumulative incidences of disease progression/relapse and TRM were 19% and 29%, respectively. This latest analysis confirms in a very large patient cohort, the extremely low rate of disease recurrence occurring after allogeneic transplant. The authors also conducted a multivariate analysis and found that chemoresistant disease, older age, heavy pretreatment, poor

performance status (PS), and myeloablative protocols were predictors for worse survival. In contrast to a prior analysis [52], they did not find an increase in relapse rate associated with reduced intensity conditioning. As the authors acknowledge, this multivariate analysis has considerable limitations, mainly related to the large proportion of patients with missing data on performance score (32%), histology (26%), prior treatment (45%), GVHD prophylaxis (18%), etc.

The ravages of chronic GVHD have led many to investigate the use of in vivo T-cell depletion. In a British study, including 88 patients with relapsed-refractory B-cell lymphoma, T-cell depletion with alemtuzumab resulted in a grade ≥ 2 chronic GVDH rate of 7%, a transplant-related mortality of 8%, and a 3-year progression-free survival of 65% (including patients who needed donor lymphocyte infusion) [58]. The same approach was subsequently used in a multicenter prospective trial including 82 young patients treated with allogeneic stem cell transplant (half of which from unrelated donor) at time of remission, between 1998 and 2009 [59]. After a median follow-up of almost 4 years, grade ≥ 2 chronic GVHD rate was 20%, transplant-related mortality rate 15%, and 4-year PFS was 76% (including patients who needed donor lymphocyte infusion). In a European study, the outcome of 88 patients receiving alemtuzumab or ATG was compared to that of 76 patients who did not undergo any T-cell depletion [60]. Patients receiving T-cell depletion had a significantly lower incidence of grade ≥ 2 acute GVHD (17% vs 31%, $p = 0.04$) and chronic GVHD (33% vs 73%, $p < 0.001$), but similar progression-free survival (including patients who needed donor lymphocyte infusion as salvage strategy).

Role of Donor

For many patients who do not have a matched sibling donor available at the time of allogeneic transplant, alternative options are matched unrelated donors, cord blood, and haploidentical donors.

In a retrospective analysis of the EBMT registry, including 131 patients treated with a matched unrelated donor transplant between 2000 and 2005, at a median follow-up of 3 years, grade ≥ 2 acute GVHD was reported in 37% of patients, chronic GVHD in 48%, and 3-year progression-free and overall survival were 47% and 51%, respectively [61]. Of interest, reduced intensity conditioning was the preferred regimen, and about half of patients had progressed after autologous stem cell transplant.

The clinical outcome associated with the use of umbilical cord blood in patients with chemo-sensitive lymphoma has also been retrospectively analyzed by the EBMT registry [62]. The majority of patients received single cord transplant after a reduced intensity regimen. Among patients with low-grade lymphoma, 1-year transplant-related mortality was 20%, 1-year progression-free survival 60%, and 1-year overall survival 68%.

Haplo-identical transplantation is also increasingly being utilized and provides a readily available treatment option for many patients lacking unrelated donors. Several

registry studies show outcomes in lymphoma patients that are comparable with those of related and unrelated donor transplant [63, 64]. We and others have explored the use of haplo-cord transplantation; by combining a mismatched graft with a cord blood unit, we observe rapid engraftment and very limited risks of GVHD [65]. We recently reported excellent outcomes in 42 patients with lymphoma (Vignette 9.2) [66]. This procedure – though technically more complex than haplo-identical transplantation – results in faster engraftment and lower rates of chronic GVHD [67]. By using an unrelated mismatched donor, it may also provide a transplant option for the approximately twenty percent of patients who lack suitable haplo-identical donors [68]. Collectively- despite rapidly evolving technologies- these data point to rapid improvements in outcome for alternative donor transplant for lymphoma. A donor can be identified for all and in the recommendation for transplant, and donor availability or donor type should no longer be a major determinant in the formulation of a transplant recommendation for patients with lymphoma [69].

Role in Transformed Lymphoma

About 3–5% of patients with follicular lymphoma experience transformation to large B-cell lymphoma every year, with a subsequent median survival of less than 2 years [70, 71]. Autologous stem cell transplant is an effective consolidation strategy for these patients, with a 5-year progression-free survival of 30% and a 5-year overall survival of 51% reported in a EBMT retrospective analysis of 50 chemosensitive patients [72], and similar findings observed in smaller studies [73–76].

A Canadian group has recently retrospectively compared the outcome of 22 patients with chemo-sensitive non-bulky transformed follicular lymphoma treated with autologous transplant, to that of 97 patients treated with allogeneic transplant and 53 receiving rituximab-based chemotherapy only [77]. No difference in 5-year overall survival was observed among the 3 groups (65% vs 46% vs 61%, $p = 0.24$). However, after adjusting for confounding factors, such as type of salvage chemotherapy regimen, a modest survival benefit was observed for autologous stem cell transplant over chemotherapy alone, while allogeneic stem cell transplant remained associated with a high rate of transplant-related mortality.

CIMBTR retrospectively analyzed the outcome of 108 patients with transformed follicular lymphoma treated with autologous transplant to that of 33 patients treated with allogeneic transplant [78]. While autologous transplant was associated with prolonged survival in the majority of patients, among patients treated with allogeneic stem cell transplant, a survival benefit was observed only for those receiving reduced intensity conditioning, likely as a consequence of decreased treatment-related mortality.

At this time, the optimal treatment of patients with transformed follicular lymphoma remains an active field of investigation. Multiple factors, including disease burden, chemo-sensitivity, and patient general conditions need to be weighed, both autologous and allogeneic stem cell transplant representing potential therapeutic options.

CAR-T Cells

CAR-T cell technology (Chimeric antigen receptor) has revolutionized the management of B-cell ALL and of refractory diffuse large B-cell lymphoma. CAR-T cells are patient-derived lymphocytes that are transduced in vitro with a chimeric receptor, part antibody, part co-signaling domain, part T-cell signaling domain. Anti-CD19 CAR-T have resulted in impressive and durable responses in patients with refractory ALL and large cell lymphoma [79]. The CAR-T cell field is developing rapidly with studies of modified CARs and new targets being reported daily. Most of the studies have been conducted in aggressive and transformed lymphoma, where approximately 50% of treated patients obtain durable remissions – a rate of response that is unheard of with other therapies. Experience in untransformed follicular lymphoma remains limited at present although some of the initial observations were made in this setting [80]. The toxicity of CAR-T cell therapy is considerable and includes severe cytokine release syndrome and neurological toxicity [81]. Commercial products have only recently been approved and the use of CAR-T cells is – for now – restricted to experienced centers [82].

Case Vignettes

Case Vignette 9.1
A 35-year-old woman presented with right-sided dull abdominal and flank pain. CT chest, abdomen, and pelvis showed retroperitoneal, mesenteric, pelvic, and inguinal adenopathy. A left inguinal lymph node biopsy showed grade 1 follicular lymphoma. She initially was observed, but 4 years later, because of slow disease progression, started Rituxan × 4 weekly treatments and obtained a partial response. Shortly thereafter she presented with chin numbness and subtle MRI abnormalities, but negative lumbar puncture. Because of concern over possible CNS involvement, she was treated with R-CHOP x6 and high-dose methotrexate. One and a half year later, she presented with widespread adenopathy, splenomegaly, and lytic lesions in the femur. Repeat biopsy was consistent with follicular lymphoma.

She then received 6 cycles of bendamustine-rituximab with excellent clinical response. This was followed by autologous stem cell collection and autologous stem cell transplantation using BEAM conditioning. She remains in remission 2 years after autologous stem cell transplantation.

Case Vignette 9.2
A 44-year-old male presented with extreme lymphocytosis of 250,000 per microliter, marrow replacement, splenomegaly, and diffuse PET-positive lymphadenopathy (highest SUV 9.2). LDH was elevated at 284 (ULN 192). He also had massive pleural effusions with lymphoma involvement. FISH on the bone marrow showed Ig gene rearrangement; PCR showed bcl2-IgH rearrangement. He received six cycles of bendamustine-rituximab and obtained a remission.

Within 3 months of completing chemotherapy, he had disease progression. Lymph node biopsy showed grade 3A follicular lymphoma. He was treated with RICE chemotherapy (rituximab-ifosfamide-carboplatin-etoposide) and obtained a partial remission. He was then offered allogeneic transplantation. Since no HLA-identical donor could be identified, he underwent haplo-cord transplantation using third-party donor cells from his sibling and an umbilical cord blood graft. Conditioning consisted of fludarabine-melphalan and TBI 400 cGray. He remains in ongoing remission more than 2 years after transplant, without graft vs host disease or other long-term sequelae.

Note: This chapter is based in part on an earlier publication: Stem cell transplantation in follicular lymphoma, by Satyajit Kosuri and Koen Van Besien, in Clinical Guide to Transplantation in lymphoma. Eds. Bipin Savani and Mohammed Mohty (Wiley Blackwell, 2015) [83].

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Part III
Emerging Therapy in Follicular Lymphoma

Chapter 10
Antibody Therapy in Follicular Lymphoma

J. C. Villasboas and Grzegorz S. Nowakowski

Introduction

The use of monoclonal antibodies (mAbs), alone or in combination with chemotherapy, is commonplace in the management of patients with B-cell lymphomas and many other cancers. With the growing number of novel therapeutic strategies developed in the recent years, it is easy to take for granted the major advance that monoclonal antibody therapy represents for the field of Oncology. This chapter will review the scientific evidence supporting the use of mAbs in follicular lymphoma (FL) with a focus on molecules targeting CD20, CD19, and CD22. We will also provide an overview of antibody-drug conjugates and look at the prospects of novel targets for mAb therapy in FL. The studies discussed in this section include patients with indolent B-cell NHL, primarily those with follicular lymphoma histology grades 1 to 3a. Studies focused on patients with grade 3b or transformed follicular lymphomas are excluded from this review. When possible, studies are discussed in chronological order of publication to facilitate understanding of how practice patterns changed as new scientific evidence emerged.

Monoclonal Antibodies Targeting CD20

Rituximab

In the fall of 1997, Rituximab received regulatory approval in the United States and became the first monoclonal antibody available for the treatment of human

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cancer. This landmark approval inaugurated a new paradigm of cancer treatment, fueled by the discovery of targetable cancer antigens and supported by technological advances in antibody production systems. The impact of rituximab – a type I chimeric IgG1 monoclonal antibody with specificity for CD20 – is such that the history of lymphoma treatment is dichotomized between pre- and post-rituximab eras [1]. The experience with the use of rituximab in FL is extensive and dates back to the very first publication that led to FDA approval of the drug in the United States as summarized in Figs. 10.1 and 10.2. Despite numerous attempts at challenging its role, rituximab retains center stage position in the management of FL which is anchored on solid scientific evidence reviewed here.

