Blood First Edition Paper, prepublished online April 17, 2018; DOI 10.1182/blood-2017-11-764365

Critical influences on the pathogenesis of follicular lymphoma

Ralf Küppers[1] and Freda K. Stevenson[2]

[1]Institute of Cell Biology (Cancer Research), University of Duisburg-Essen, Medical Faculty, Essen, Germany

[2]Cancer Research UK Centre, Cancer Sciences Unit, University of Southampton Faculty of Medicine, Southampton General Hospital, Southampton, UK

Correspondence: F. K. Stevenson, Cancer Research UK Centre, Cancer Sciences Unit, University of Southampton Faculty of Medicine, Southampton General Hospital, Southampton, SO16 6YD, UK; e-mail: fs@soton.ac.uk

Scientific category: Lymphoid neoplasia

Short title: Follicular lymphoma

Words: Abstract: 250, Text: 3998

5 Figures, 2 Tables, 95 references

Abstract

The development of follicular lymphoma (FL) from a founder B cell with an upregulation of BCL2, via the t(14;18) translocation, to a proliferating clone, poised to undergo further transformation to an aggressive lymphoma, illustrates the opportunistic Darwinian process of tumorigenesis. Protection against apoptosis allows an innocent cell to persist and divide, with dangerous accumulation of further mutational changes, commonly involving inactivation of chromatin-modifying genes. But this is not all. FL cells reflect normal B cells in relying on expression of surface Ig. In doing so, they add another supportive mechanism by exploiting the natural process of somatic hypermutation of the *IGV* genes. Positive selection of motifs for addition of glycan into the antigen-binding sites of virtually all cases, and the placement of unusual mannoses in those sites, reveals a post-translational strategy to engage the microenvironment. A bridge between mannosylated surface Ig of FL cells and macrophage-expressed DC-SIGN produces a persistent low-level signal which appears essential for life in the hostile germinal center. Early stage FL therefore requires a triad