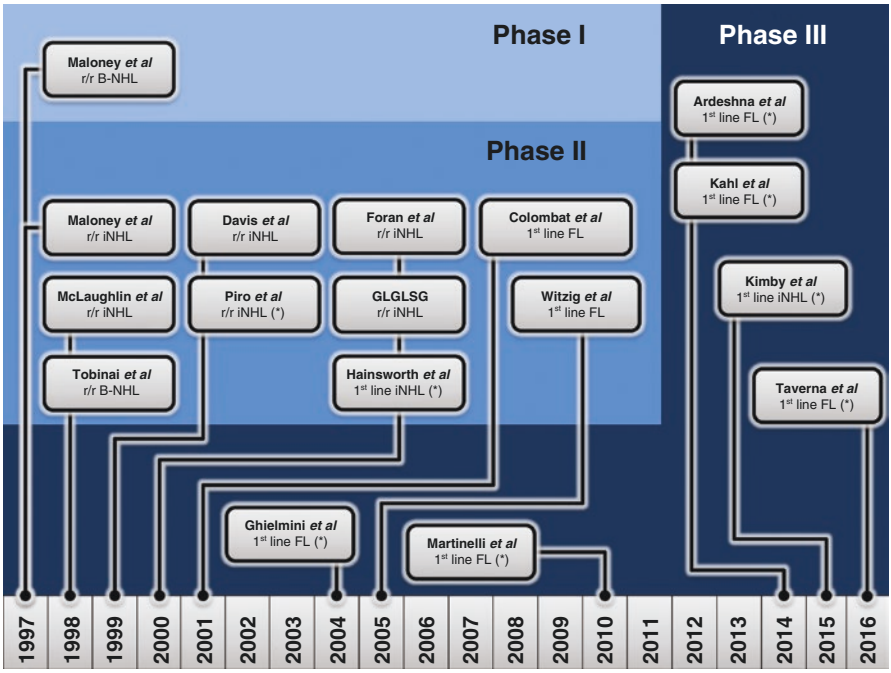

Fig. 10.1 Chronological overview of rituximab monotherapy clinical trials in follicular lymphoma. Individual studies are identified by lead author, setting, and primary population enrolled. Trials are stratified by phase of development (phase I, phase II, phase III) and connectors indicate the year of publication of original manuscript. Asterisks (∗) indicates studies with maintenance or extended rituximab treatment schedules. Publications with updated results of previously published studies were excluded. r/r relapsed/refractory, B-NHL B cell non-Hodgkin lymphoma, iNHL indolent non-Hodgkin lymphoma, FL follicular lymphoma

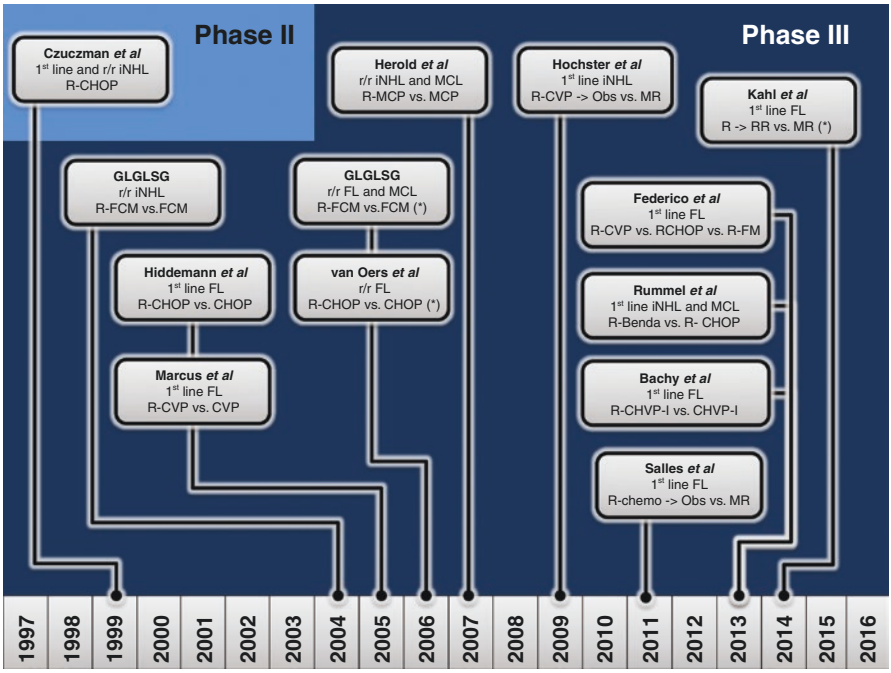

Fig. 10.2 Chronological overview of clinical trials combining rituximab with chemotherapy in follicular lymphoma. Individual studies are identified by lead author or group, setting, and primary population enrolled. Trials are stratified by phase of development (phase II, phase III) and connectors indicate the year of publication of original manuscript. Asterisks (∗) indicates studies with maintenance or extended rituximab treatment schedules. Publications with updated results of previously published studies were excluded. r/r relapsed/refractory, B-NHL B cell non-Hodgkin lymphoma, iNHL indolent non-Hodgkin lymphoma, FL follicular lymphoma, MCL mantle cell lymphoma, R rituximab, CHOP cyclophosphamide, doxorubicin, vincristine, and prednisone, GLGLSG German Low-Grade Lymphoma Study Group, FCM fludarabine, cyclophosphamide, and mitoxantrone, CVP cyclophosphamide, vincristine, and prednisone, MCP mitoxantrone, chlorambucil, and prednisolone, RR rituximab retreatment, MR maintanance rituximab, FM fludarabine, mitoxantrone, Benda bendamustine, CHVP cyclophosphamide, adriamycin, etoposide, and prednisolone, I interferon

Initial Studies of Rituximab Monotherapy in Relapsed/Refractory FL

The first trials evaluating the clinical efficacy of rituximab in FL consisted of single-arm phase I/II studies in patients with relapsed or refractory disease [2–4]. The seminal paper which led to regulatory approval of the drug in the United States was published by McLaughlin and colleagues in 1998 [5]. In this multicenter study, a total of 166 patients with relapsed or refractory indolent B-cell lymphomas expressing CD20 were treated. Patients with tumors equal or greater than 10 cm were excluded from participation. The majority of enrolled patients had follicular

histology (71%) and all received four weekly doses (375 mg/m^2) of the antibody, at the time known as IDEC-C2B8. The objective response rate for the entire cohort was 48% (6% complete responses) with 60% of the FL patients responding to treatment. The projected median time to progression was 13 months and the drug was associated with very few high-grade adverse events (beyond what is now known to be typical infusion reactions).

A companion study, published in 1999 by Davis and colleagues, extended the evaluation of rituximab's efficacy to patients with bulky relapsed/refractory indolent B-cell lymphomas [6]. A total of 31 patients were treated in this small single-arm study including 22 patients (71%) with follicular histology. The same treatment schedule and dosage was used and once again rituximab proved safe. The overall response rate for the entire cohort was 39% (12 patients) including one complete response.

By the end of the year 2000, three additional phase II studies had been published confirming the safety and efficacy of rituximab monotherapy in the treatment of relapsed/refractory FL around the world. The German Low-grade Lymphoma Study group treated a total of 38 patients with indolent histologies and showed a 47% overall response rate after 4 weekly infusions [7]. In the United Kingdom, Foran and colleagues reported a similar overall response rate (46%) using the same treatment schedule [8]. In the United States, Piro and colleagues demonstrated an overall response rate of 57% after treating 37 patients with an extended regimen (8 weekly doses of 375 mg/m^2) [9].

Collectively, these initial studies served as a consistent display of rituximab's clinical performance and safety, leaving no doubt that it was here to stay.

Studies of Rituximab Monotherapy in Untreated FL

A second wave of trials testing the efficacy of single-agent rituximab in the frontline treatment of FL followed these seminal studies. Hainsworth and colleagues published in 2000 the first report of a phase II study using single-agent rituximab as initial systemic therapy for patients with indolent B-NHL [10]. The original publication reported on the first 39 patients (64% with follicular histology) who were treated with the standard rituximab induction schedule (four weekly infusions of 375 mg/m^2). At the time of first evaluation (6 weeks), 54% of patients had achieved an objective response including 5% complete remissions. Patients without primary refractory disease were treated with an extended regimen consisting of additional 4-week courses repeated every 6 months for up to 2 years. Updated results were published 2 years later on a total of 60 evaluable patients [11]. At the time of extended follow-up, the objective response rate following induction (at the 6-week mark) for the cohort was 47%, which increased to 73% with additional infusions of rituximab in the extended regimen.

A second report on the use of frontline single-agent rituximab was published by Colombat and colleagues in 2001 [12]. A total of 49 patients with follicular lymphoma and low tumor burden received standard rituximab induction followed by observation. The overall response rate was 73% including 26% achieving a complete remission (of which 6% was unconfirmed). At the 1-year post-treatment mark, response was maintained by most (92%) of the patients who achieved a complete remission and many

(61%) of the patients with a partial response. These outstanding results were updated in 2012 when the 7-year follow-up report was published [13]. With longer follow-up, best overall response was reported at 80% including 52% with complete remissions. Median progression-free survival was 23.5 months and overall survival 92%. Seven patients (15%) remained free of progression, demonstrating that long-term responses are possible in select FL patients after a single 4-week course of frontline rituximab.

A third phase II study of frontline single-agent rituximab was published by the North Central Cancer Treatment Group (NCCTG) in 2005 [14]. Enrollment in this trial was restricted to untreated advanced stage (III/IV) grade 1 FL who received standard rituximab induction followed by observation. A total of 36 patients were treated with overall response rate of 72% and 36% complete remissions. Median time to progression was 2.2 years.

By the end of 2012, a couple of additional studies had reported similarly high response rates with mild toxicity for the frontline use of rituximab in FL [15, 16]. Collectively, these studies created significant equipoise for a comparison between observation and rituximab monotherapy as a standard strategy for the management of newly diagnosed FL.

The much-expected head-to-head comparison between observation (watch-and-wait) and rituximab in untreated FL was published in 2014 [17]. In this international landmark phase III study, Ardeshna and colleagues randomized 379 newly patients with asymptomatic, advanced stage, non-bulky FL to one of three treatment groups: (1) watch-and-wait, (2) standard rituximab induction (375 mg/m^2 weekly for 4 weeks), and (3) standard rituximab induction followed by maintenance (12 additional infusions repeated every 2 months). The original design of the trial was set out to answer two fundamental questions: (1) Is rituximab superior to observation in the management of advanced stage, non-bulky, asymptomatic FL? (2) Is an extended rituximab schedule (maintenance) superior to a single 4-week course in these patients? Unfortunately, the arm consisting of standard rituximab induction was prematurely closed due to poor accrual and the trial was amended for a 2-arm study (observation versus rituximab induction followed by maintenance). A total of 379 patients were assigned (1:1) between the watch-and-wait and maintenance rituximab arms. At the 3-year mark, 46% of patients in the watch-and-wait group remained without treatment compared to 88% in the maintenance arm. Quality-of-life measurements obtained midway through the first year of rituximab maintenance (measured at month 7 and compared to baseline) favored the treatment arm. Overall survival was the same for both groups with greater than 90% of the patients alive at 3 years regardless of assignment. Serious adverse events were rare and no treatment-related deaths occurred. In the absence of an overall survival benefit, the results of this study leave plenty of room for argument on both sides of the aisle. Proponents of the watchful-wait approach are quick to point out that almost half of the patients in the observation group did not require treatment by 3 years, arguing that unnecessary treatment will be given to roughly half of all FL patients if rituximab is administered universally to newly diagnosed cases. On the other hand, pro-treatment proponents highlight the improvement in the quality of life after initiation of rituximab treatment, often labeling the observation strategy as “watch-and-worry.” Ultimately, the debate of the optimal management of asymptomatic newly diagnosed FL is still ongoing [18]. This underscores the need for an individualized

approach, taking into account both clinical and biological factors along with patients' preferences and values.

Rituximab in Combination with Chemotherapy

Studies combining rituximab to multiagent chemotherapy (Fig. 10.2) were the natural step once the efficacy and safety of monotherapy was demonstrated. The first published report in indolent lymphomas was authored by Czuczman and colleagues in 1999 [19]. In this phase II trial, 40 patients (majority untreated) with indolent lymphomas received 6 cycles of rituximab in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). An impressive response rate of 95% (with 55% complete responses) was shown with no obvious toxicity beyond that expected for CHOP alone. The 9-year follow-up report was published in 2004 demonstrating 100% response rate and 87% complete responses. A long duration of response was evident with the extended follow-up where the median time to progression exceeded 80 months.

Additional support for the combination of rituximab to multiagent chemotherapy in relapse/refractory FL came from two phase III trials. In the first study by the German Low-Grade Lymphoma Study Group, a cohort of 147 patients with relapsed indolent lymphomas (44% of which was FL) was randomly assigned for treatment with FCM (fludarabine, cyclophosphamide, and mitoxantrone) plus or minus rituximab [20]. The addition of rituximab was associated with increase in response rate and superior progression-free as well as overall survival. The second study randomized 465 patients with relapsed/refractory FL to receive CHOP plus or minus rituximab. The addition of rituximab to CHOP in this study increased response rate and prolonged progression-free survival. Eligible responding patients underwent a second randomization between observation and maintenance rituximab consisting of infusions every 3 months for a total of 2 years. Extended treatment with rituximab was associated with longer progression-free and overall survival with larger treatment effect observed in patient who did not receive rituximab as part of the induction regimen.

Four large phase III studies evaluated the benefit of adding rituximab to chemotherapy into the frontline treatment of patients with FL. The first study by Marcus and colleagues reported on 321 patients randomized for treatment using CVP (cyclophosphamide, vincristine, and prednisone) with or without rituximab [21]. The addition of the monoclonal antibody increased overall and complete response rates and prolonged progression-free survival. Extended follow-up showed that the benefit translated in improvement in all measured clinical outcomes including overall survival [22]. The second study by Hiddemann and colleagues reported on 428 patients with untreated FL randomly assigned for treatment between CHOP and R-CHOP [23]. Once again, the addition of rituximab improved all measures of treatment efficacy including overall survival without increased incidence of serious adverse reactions. The third study, authored by Herold and colleagues, reported on 201 patients with untreated FL randomized to receive MCP (mitoxantrone, chlo-

rambucil, and prednisolone) with or without rituximab. Response and survival rates were improved with rituximab. The fourth study by Salles and colleagues reported on 358 patients randomly assigned for treatment with CHVP (cyclophosphamide, adriamycin, etoposide, and prednisolone) plus interferon with or without the addition of rituximab. Response and progression-free survival was significantly increased with rituximab. This was the only study out of the four that failed to show a statistically significant improvement in overall survival with the addition of rituximab. Although each trial used a different chemotherapy backbone, they all delivered a similar underlying message: rituximab + chemotherapy is superior to chemotherapy alone in the frontline treatment of FL.

At this point the question was no longer whether rituximab should be added to chemotherapy but rather which chemotherapy backbone would be the best partner for rituximab in the frontline management of FL. Two large phase III studies attempted to answer this question by providing a series of head-to-head comparisons. Study FOLL05, conducted by the Fondazione Italiana Linfomi, randomized 534 patients with untreated FL to treatment with rituximab added to one of the 3 chemotherapy backbones: CHOP, CVP, or FM (fludarabine, mitoxantrone) [24]. All arms achieved overall responses greater than 88% which was not statistically different between the groups. R-CVP performed inferior to the other 2 arms in terms of progression-free survival while R-FM was associated with higher incidence of severe neutropenia. Although no clear winner was declared, it seemed that R-CHOP combined the best risk-benefit ratio of the three regimens studied. The landmark study by Rummel and colleagues of the StiL group reported on 514 patients with untreated indolent lymphoma (54% of which had FL) randomized for treatment with either bendamustine plus rituximab (BR) or R-CHOP. A non-inferiority design was applied with a 10% prespecified margin for the primary outcome of progression-free survival (PFS). Ultimately, treatment with BR proved superior to R-CHOP with a median PFS exceeding 69 months in the BR groups compared to 31.2 months in the R-CHOP group. In addition, the adverse event profile of BR proved more favorable compared to R-CHOP. As a direct result of this study, bendamustine plus rituximab has been incorporated as the preferred frontline regimen for patients with FL requiring treatment by many guidelines around the globe.

Rituximab Maintenance in FL

The concept of rituximab maintenance therapy has been tested in a number of randomized trials in FL, all of which demonstrated improvement in progression-free survival (or related time-to-event outcome) but failed to show a consistent measurable improvement in overall survival. The first two phase III studies looking at this question in the relapsed/refractory population were published in 2006. Using a two-stage design, Forstpointner and colleagues randomized 105 patients with relapsed/refractory FL who had responded to induction therapy using the FCM or R-FCM (part of a separate randomization) to observation or extended rituximab (2 additional 4-week courses after 3 and 9 months) [25]. Duration of response was increased

with the extended schedule. Using a similar design, van Oers and colleagues randomized 334 patients with relapsed/refractory FL who achieved a response to induction (CHOP or R-CHOP) to receive rituximab maintenance (every 3 months for 2 years) or observation [26]. The extended rituximab arm experienced longer progression-free survival and overall survival in their first report published after a median follow-up of 33 months. In an updated report with mature follow-up (median 6 years), the PFS benefit was maintained but the 5-year overall survival did not reach a statistically significant difference between the groups [27].

The first phase III study to examine the benefit of rituximab maintenance in the frontline setting of FL was published in 2009 by Hochster and colleagues from the Eastern Cooperative Oncology Group (ECOG) [28]. E1496 randomized 311 patients with indolent lymphoma (90% FL) who had stable disease or response after CVP induction to rituximab maintenance (4-week course every 6 months for 2 years). Median progression-free survival was improved with maintenance but overall survival failed to reach statistical significance.

Investigators from the Swiss Group for Clinical Cancer Research (SAKK) tested a different rituximab maintenance schedule consisting of 4 doses every 2 months in a mixed cohort of patients with FL (64 untreated and 138 relapsed) [16]. On the SAKK 35/98 study, all patients received induction treatment with rituximab monotherapy and the 151 individuals who did not progress proceeded to randomization between observation and extended treatment. Again, additional doses of rituximab were associated with longer PFS but not with a statistically significant difference in overall survival.

The Prima study, published in 2011, was designed to be the definitive answer to the debate over rituximab maintenance in untreated FL in the post-rituximab era [29]. In this multicenter international phase III trial, 1217 patients with untreated FL and high tumor burden were induced with one of three rituximab-containing chemotherapy protocols according to investigator choice (R-CVP, R-CHOP, or R-FCM). The 1019 patients who achieved at least a partial response went on to be randomized between observation and rituximab maintenance (every 2 months for 2 years). After a median follow-up of 36 months, PFS improved from 57.6% in the observation group to 74.9% in the maintenance group. Survival was excellent in both groups and no statistically significant difference was detected. Updated results after almost 10 years of follow-up were presented at the American Society of Hematology annual meeting in December 2017, confirming a sustained PFS benefit for the maintenance arm and an identical overall survival between groups.

ECOG 4402 (also known as the RESORT trial) examined a different question by randomizing patients with untreated FL to either maintenance rituximab or re-treatment rituximab at the time of relapse [30]. In this study, 289 patients with untreated FL and low tumor burden who had achieved a response to induction rituximab monotherapy were randomly assigned to either group. A composite primary endpoint of time-to-treatment failure was used. At 3 years, more patients in the maintenance group remained free of cytotoxic chemotherapy treatment but no significant difference was noted in the primary outcome. The authors concluded that re-treatment with rituximab provided similar degree of disease control with less rituximab usage.

In the absence of a consistent overall survival benefit, there is currently no agreed universal consensus on the advantages of rituximab maintenance compared to observation in the management of FL patients who responded to induction treatment. Curiously, the most popular use of rituximab maintenance is following a successful induction using BR (bendamustine and rituximab). Since none of the phase III maintenance trials utilized BR as their backbone, this application is largely based on extrapolation from other multiagent chemoimmunotherapy bundles and non-randomized data. A reasonable approach is to offer extended treatment for patients with high burden symptomatic disease, in whom prolongation of the disease-free interval is expected to translate into improved quality of life and outweigh the risks of prolonged rituximab treatment. Ultimately, treatment individualization returns to center stage in the discussion of treatment strategies for patients with FL who require active treatment.

Obinutuzumab

Obinutuzumab (GA101 or Gazyva) is a type II humanized IgG1 monoclonal antibody targeting CD20 displaying enhanced cytolytic activity against B cells in preclinical studies [31, 32]. This glycoengineered molecule is the most active contender in the challenge to overthrow rituximab from its dominant position in the treatment of follicular lymphomas. In early phase I studies, obinutuzumab monotherapy showed overall response rates ranging from 32% to 48% in B-NHL patients with relapsed/refractory disease [33–35]. No concerning safety signals were detected and the drug moved forward in the development pipeline.

Three phase II studies (GAUDI, GAUGUIN, and GAUSS) further examined the clinical efficacy of obinutuzumab in patients with follicular lymphoma. The GAUDI study (BO21000) published in 2013 evaluated the safety and efficacy of obinutuzumab (G) added to two different chemotherapy backbones (G-CHOP or G-FC) using a randomized phase II design [36]. Responders were eligible for extended treatment with the antibody every 3 months for up to 2 years. A total of 56 patients with relapsed/refractory FL were treated and response rates after induction were encouraging in both the G-CHOP (ORR 96%; CR 39%) and G-FC (ORR 93%; CR 50%) arms. Safety profile was compatible to that expected for multiagent chemoimmunotherapy protocols. The phase I/II GAUGUIN study tested the safety and efficacy of single-agent obinutuzumab in patients with relapsed/refractory B-NHL [37]. A total of 40 patients were treated in the indolent NHL cohort (85% FL) and randomized between two dosages of the drug (400 mg on days 1 and 8 of cycle 1 followed by 400 mg on day 1 of cycles 2–8; or 1600 mg on days 1 and 8 of cycle 1 and 800 mg on day 1 of cycles 2–8). The highest dose cohort had greater activity, with an overall response rate of 55% (9% complete responses) including some patients with rituximab-refractory disease. The first head-to-head comparison between rituximab and obinutuzumab came from the GAUSS study [38]. In this phase II trial, 175 patients with rituximab-sensitive relapsed indolent B-NHL (85%

FL) were randomized for treatment with either rituximab or obinutuzumab. Patients without disease progression continued on a maintenance schedule of their assigned antibodies for up to 2 years. Overall response to obinutuzumab was 45%, which was not statistically different compared to rituximab. Progression-free survival did not differ between the groups and the trial was essentially negative. Despite the loss in this first round, obinutuzumab proceeded to challenge rituximab in phase III trials.

Two phase III trials (GADOLIN and GALLIUM) further examined the activity of obinutuzumab in the management of FL patients, each leading to an approved indication of this agent in FL. The GADOLIN study, published in 2016 by Sehn and colleagues, was a phase III study evaluating the activity of obinutuzumab combined to bendamustine in patients with rituximab-refractory indolent B-NHL [39]. Included in the definition of rituximab refractoriness were patients who failed to respond or progressed during treatment with a rituximab-containing regimen, along with patients whose disease relapsed within 6 months of the last dose of rituximab. A total of 396 patients (81% FL) were randomly assigned for treatment with either single-agent bendamustine (B) or bendamustine plus obinutuzumab (BG). Non-progressing patients in the obinutuzumab arm continued on maintenance therapy for up to 2 years. The trial was stopped early for benefit after it had met its primary endpoint of progression-free survival on planned interim analysis (median PFS of 14.9 months for the bendamustine group and not reached for the obinutuzumab plus bendamustine arm). No difference in overall survival was detected at the time of analysis. The safety profile of the BG arm was comparable to the B arm. Based on the results of GADOLIN the FDA approved in February of 2016 obinutuzumab for use in combination with bendamustine followed by binutuzumab monotherapy in patients with FL who relapsed after, or are refractory to, a rituximab-containing regimen. The GALLIUM study, published in 2017 by Marcus and colleagues, enrolled 1202 patients with untreated advanced-stage FL [40]. In this multicenter trial, patients were randomly assigned for treatment with chemotherapy added to either obinutuzumab or rituximab. Three chemotherapy regimens were used (CHOP, CVP, or bendamustine) and the choice of regimen was stipulated individually at each site where all patients received the same induction protocol. Responding patients continued with maintenance therapy of the same antibody contained in their induction regimen for up to 2 years. The trial was also stopped early for benefit after meeting its primary endpoint of progression-free survival in a pre-planned interim analysis showing an absolute increase of 6.7% in the estimated 3-year PFS in favor of obinutuzumab. Overall response rate was greater than 86% and did not differ between groups. High-grade (grades 3–5) and serious adverse reactions were more frequent in the obinutuzumab group and overall survival did not differ between groups. Based on the results of GALLIUM, the FDA extended the indication of obinutuzumab (in combination with chemotherapy and followed by maintenance) to include patients with untreated advanced stage FL.

The approval of obinutuzumab in FL extends the treatment portfolio for these patients but leave plenty of room for discussion of the optimal monoclonal antibody of choice in FL. Because GALLIUM did not include an arm with rituximab monotherapy, its results mainly apply for those patients in whom their treating physician

deem eligible and requiring of chemotherapy. Additionally, because both GALLIUM and GADOLIN included mandatory maintenance therapy for responding patients, the applicability of results are questionable in patients who do not desire or are not eligible for extended therapy. There is an ongoing concern for the long-term safety of rituximab maintenance (2 or 4 years) following BR induction as new data emerges. In the absence of an overall survival benefit and in the presence of a signal for increased toxicity noted on the results of GALLIUM, the battle for the title of monoclonal antibody of choice in FL continues.

Ofatumumab

Ofatumumab, a fully human monoclonal antibody targeting a different epitope of CD20, is the other member of the second-generation class of monoclonal antibodies being tested in B-NHL. Three early-stage trials testing the safety and activity of ofatumumab in FL have been published thus far. The first study, published in 2008 by Hagenbeek and colleagues, was a phase I/II trial testing different single-agent dosages of the drug in a total of 40 patients with relapsed/refractory FL [41]. No concerning safety signals were found and response rates varied from 20% to 63% in the different dose-level cohorts. The subsequent 2 studies were published in 2012 and both authored by Czuczman and colleagues. One of the studies focused on patients with untreated FL which were treated in a phase II design with ofatumumab CHOP and randomized to one of two dose levels of ofatumumab (O-CHOP) [42]. A high response rate was demonstrated (greater than 90% in both arms) and no excessive or unexpected toxicity was identified. The other study focused on patients with rituximab-refractory disease and enrolled a total of 116 patients with heavily pretreated FL [43]. Patients were treated with ofatumumab monotherapy at two randomly assigned dose levels. Overall response rates were 10% and 13% at the lower and higher dosages respectively. Compared to obinutuzumab, the experience with ofatumumab is more limited and preliminary. As obinutuzumab continues to actively challenge rituximab and gain ground as with approved indication for the treatment of FL the future of ofatumumab in this disease remains uncertain.

Monoclonal Antibodies Targeting CD19

Monoclonal antibodies targeting CD19, another surface marker commonly expressed by B-lineage NHL cells, have been recently developed. Although no approved indications for this class of molecules exist in lymphoma, ongoing studies are evaluating the role of targeting CD19 in this disease. MOR208 (XmAb 5574) is a humanized monoclonal antibody against CD19 with an engineered FC receptor for enhanced cytotoxic activity. Preclinical studies have shown promising activity [44–47] but clinical studies are still early in development and primarily focused in

chronic lymphocytic leukemia [48]. Ongoing studies combining MOR208 with the immunomodulatory drug lenalidomide seem particularly promising. Another molecule of the anti-CD19 class is inebilizumab (MEDI-551), a glycoengineered molecule with potent B-cell depleting activity in vitro [49, 50]. Trials with the drug are ongoing but published results in indolent B-cell lymphoma patients are still lacking. The recent development of chimeric antigen receptor (CAR) T-cell therapy targeting CD19 poses a logistical challenge for further development of monoclonal antibodies targeting CD19 in lymphoma. There is at least the theoretical concern that using anti-CD19 antibodies prior to CAR-T cell therapy may decrease the efficacy of the chimeric lymphocytes by exerting selective pressure on CD19-expressing malignant cells. Despite its logical appeal, targeting CD19 with monoclonal antibodies has no current role in the clinical management of follicular lymphoma and remains an area of scientific investigation.

Monoclonal Antibodies Targeting CD22

Utilizing monoclonal antibodies to target additional antigens on the surface of malignant B cells was a natural unfolding in the field of cancer immunotherapy inaugurated by the introduction of rituximab. Epratuzumab, a humanized IgG1 against CD22, targets a molecule strongly expressed in follicular, marginal zone and mantle B cells as well as many B-NHL cells. The first report evaluating the safety and activity of epratuzumab in B-NHL was published by Leonard and colleagues in 2003 [51]. In this phase I/II study, 50 patients with relapsed indolent B-NHL (72% FL) were treated in escalating dose-level cohorts of the antibody weekly for four treatments. No dose-limiting toxicity was identified and an objective response was seen in 18% of patients – all with follicular histology. Median duration of response was 79.3 weeks which provided a good lead for further research of this agent in FL. Due to their favorable toxicity profile and potential synergistic activity, further studies of epratuzumab evaluate the anti-CD22 antibody in combination with rituximab. The first report of dual monoclonal antibody therapy was published in 2005 by Leonard and colleagues [52]. In this phase II study, 23 patients with recurrent B-NHL (68% FL) received epratuzumab and rituximab weekly for four doses. The combination was well tolerated and 67% of patients with FL achieved a response (including 60% complete remissions). Responses seemed durable, with median time to progression of 17.8 months in patients with indolent histologies. A second phase II study with almost identical design was published in 2006 by Strauss and colleagues [53]. A total of 65 patients (52% FL) with relapsed/refractory B-NHL were treated with the dual monoclonal antibody strategy and 64% of patients with FL showed an objective response. A third multicenter phase II trial further solidified the notion that combined anti-CD19 and anti-CD22 antibody therapy was safe and efficacious in indolent B-NHL [54]. This report, published in 2008 by Leonard and colleagues, focused on indolent histologies and showed an overall response rate of 54% in the 41 patients with relapsed FL including 24% complete responses. A remarkably durable response was shown in this study with median duration of

13.4 months in FL patients. These preliminary studies in relapsed FL patients provided the rationale for the CALGB 50701 study, testing the activity of epratuzumab combined with rituximab in untreated FL patients [55]. In this phase II study, a total of 59 patients with FL were treated with an induction regimen combining the two antibodies followed by repeated infusions every 8 weeks for four additional treatments. Toxicities were mild and compatible with that of rituximab monotherapy. An overall response rate of 88% was shown, including 42% with complete responses. Responses once again seemed durable with 60% of patients maintain a response by 3 years. Despite a very strong signal in FL patients, favorable toxicity profile and potential for synergistic treatment combined with rituximab, drug development for epratuzumab in follicular lymphoma came to a halt likely due to competing strategies that emerged around the same time period. Epratuzumab currently has no active role in the management of FL outside a clinical trial.

Antibody-Drug Conjugates in Follicular Lymphoma

The term antibody-drug conjugates (ADCs) is used to describe therapeutic molecules that combine the specificity of monoclonal antibodies with a cytotoxic payload that is meant to be delivered precisely to the interior of cells expressing the target. Many ADCs are already in clinical use currently such as trastuzumab emtansine (breast cancer) and brentuximab vedotin (Hodgkin lymphomas and other CD30+ lymphoid malignancies). A variety of ADCs have been tested in B-cell NHL but for the most part only a few have shown a strong enough signal to proceed to later stages of clinical experimentation. Two such molecules deserve special mention in the context of FL and will be discussed here: coltuximab ravtansine, and inotuzumab ozogamicin.

Coltuximab ravtansine (SAR3419 or huB4-DM4) is an ADC combining a humanized IgG1 monoclonal antibody against CD19 (huB4) to a potent microtubule inhibitor (DM4). In a first-in-human phase I trial, the drug was given in escalating doses to a cohort of 39 patients (44% FL) with relapsed/refractory B-NHL [56]. The maximum tolerated drug was determined to be 160 mg/m^2 every 21 days and 74% of all patients demonstrated a reduction in tumor size. Toxicity was generally mild and the drug was felt to have a favorable adverse event profile. A separate phase I study evaluating a weekly dosing schedule found increased toxicities, likely due to its long terminal half-life, and anti-lymphoma activity in the order of 30% [57]. Despite a signal of activity in FL, no active trials currently exist for this drug in this population. Drug development for coltuximab ravtansine continues in diffuse large B cell lymphoma.

Inotuzumab ozogamicin (CMC-544) is an ADC that combines a humanized IgG4 monoclonal against CD22 (G544) with a cytotoxic antibiotic (calicheamicin). The drug is currently approved for the treatment of relapsed/refractory pre B-cell acute lymphoblastic leukemia. Early indication of its activity in relapsed/refractory FL patients was noted on the phase I trial designed to establish the safety of the drug and determine the maximum tolerated dose [58]. The study enrolled 79 patients with relapsed/refractory B-NHL of various histologies. The objective response rate at the

end of treatment was 39% for the entire cohort and 68% for all patients with follicular NHL treated at the MTD. A parallel phase I study in a Japanese cohort of relapsed/refractory FL lymphoma patients found a similar maximum tolerated dose. Overall response rate in the Japanese study was 85% (54% complete responses) confirming a strong early signal of activity in this population. A subsequent phase I/II study evaluated the safety and efficacy of inotuzumab in combination with rituximab in patients with relapsed/refractory B-NHL. A total of 119 patients were enrolled (35% FL) and the study confirmed the maximum tolerated dose found in single-agent studies. An objective response rate of 87% was seen in FL patients and the median progression-free survival for the FL cohort had not been reached at the time of the publication. At this time inotuzumab ozogamicin remains an investigational drug in FL and drug development has halted in this particular population of lymphoma patients.

Conclusion

Monoclonal antibodies are a vital part of the armamentarium used to treat patients with follicular lymphomas. Dozens of studies, thousands of patients, and decades of clinical experience strongly support the use of these drugs in the treatment of patients with follicular lymphoma and other hematological malignancies. Since the introduction of rituximab, the first mAb approved for the treatment of human cancer, the evolution in the treatment FL mirrors the trajectory of drug development in the field of monoclonal antibody therapy as we hope to have shown in this chapter. While rituximab remains a centerpiece in the management of most patients with FL, next-generation molecules with enhanced properties begin to claim their own space in the drug portfolio, as evidenced by recent FDA approvals. As the field of oncology embarks on the next phase of cancer immunotherapy, which brings checkpoint inhibitors and CAR-T cells, it will be critical to examine how these new drugs can build on the solid advances gained with the advent of mAb therapy. In a fast-paced and ever-changing field, careful examination of the immunological ecosystem where the tumor is inserted will be critical to increase our chances of success.

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Chapter 11
Molecular Targeting in Follicular Lymphoma

Loretta J. Nastoupil

Introduction

Follicular lymphoma (FL) is typically an indolent disease that is sensitive to a variety of immunotherapy and chemotherapy agents; however, FL exhibits a continuous pattern of relapses with decreasing sensitivity to treatment over time [1–3]. Common initial management strategies for FL vary from watchful waiting for low tumor burden or asymptomatic patients to rituximab (chimeric monoclonal CD20 antibody) monotherapy to chemo-immunotherapy for high tumor burden patients with common chemotherapy regimens consisting of bendamustine, CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or CVP (cyclophosphamide, vincristine, prednisone) in combination with rituximab or obinutuzumab (glycoengineered humanized monoclonal CD20 antibody) followed by maintenance therapy with the monoclonal antibody [1, 4–8]. Choice of treatment depends on multiple factors including patient-specific factors such as age, performance status, and comorbidities as well as tumor-specific factors such as tumor burden, histologic grade, and treatment objectives. Prognosis and quality of life including treatment-related side effects also play a role in decision making.

In the relapsed setting, treatment options are equally abundant and dependent on similar patient- and disease-specific characteristics with one exception, which includes the consideration of prior therapy and the outcomes associated with prior therapies. Despite a paucity of drug approvals for a number of years for relapsed indolent lymphoma, the past few years have resulted in rapid development with several drugs approved for the management of FL. Outcomes for patients with FL are improving and likely reflect the expanding treatment landscape, improved understanding of lymphoma biology, and improvement in supportive care. This is

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good news for our patients. Despite these advances there are still unmet needs in the management of FL in modern times including improvement in risk stratification and development of novel therapy that will result in indefinite remission without a detrimental impact on the quality of life.

This chapter will highlight our current understanding of the B-cell receptor (BCR) signaling pathway and the application of successful therapeutic targets that have resulted in FDA approval, including idelalisib and copanlisib. In addition, we will discuss potential mechanisms of resistance and rationale combinations that may overcome these resistance pathways. Lastly, we will discuss new agents currently in early phase development that may shape the treatment landscape in the near future.

The B-Cell Receptor Signaling Pathway

Targeted therapy has been considered to be the preferred approach to cancer therapy with the potential to reduce toxicity and improve upon efficacy. The success story of targeted therapy in chronic myelogenous leukemia (CML) has generated enthusiasm and dedication to identifying critical pathways for lymphomagenesis and survival. B cells are defined by the rearrangement of immunoglobulin heavy (IgH) and light (IgL) chain genes, leading to the expression of a unique BCR. Signals from the BCR act through downstream signaling pathways that drive proliferation, growth, and survival of a normal B cell. BCR signaling has also been shown to be manipulated and critical to most B-cell malignancies and provides a number of therapeutic targets (Fig. 11.1). Antigen-dependent and antigen-independent activation of the BCR pathway are fundamentally different mechanisms of BCR signaling [9, 10], although the contribution of these two types of signaling in FL and across chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma (NHL) subtypes is debatable. Though there remains much to be learned about targeting the BCR pathway in FL lymphoma, this appears to be an attractive approach with a number of BCR tyrosine kinase inhibitors with proven efficacy and several more in development for patients with relapsed FL.

Phosphoinositide 3-Kinase (PI3K) Inhibitors

Idelalisib is an oral PI3Kδ inhibitor, and was FDA approved in 2014 for the treatment of relapsed FL patients who have received at least two prior systemic therapies. This approval was based on the results of a single-arm, phase II multicenter study enrolling 125 patients with indolent NHL who were refractory to rituximab (monoclonal CD20 antibody) and an alkylating agent or were refractory to their most recent therapy [11]. These patients were heavily pretreated with a median of four prior therapies (range, 2–12). This study included 72 subjects with relapsed

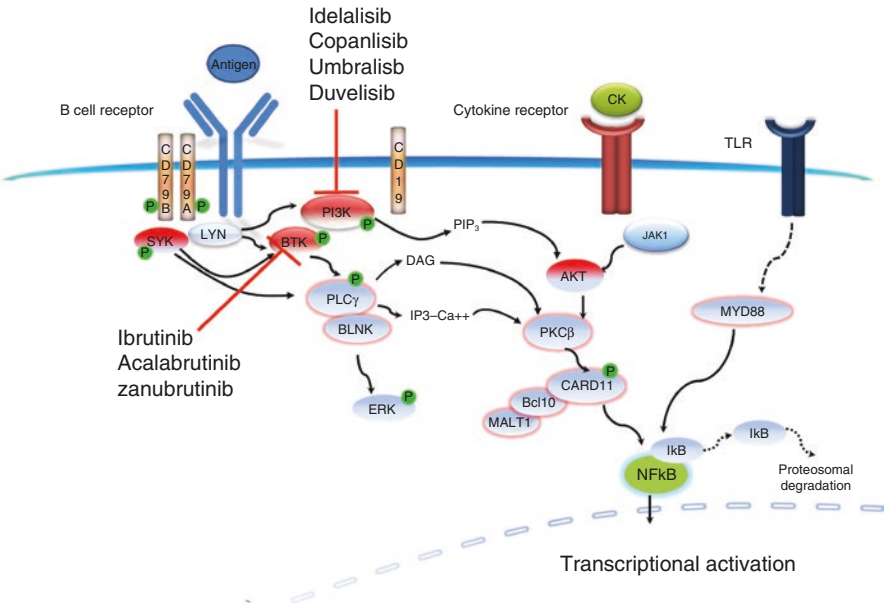

Fig. 11.1 B-cell receptor signaling pathway and therapeutic targets

FL. Subjects received 150 mg of idelalisib by mouth twice daily until disease progression or unacceptable toxicity. The overall response rate (ORR) was 57% with 6% achieving a complete response (CR). The median time to response was 1.9 months with a median duration of response of 12.5 months and median progression-free survival (PFS) of 11 months. The most common adverse events of grade 3 or higher were neutropenia (27%), elevation in liver enzymes (13%), diarrhea (13%), and pneumonia (7%). Prior to the introduction of idelalisib, outcomes for patients with relapsed FL who had failed at least two prior therapies were poor with remissions and responses diminishing with each relapse and ultimately patients were dying as a result of refractory or transformed lymphoma. Idelalisib was truly a breakthrough in the management of relapsed FL.

There are four different isoforms of PI3K. PI3Kδ and PI3Kγ expression is largely restricted to hematopoietic cells whereas PI3Kα and PI3Kβ are ubiquitously expressed [12, 13]. Key signal transduction from the BCR in B cells is largely dependent on PI3Kδ, whereas both PI3Kδ and PI3Kγ are responsible for signaling in T cells. Sole inhibition of PI3Kδ impairs CD8+ cytotoxic T cells and blocks the ability of naïve CD4+ cells to proliferate, expand, and differentiate into helper T cell subsets [14, 15]. In addition, PI3Kδ is critical for T reg survival and function [16]. Knockout mice, p110δ deficient, develop an autoimmune colitis thought to be due to an impaired ability of T regs to restrain inflammation in response to the colon microbiome [17, 18]. Inhibiting PI3Kδ with an agent such as idelalisib can, in addition to impairing BCR signaling in malignant B cells, impair the function of

conventional T cells, which is often counterbalanced by a decrease in T reg function resulting in unrestrained immune-mediated adverse effects such as rash, colitis, transaminitis, and pneumonitis, which may explain the unique toxicity profile associated with idelalisib. In addition, increased rates of infection have been associated with idelalisib, which may be secondary to drug-induced neutropenia and increased risk for opportunistic infections due to impairment of T-cell function. Patients should be monitored closely for toxicity while on idelalisib. Early intervention may be critical to avoiding untoward morbidity and mortality. In addition, introducing idelalisib to patients that are previously untreated or with few prior therapies (<) may result in unacceptable toxicity and is not advised.

Preclinical data suggest that complete abrogation of signaling from the BCR may require inhibition or both PI3Kα and PI3Kδ [19]. Furthermore, p110α gene amplification and PTEN loss of expression are commonly seen in mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL) suggesting that efficacy may be enhanced with targeting more than one isoform of PI3K in B-cell lymphomas [20–22]. Copanlisib is an intravenous (IV) pan-PI3K inhibitor, though the predominant activity is through inhibition of PI3Kα and PI3Kδ isoforms.

Copanlisib was FDA approved in 2017 for the treatment of adult patients with relapsed FL who have received at least two prior systemic therapies. Approval was based on the results of a phase II study, CHRONOS 1 part B. Copanlisib (60 mg IV) was administered on days 1, 8, and 15 of a 28-day cycle and continued until disease progression or unacceptable toxicity. In the FL subset (N = 104), the ORR was 58.7% (14.4% CR and 44.2% PR [partial response]) [23]. With a median duration of treatment of 22 weeks, the median PFS was 11.2 months. The most common treatment-related adverse events (all grade) were transient hyperglycemia (49%) and hypertension (29%). Other adverse events grade ≥3 included neutropenia (19%), diarrhea (4%), lung infection (11%), pneumonitis (1.4%), and colitis (0.7%). There were two nonfatal opportunistic infections. Elevated ALT and AST were observed in 23% and 28%. Based on the efficacy and manageable safety profile, copanlisib was approved for the management of relapsed or refractory FL in third line or greater.

The toxicity profile of copanlisib (PI3Kα and PI3Kδ inhibitor) is different in comparison to idelalisib (PI3Kδ inhibitor) which can be attributed to the addition of inhibiting the alpha subunit, but may also be attributed to differences in administration. Hyperglycemia is a known side effect of PI3Kα inhibition. Hypertension has been observed with other tyrosine kinase inhibitors, but not with other PI3K inhibitors to date. Both the hyperglycemia and hypertension are transient, resolving within 24 hours of the infusion. The lower incidence of diarrhea, colitis, and transaminitis may be attributed to the intermittent IV dosing schedule of copanlisib and bypass of hepatic first-pass metabolism. Longer follow-up will be necessary to gauge whether copanlisib truly is associated with less immune-mediated adverse effects as the onset of colitis has been described after 6 months of drug exposure with idelalisib. Nonetheless, confirmatory phase III trials of copanlisib-based therapy are currently enrolling patients with indolent NHL.

Additional PI3K inhibitors under development include umbralisib, PI3Kδ inhibitor with a unique chemical structure in comparison to other PI3Kδ inhibitors and a differentiated safety profile which may be the result of the chemical structure. Safety data from the early phase studies with umbralisib suggest rates of transaminitis rate of 6% (grade ≥3 3%), pneumonitis 1%, neutropenia 19% (grade ≥3 16%), and diarrhea 42% (grade ≥3 2%) [24], lower rates than what has been reported with other PI3Kδ inhibitors. Preliminary efficacy also appears promising with umbralisib. Phase III studies with umbralisib-based regimens in NHL and CLL are ongoing.

Duvelisib, a dual inhibitor of PI3Kδ and PI3Kγ, was reported to have an ORR of 41% in patients with relapsed/refractory FL in the phase II, single-arm DYNAMO study [25]. The median PFS was 8.4 months and the toxicity profile was similar to what had been previously described with idelalisib. The addition of the PI3Kγ inhibition did not seem to improve upon the efficacy observed with idelalisib and with a similar safety profile, these results were disappointing. Despite this, duvelisib is still under clinical development in combination with monoclonal CD20 antibodies and chemotherapy.

Bruton Tyrosine Kinase (BTK) Inhibitors

BTK is a nonreceptor kinase in the BCR signaling pathway (Fig. 11.1). A functional BTK is essential to normal B-cell development as demonstrated by the absence of B cells in patients with Bruton agammaglobulinemia who have inactivating BTK mutations [26]. Ibrutinib is an orally available, first in class, irreversible inhibitor of BTK. In the initial phase I study of ibrutinib, in patients with relapsed/refractory B-cell malignancies, no maximum tolerated dose was reached and the side effect profile was favorable with minimal myelosuppression and no significant reduction in normal B cells or immune globulin levels suggesting that blockade of BTK as opposed to an inactivating mutation had minimal impact on normal B-cell development [27]. Notable responses were also observed across histologies in this phase I dose escalation study, leading to further development and FDA approval of ibrutinib for several B-cell malignancies including CLL, MCL, marginal zone lymphoma (MZL), and Waldenstrom's macroglobulinemia.

Six of 16 (37.5%) subjects with FL in the phase I study achieved an objective response to ibrutinib, with three subjects achieving a CR (18.8%). A multi-center, phase II study was conducted to examine the efficacy and safety of 560 mg of ibrutinib administered daily in patients with relapsed FL [28]. Ibrutinib was continued until disease progression or intolerance. The ORR was 37.5% (CR 12.5%), and median PFS was 14 months. Patients with rituximab-sensitive disease were much more likely to respond to ibrutinib in this study (52.6% versus 16.7%; $P = 0.04$). CARD11 mutations were present in 16% (5/31) of patients and predicted resistance to ibrutinib with only wild-type patients responding. The toxicity profile was

manageable and similar to prior studies. However, the modest activity of ibrutinib as a single agent in the relapsed setting was disappointing and suggests ibrutinib as monotherapy in relapsed FL should not be pursued.

Patients with newly diagnosed FL often present with advanced stage and remain incurable with standard therapy. There is no agreed-upon standard of care for previously untreated FL. FL often impacts elderly patients with comorbidities who may not be optimal candidates for chemotherapy-based approaches. In addition to clinical prognostic factors, biologic or immune signatures suggest the microenvironment plays a pivotal role in FL pathogenesis and prognosis. Novel, well-tolerated therapies that effectively target the microenvironment would be advantageous. In addition to targeting BTK and inhibiting BCR signaling, ibrutinib has been shown to impact the microenvironment through disruption of cell adhesion, chemotaxis, stromal-tumor interaction, decrease in inflammatory cytokines, and changes in T-cell subsets, and T-cell activation and pseudoexhaustion [29]. Therefore, examination of ibrutinib in combination with rituximab in untreated FL patients, particularly those not suitable for chemotherapy-based regimens, was pursued.

A multicenter, phase II study with treatment-naïve FL (grade 1, 2, and 3a, stage II–IV disease) were treated according to 1 of 2 treatment schedules [30, 31]. In Arm 1, patients received 560 mg of ibrutinib daily, until progressive disease (PD) or unacceptable toxicity, combined with rituximab 375 mg/m^2 IV once weekly for 4 doses for the first 4 weeks of the study. In Arm 2, the two agents were staggered with patients receiving a lead-in of ibrutinib 560 mg for 8 weeks, and then concurrently with rituximab 375 mg/m^2 once weekly for 4 doses, followed by continuous ibrutinib until PD or unacceptable toxicity. The purpose of the Arm 2 design was to identify biomarkers that may predict ibrutinib sensitivity or resistance. At a median time on study of 22 months for Arm 1, the ORR was 85%, 35% (21/60) CRs and 50% (30/60) PRs. At a median time on study of 15 months for Arm 2, the ORR was 75%, 35% (7/20) CRs and 40% (8/20) PRs. The 12-month PFS rates were 87% (Arm 1) and 77% (Arm 2). Common grade ≥3 adverse events in either Arm (1 or 2) included maculopapular rash (5% and 10%, respectively), fatigue (7% and 5%), pyrexia (3% and 10%), and diarrhea (Arm 2 only-10%). As a result of the high ORR and no new safety findings, a randomized study investigating ibrutinib in combination with rituximab versus rituximab monotherapy in treatment naïve FL patients is currently enrolling. High response rates in untreated FL is not uncommon, but the favorable safety profile of this non-chemotherapy approach if superior in efficacy to rituximab may provide a treatment option for elderly patients with untreated FL that are not suitable for chemotherapy-based approaches.

Additional BTK inhibitors are currently under investigation in FL. Acalabrutinib is a more selective BTK inhibitor than ibrutinib with no effect on ITK, Tec, and EGFR which may explain a differentiated safety profile. Acalabrutinib has been associated with favorable ORR in relapsed CLL (95%) [32] and MCL (81%) [33]. Acalabrutinib was FDA approved for patients with MCL who have received at least one prior therapy in 2017 and is under investigation in B-cell malignancies including FL. BGB-3111 (BeiGene, Ltd) is a highly selective and more potent BTK inhibitor than ibrutinib with superior oral bioavailability and higher BTK specificity [34].

Preliminary results of the phase Ib study of BGB-3111 in relapsed or refractory B-cell malignancies reported an ORR in FL patients of 41% [35]. Further study of BGB-3111 in combination with obinutuzumab in FL is ongoing.

Though caution must be taken when making cross-trial comparisons, BTK inhibition in FL is associated with only modest efficacy. Future development will require combination therapies. The simplest combination includes the addition of a monoclonal antibody given studies thus far have demonstrated promising efficacy and no additive or concerning toxicity profiles. Rationale combinations developed to enhance synergism require additional molecular profiling and interrogation of the microenvironment. Synthetic lethality has been described in DLBCL cell lines with ibrutinib in combination with the immune modulator lenalidomide (derivative of thalidomide) suggesting a synergistic combination [36]. A phase I study of rituximab in combination with ibrutinib and lenalidomide has been completed in treatment naïve FL and though associated with a high ORR (95%), further investigation was felt to be unwarranted due to increased toxicity and no significant improvement in efficacy beyond two drug combinations [37]. Half the study population required dose modification; the majority due to rash (all grades 82%, grade 3 36%). This study highlights how challenging clinical development of rationale combinations in FL can be and emphasizes the critically important role safety plays in drug development.

Targeting BCL-2

Dysregulation of apoptosis via overexpression of the anti-apoptotic protein B-cell leukemia/lymphoma -2 (BCL-2) is fundamental to the pathophysiology of several subtypes of NHL. The hallmark of FL is t(14,18) which leads to overexpression of BCL-2 promoting cell survival in the harshly pro-death environment of the germinal center [38]. Venetoclax is a highly selective BCL-2 inhibitor with potent activity against FL, DLBCL, and MCL cell lines [39]. A phase I study of venetoclax in patients with relapsed or refractory NHL reported an ORR of 38% in FL patients, with 14% achieving a CR and a median PFS of 11 months [40]. The response rate in FL in this dose escalation study appeared to be associated with higher dose responses observed at the 1200 mg dose versus ≤900 mg (44% versus 27%). Adverse events were most commonly grade 1 or 2. The most common grade 3 or 4 events were hematologic including anemia (15%), neutropenia (11%), and thrombocytopenia (9%). Despite BCL-2 being the hallmark of FL, inhibiting BCL-2 with venetoclax in relapsed/refractory FL was associated with modest activity. BCL-2 expression alone was not an adequate biomarker to predict clinical response to venetoclax, and functional assessments of the relative balance of the anti- and pro-apoptotic BCL-2 family proteins may be more functional biomarkers and warrant further exploration. Unfortunately, further development of venetoclax monotherapy in FL is not being pursued.

It is possible that combination regimens incorporating venetoclax may result in improvement in efficacy. However, the optimal combination partners for venetoclax

in FL remain to be defined. Studies are under way to assess the safety and efficacy of venetoclax in combination with chemotherapy (bendamustine), monoclonal antibodies (obinutuzumab), and BCR signaling inhibitors (ibrutinib, BGB-3111). The impact of these studies will be defined by enhancement in efficacy without compromising safety.

Enhancer of Zeste-Homolog 2 (EZH2) Inhibitors

Efforts aimed at understanding lymphomagenesis mechanisms have been emphasized to identify therapeutic targets that may change the natural history of FL and identify potentially curative therapy. Next-generation sequencing (NGS) studies have demonstrated frequent mutations in epigenetic regulators in nearly all cases of FL [41, 42]. The m7-FLIPI score is a prognostic model, which incorporates known clinical prognostic factors such as FLIPI (Follicular Lymphoma International Prognostic Index) score and performance status with the mutational status of seven genes resulting in an improvement in risk stratification for FL patients of high tumor burden [42]. These genes include the histone methyltransferase *EZH2* gene. EZH2 is often constitutively activated in germinal center-derived NHLs by gain-of-function mutations. Somatic heterozygous mutations have been described in approximately 25% of FL cases [43, 44]. Dysregulation of the germinal center reaction by constitutively active EZH2 may promote lymphomagenesis and may be a promising therapeutic target [45]. Patients with EZH2 gain-of-function mutations may be ideal candidates for EZH2 inhibition. However, EZH2 non-mutated patients may also benefit and in addition to alterations, copy-number status of EZH2 should be considered when evaluating patients for EZH2 inhibitor therapies [46].

A phase I dose escalation study of tazemetostat included patients with relapsed/refractory NHL including five with FL, 13 with DLBCL, and one patient with MZL [47]. The most common adverse events were asthenia, anorexia, constipation, nausea, dysgeusia, vomiting, and muscle spasms. Grade 3 or greater related adverse events though included thrombocytopenia, neutropenia, hypertension, anorexia, and transaminase elevation. Though small sample size, objective responses were seen in 3/5 (60%) FL, 5/9 (56%) DLBCL, and 1/1 (100%) MZL patients. The majority of the responses occurred at the recommended phase II dose of 800 mg BID. EZH2 status in patient tumors was determined for 14/19 NHL patients with 13/14 found to be wild-type and one patient, who experienced an ongoing PR at week 16, expressing an Y646H mutation. These preliminary findings of the phase I dose escalation study of tazemetostat demonstrated a tolerable safety profile and promising efficacy.

Based upon the observed safety and efficacy profile of tazemetostat, a phase II trial in relapsed or refractory FL and DLBCL patients, stratified by EZH2 mutation status, was pursued. Interim data from the phase II study of tazemetostat (800 mg orally BID) reported an ORR of 92% in the subset of patients with FL with EZH2-activating mutations [48]. Patients with follicular lymphoma with EZH2 wild-type

had an ORR of 26%. Tazemetostat also was associated with a favorable safety profile across all patient populations in this study with no new safety concerns identified. The preliminary findings of this phase II study suggest tazemetostat is associated with a favorable safety profile and preferential benefit in patients with FL whose tumors bear activating EZH2 mutations. The response rates among those with EZH2 wild-type status is intriguing in that responses were observed, but the low to modest activity suggests further investigation is necessary to understand the predictors of response and inform clinical trial design with rationale combinations for these patients.

Conclusions

FL is characterized as an indolent or slow-growing tumor that often times can be safely observed. However, clinical outcomes remain heterogeneous. Despite high initial response rates to frontline therapy, patients with FL will continue to experience disease relapse and will ultimately succumb to their disease. Chemotherapy had been the mainstay of therapy of many years; however, the clinical benefit of chemotherapy declines beyond two lines of therapy, and the toxicity can be prohibitive particularly as patients age and develop comorbidities. Effective targeted therapy such as PI3K inhibitors, BTK inhibitors, BCL-2 inhibitors, and EZH2 inhibitors that are associated with a favorable toxicity profile appear promising particularly as patients start to experience multiple relapses. Targeted therapy is often associated with mechanisms of action that differ and may overcome chemotherapy resistance. In addition, as we learn more about the biology of FL these targeted agents may provide a more rational approach in a disease in which chemotherapy has failed to achieve a cure. Predictive biomarkers that will identify suitable candidates for monotherapy versus those that will benefit from a combination approach are necessary to influence practice patterns and improve cost-effectiveness in a disease with a prolonged natural history.

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Chapter 12
Targeting the Tumor Microenvironment

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Introduction

It is increasingly clear that the tumor microenvironment plays an essential role in the pathogenesis, survival, and progression of follicular lymphoma. The impact of the host immunity has long been hypothesized to be responsible for the "waxing and waning" nature of follicular lymphoma, as well as the significant percentage of patients who achieve remission without therapy. Unfortunately, early attempts to modulate the microenvironment with agents such as interferon were met with only moderate success. Over the past several years, a greater understanding of the complex biology that make up the microenvironment has led to an entire field of study dedicated to modifying or modulating the cellular interactions present in the malignant lymph node and marrow.

Innovative strategies targeting the microenvironment have resulted in impressive clinical results. In addition, we are now learning that several agents targeting key cellular pathways in B-cell lymphoma also may have a profound impact on the benign cellular infiltrate present in the malignant niche. Specific examples of recent advances include novel monoclonal antibodies, immunomodulatory drugs (IMiDs), and B-cell receptor inhibitors. In the following chapter, we will highlight recent therapeutic advances, focusing on agents whose primary or secondary mechanism results in modulation of the immune microenvironment, and discuss potential ways to combine emerging drugs to improve clinical outcomes.

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Monoclonal Antibodies in FL

The complexity of the tumor microenvironment prior to the development of anti-CD20 therapy (e.g., rituximab) for follicular lymphoma has been well characterized. The presence of CD8+ tumor-infiltrating lymphocytes [1, 2] and regulatory T cells has been demonstrated to be associated with a more favorable prognosis [3]. Macrophages expression, on the other hand, has been shown to contribute to tumor dissemination [4], with increased expression of the CD163+ M2 macrophage phenotype conferring a poor prognosis while promoting tumor angiogenesis [4–6]. This negative prognostic effect of infiltrating macrophages in the pre-rituximab era was later confirmed through gene-expression profiling [7]. The advent of rituximab, however, brought a new era of effective tumor destruction through antibody-dependent cell-mediated cytotoxicity (ADCC), which relies on the presence of Fc receptors on macrophages, natural killer cells, and neutrophils, in addition to direct apoptotic effects [8, 9]. Recent studies have shown evidence that the addition of rituximab reverses the negative prognostic effect of high tumor-associated macrophages within the microenvironment; an increased number of macrophages were associated with improved progression-free survival in those treated with R-CHOP but not CHOP, for example [10]. Despite significant heterogeneity in macrophage subtype and function, this reversal in the prognostic effect of macrophages within the tumor microenvironment has been seen with both the CD163+ M2 macrophage phenotype and CD68+ macrophages, and confirmed most recently through gene expression profiling [11–13]. This effect may be dependent upon the specifics of the concurrent chemotherapy regimen, however, as patients who receive a doxorubicin containing regimen were shown to have a 5-year PFS of 60% versus 44%, $p = 0.01$, in one study [13].

Give the mechanism and proven efficacy of rituximab, several studies have demonstrated that single-agent rituximab for follicular lymphoma and low tumor burden is an effective therapy. Four single weekly doses of rituximab followed by every other month dosing for 8 months was shown to induce a complete or partial response in 73% of untreated follicular lymphoma patients and reduced the need for cytotoxic therapy at 3 years [14]. While there does not appear to be an effect on overall survival, another study of both untreated patients with follicular lymphoma and those with relapsed/refractory disease showed a similar response rate, and those randomized to rituximab maintenance had a 23-month median event-free survival with 35 months of follow-up [15].

The advent of targeted therapies against different antigens commonly expressed in follicular lymphoma will likely affect the tumor microenvironment in a manner that remains to be fully elucidated. Anti-CD19 antibodies are a promising target in follicular lymphoma and have been conjugated to toxins or engineered with a Fc domain that increases cytotoxicity; most recently the CD19/CD3 bispecific T-cell engager antibody blinatumomab has been approved for use in acute lymphoblastic leukemia. In one phase II study of an Fc-engineered CD19 antibody MOR208 in relapsed/refractory lymphoma, partial or complete treatment response

was seen in 29% of follicular lymphoma patients, with 4 of 9 responses lasting greater than 12 months [16]. This CD19 antibody works both through antigen-dependent cell-mediated cytotoxicity and direct cytotoxicity through the disruption of the B-cell receptor signaling pathway, as demonstrated in chronic lymphocytic leukemia models [17, 18]. The combination of rituximab and blinatumomab in in-vitro B-cell lymphoma models has also been tested, but it is unclear at this time if their combined effects will be additive versus synergistic in the setting of different mechanisms of actions and their effects on NK cells, macrophages, and T cells, respectively [19].

Similarly, CD47 has been an attractive target as binding of CD47 by macrophages results in inhibition of phagocytosis [20]. CD47 expression is increased in non-Hodgkin lymphoma and follicular lymphoma and has been demonstrated to act synergistically with rituximab in mouse models to increase macrophage phagocytosis through both FcR-dependent and -independent mechanisms [21, 22]. In a phase I study of CD47 blockade and rituximab in relapsed/refractory non-Hodgkin lymphoma, the objective and complete response rates were 71% and 43%, respectively, for those with follicular lymphoma, with median duration of response note reached after 8.1 months of follow-up [23]. Future studies will no doubt identify how to combine these novel targeted therapies with existing chemotherapy and anti-CD20 therapy to better affect a durable cytotoxic response while minimizing toxicities.

Immunomodulatory Drugs

Immunomodulatory drugs (IMiDs) represent an emerging class of agents with exciting activity across multiple B-cell malignancies. The parent drug, thalidomide, was a sedative initially prescribed to treat morning sickness, but was found to have devastating effects on the unborn fetus. Decades later, thalidomide and its progeny have reemerged as active anticancer drugs with the ability to modulate the immune microenvironment across a range of B-cell cancers. In 2010 Ito and colleagues described cereblon, an E3 ubiquitin ligate complex, as a key target for IMiDs [24]. Binding of thalidomide to cereblon results in alterations in multiple substrate levels across various cell types, both benign and malignant. Specifically, IMid binding has been shown to result in rapid ubiquitination and degradation of Ikaros and Aiolos, transcription regulators of B- and T-cell development [25].

Lenalidomide, a second-generation IMiD, inhibits tumor cell proliferation and stimulates cytotoxic T and NK cells. Preclinically, lenalidomide has antineoplastic effects on malignant B cells, with sparing progenitor and normal B cells [26]. Lenalidomide has also been shown to increase NK cell number, NK cell cytotoxicity, and improve antibody-dependent cellular cytotoxicity (ADCC) when used with rituximab [27, 28].

As a single agent, lenalidomide has shown modest activity in indolent lymphomas, including follicular. In 2009, Witzig and colleagues reported the results of a

phase II study of 22 patients with relapsed follicular lymphoma showing a 27% overall response rate (ORR), with 9% of patients attaining complete remission [29]. Based on earlier preclinical work demonstrating an IMiD's potential to increase ADCC in NHL animal models, investigators at MD Anderson initiated a phase I study of lenalidomide plus the anti-CD20 antibody, rituximab in relapsed lymphoma (Wang et al). After demonstrating encouraging safety and activity, a subsequent combination pilot study was launched in 110 patients with untreated indolent lymphoma. This single-center trial demonstrated a remarkable xx percent overall response rate with xx % of patients achieving a complete remission. Recent long-term follow-up showed xx % of patients remained in remission at 5 years following initial exposure. Adverse events were well managed and generally low grade, with fatigue, cytopenia, and rash commonly reported. Subsequent cooperative group trials with the same combination confirmed high overall response rates and durable remissions [30].

Based upon these results, two pivotal phase III trials were launched in both the relapsed and untreated setting. The RELEVANCE trial randomized over 1000 newly diagnosed follicular lymphoma patients with high tumor burden to receive standard chemotherapy (CHOP, bendamustine, CVP) plus rituximab or lenalidomide plus rituximab using the dose and schedule previously published by Fowler and colleagues. The study demonstrated similar overall and complete response rates between the two regimens as well as nearly identical progression-free survival rates at 3 years for lenalidomide plus rituximab compared to rituximab plus chemotherapy (77% vs 78% respectively). Furthermore, neither regimen appeared superior regardless of subgroup or initial tumor characteristic (bulk, stage, FLIPI, etc.) analyzed. As predicted, the adverse event profile was different between arms, with neutropenia occurring with higher frequency in chemotherapy backbones compared to rash with lenalidomide. Increased secondary cancers were not higher in either arm. The AUGMENT trial.

In the relapsed setting, lenalidomide and rituximab has also shown promising activity. The CALGB 50401 study demonstrated improved efficacy and similar tolerability when a monoclonal antibody was added to lenalidomide. In this phase II randomized study, 91 patients with relapsed follicular lymphoma received lenalidomide alone ($n = 45$) or lenalidomide plus rituximab ($n = 46$). The overall response rate was significantly higher in the combination arm compared to single agent lenalidomide (76% vs. 53%). Importantly, the median time to progression was also longer when rituximab was added to lenalidomide (1.1 year vs. 2 years, respectively). Grade 3 events were similar in both arms, including cytopenias and fatigue [31, 32].

The activity of the combination was further explored in the recent randomized phase III AUGMENT study. Over 350 patients with relapsed, rituximab-sensitive follicular and marginal zone lymphoma were enrolled and randomized to receive single agent rituximab weekly for 4 weeks followed by four monthly doses versus the same regimen with lenalidomide given on days 1–21 or each 28-day cycle. Progression-free survival was significantly improved for the combination versus rituximab alone with a duration of 39.4 months versus 14.1 months, respectively.

Table 12.1 Select lenalidomide combination studies in follicular lymphoma

Combination	Phase	Population	Status	Sponsor
Lenalidomide + obinutuzumab	I/II	Relapsed follicular	ongoing	LYSA (France)
Lenalidomide + obintuzumab	I/II	Relapsed follicular	completed	MD Anderson
Lenalidomide + obinutuzumab	II	Untreated follicular	completed	MD Anderson
Lenalidomide + rituximab + venetocla	I/II	Relapsed follicular	ongoing	Peter MacCallum (Australia)
Lenalidomide + rituximab + ibrutinib	I/II	Relapsed follicular	completed	CALGB
Lenalidomide + rituximab + ibrutinib	II	Relapsedollicular	ongoing	MD Anderson
Lenalidomide + rituximab + acalabrutinib	I/II	Relapsed follicular, marginal zone	Ongoing	MD Anderson

Adverse events were more common in the lenalidomide plus rituximab arm, including higher rates of neutropenia (58% vs 23%) and cutaneous reactions (32% vs. 12%). Interestingly, more patients in the combination arm completed therapy despite the increased adverse event rate. The significant improvement in disease control with the combination led to approval by the Food and Drug Administration for lenalidomide and rituximab in relapsed follicular and marginal zone lymphoma.

Based upon these encouraging results, several studies ongoing are exploring lenalidomide plus rituximab as a backbone combination strategy (see Table 12.1).

List of Other Ongoing Studies with R2 as Backbone

Checkpoint Inhibitors for the Treatment of FL

Several monoclonal antibodies targeting the immune checkpoint programmed death-1 (PD-1) receptor, such as nivolumab, pembrolizumab and pidilizumab, or its ligand (PD-L1), such as atezolizumab, have been extensively investigated for the treatment of various solid tumors and lymphoma subtypes. The interaction between PD-1 receptor and PD-L1, typically expressed on cancer cells, impairs normal signaling through the T-cell receptor, leading to T-cell exhaustion. Immune checkpoint inhibitors can block the engagement of PD-1 to PD-L1, magnifying the antitumoral activity of T cells against cancer cells [33].

FL cells can escape the antitumoral activity exerted by the autologous immune system by inducing an immunosuppressive phenotype in their tumor microenvironment [34]. However, while gene expression profile data have shown that an immune microenvironment enriched in macrophages and stromal cells is associated with worse prognosis in FL [35], the impact of T cells and their PD-1-induced exhaustion remains largely unexplored [36] To this regard, PD-1 is markedly upregulated on

intratumoral and peripheral blood $CD4^+$ and $CD8^+$ T cells of patients with FL. Similarly to what observed in solid tumors and Hodgkin lymphoma (HL), also in FL PD-1 expression is associated with impaired T-cell function, PD-1 blocking representing a promising therapeutic strategy to restore T-cell function against autologous tumor cells [34, 37]. In addition, although FL cells do not typically express PD-L1, its upregulation has been observed on T cells and other components of the tumor immune microenvironment [38], further highlighting the critical role played by the PD-1/PD-L1 pathway and its potential as target for therapeutic purposes in FL.

The safety and efficacy of nivolumab have been investigated in a large phase I trial of hematological malignancies, including 10 patients with relapsed or refractory FL. Overall response rate of 40%, including 1 complete remission (CR), with continued response in 3 out of 4 patients, after a median follow-up of 92 weeks. Of interest, the toxicities observed in the FL cohort were not separately reported [39]. Based on these results, multiple clinical trials are now ongoing, including a phase II trial of nivolumab as a single agent for patients with FL who have failed both an anti-CD20 monoclonal antibody and alkylating agent-based chemotherapy (NCT02038946), the combination of nivolumab and rituximab for patients with previously untreated FL (NCT03245021), the combination of nivolumab and lenalidomide in patients with relapsed or refractory FL (NCT03015896), and the combination of nivolumab and personalized tumor vaccine strategy in patients with relapsed or refractory FL (NCT03121677).

Good results have been observed with the combination of pidilizumab and rituximab in a phase II study, including 32 patients with relapsed or refractory FL. ORR was 66%, CR rate 52%, with a median duration of response of 22 months, and no grade 3 or higher toxicities [40].

The safety and efficacy of pembrolizumab have been investigated in a phase I study, including 18 patients with FL. ORR was only 11%, based on partial remission (PR) observed in 2 patients, but the remaining 9 patients had a durable stable disease. Up to 39% of patients had grade 3 or higher toxicities, mainly represented by thrombocytopenia (13%), anemia (13%), neutropenia (8%), and dyspnea (8%) [41]. Higher response rates have been observed in a phase II study of the combination of pembrolizumab and rituximab, including 30 patients with relapsed or refractory FL. ORR was 64%, CR rate 48%, with 15 patients still in remission after a median follow-up of 11 months, and no grade 3 or higher adverse events observed to date [42].

Of interest, use of other immune checkpoint inhibitors as a single agent has also resulted in low response rates. In a phase I study of atezolizumab, including 3 patients with relapsed or refractory FL, only 1 PR was observed [43]. Higher activity has been reported with the combination of atezolizumab and the anti-CD20 monoclonal antibody obinutuzumab [44] and the immunomodulatory agent lenalidomide [45]. This has encouraged the development of ongoing clinical trials investigating the activity of other combination strategies, including that of atezolizumab with chemoimmunotherapy, in patients with relapsed or refractory FL (NCT02596971), with promising early signals of safety and efficacy [46].

The results observed with the abovementioned clinical trials suggest that multiple steps still need to be taken to improve the efficacy of immune checkpoint inhibitors for the treatment of patients with FL.

In the first place, the identification of biomarkers predictive of response is warranted. To this regard, similarly to what already done in solid tumors and in HL, the density of tumor-infiltrating lymphocytes, the expression of PD-1 and PD-L1 by immunohistochemistry, the expression of 9p24.1 by conventional cytogenetics, and the assessment of tumor mutation burden by gene sequencing may help identify the subgroups of patients with FL who may benefit the most from the use of immune checkpoint inhibitors [47–49].

In the second place, given the significant role played by the innate immune system in the prognosis of FL, strategies aimed at combining available immune checkpoint inhibitors with agents able to favorably affect the phenotype and function of myeloid cells may lead to better efficacy [35]. These include agents targeting CD47, a surface receptor expressed on FL cells and representing a "do-not-eat-me" signal for tumor-associated macrophages (TAM), whose combination with rituximab has been associated with an ORR of 71% and a CR rate of 43% in patients with relapsed or refractory FL [50]. Another potential target is represented by the Toll-like receptor (TLR)4, whose stimulation through the intratumoral injection of its agonist, G100, has resulted in the activation of the innate immune system and increased efficacy of combined pembrolizumab, without worsening toxicity, in patients with relapsed or refractory FL [51].

The identification of new immune checkpoints, for the design of novel therapeutic strategies, remains an active area of investigation, and the use of immune checkpoint inhibitors remains experimental in FL [52, 53].

Effects of Small-Molecule Tyrosine Kinase Inhibitors on the FL Microenvironment

Multiple small molecules, able to inhibit the B-cell receptor (BCR) signaling cascade, have been developed over the last years for the treatment of B-cell lymphoid malignancies, including BTK inhibitors (BTKi) and PI3K inhibitors (PI3Ki). While the BCR pathway is crucial for the survival of malignant B cells, and its inhibition has an obvious direct antitumoral effect in patients with B-cell lymphoma, direct and off-target activity of BTKi and PI3Ki can also affect multiple components of the tumor microenvironment [54, 55].

To this regard, BTKi, can favorably affect T lymphocytes, by markedly increasing the effect of CD4+ and CD8+ T cells, and decreasing the expression of inhibitory surface markers, including PD-1 and PD-L1 [56]. In addition, they can also suppress regulatory B-cell function, through a STAT3-mediated mechanism [57]. However, BTKi may play an unfavorable role on other components of the tumor microenvironment, such as TAM and natural killer (NK) cells. Treatment with

BTKi, in fact, can reduce the phagocytic ability and increase the immunosuppressive profile of TAM, exacerbating the expression of M2 (pro-tumoral) markers [58, 59]. In addition, BTKi can impair NK cell function, antagonizing NK cell-mediated antibody-dependent cytotoxicity (ADCC) [60]. Of interest, such immunomodulatory effects are more prominent with the use of ibrutinib rather than acalabrutinib, a more selective BTKi, suggesting they may be due to off-target ITK inhibition rather than direct BTK inhibition [56].

Similarly to BTKi, also PI3K inhibitors (PI3Ki) can affect the phenotype and function of the tumor immune microenvironment, either by direct or off-target effect. By downregulating the secretion of chemokines, such as CXCL-12 and CXCL-13, PI3Ki can impair the chemotaxis and adhesion of multiple lymphocyte subtypes to stromal cells, polarizing the tumor microenvironment to a more antitumoral phenotype [61, 62]. PI3Ki can also hamper NK cell-mediated ADCC, though the effect is less pronounced than what was described for BTKi [63].

Multiple preclinical studies have investigated the specific effect of BTKi and PI3Ki on T lymphocytes, in order to improve the efficacy and safety of available agents, and potentially favor their combination with immune checkpoint inhibitors.

Ex vivo studies performed on primary T lymphocytes, obtained from patients with B-cell lymphoid malignancies, have shown that ITK inhibition exerted by less selective BTKi, such as ibrutinib, can skew the polarization of T lymphocytes toward a type 1 T helper and effector phenotype [64]. To this regard, the co-administration of ibrutinib and a TLR9 agonist, able to activate antigen-presenting cells, has resulted in increased antitumoral activity in a mouse model of lymphoma [65]. Similar findings have been reported with the co-administration of ibrutinib and an antibody targeting PD-L1 in different mouse models of lymphoma [66].

In regard to the effect of PI3Ki on T lymphocytes, it is important to note how this is strongly dependent on their selectivity for specific PI3K subunits. Idelalisib, a selective inhibitor of the δ subunit of PI3K, has minimal to no effect on T helper or effector lymphocytes [67]. However, ex vivo studies performed on primary patient blood samples have shown that it can induce defects in T regulatory lymphocytes, likely explaining the autoimmune toxicity observed with its use in different types of B-cell lymphoid malignancies [68]. In addition, ex vivo studies have shown that agents able to target also the γ subunit of PI3K, such as duvelisib, can be cytotoxic to all T lymphocyte subtypes and can impair the T-cell-mediated production of inflammatory and anti-apoptotic cytokines [69].

In light of the limited clinical activity observed as a single agent in patients with relapsed or refractory FL [70–72], and of the above-outlined preclinical evidence of favorable effects on the tumor microenvironment, the combination of ibrutinib with lenalidomide and rituximab (R^2) has been explored [73]. Preliminary results from an ongoing phase II study investigating the efficacy of this combination in patients with previously untreated FL have shown an ORR of 97% and a CR rate of 78%, significantly higher than what was observed historically with R^2 alone [74]. However, similar 2-year progression-free survival rates were observed (76%) as compared to R^2 alone, with up to 36% of patients developing grade 3 or higher autoimmune toxicities, mainly represented by skin rash and diarrhea [75].

Despite the lack of activity of acalabrutinib on T lymphocytes, given its limited off-target effects and less pronounced impairment of TAM activity, its combination with R2 has the preclinical potential to be more active and less toxic than observed with ibrutinib and is currently being investigated in an ongoing phase I study including patients with relapsed or refractory FL (NCT02180711).

The effect of PI3Ki on T regulatory lymphocytes, and the serious immune-mediated toxicities observed with their use, have induced the US Drug and Food Administration to approve their use only for the treatment of patients having failed 2 previous lines of therapy, when the host immune system may be weaker and then less prone to hyper-activation. In addition, their clinical development in combination with other agents able to target the immune microenvironment has been limited [55]. To this regard, studies investigating the safety and efficacy of idelalisib in combination with lenalidomide or the SYK inhibitor entospletinib, in patients with relapsed refractory FL, have been stopped early because of excessive toxicity [76–78]. Future studies, investigating different doses and schedules of PI3Ki, may better harness their effects on the tumor microenvironment, and further favor their development for the treatment of patients with FL.

Conclusions

Current treatment options for patients with untreated and relapsed follicular lymphoma are changing. Therapeutics targeting the immune microenvironment represent a potential paradigm shift in drug development for B-cell malignancies, but as single agents have yet to radically change the natural history of follicular lymphoma. This lack of dramatic single agent effect is likely secondary to multiple causes including the natural redundancy in essential immune pathways, the rapid emergence of immuno-resistant clonal phenotypes following treatment, and heterogeneity in the hosts' immune response. Combination approaches have shown greater promise, and recent randomized studies have for the first time demonstrated outcomes with immune-based approaches which are equivalent to those see with traditional cytotoxic backbones. In order to move to the next level, advanced genomic studies are needed to further elucidate resistance mechanisms to immunotherapy and to understand the variability between various patient's microenvironment.

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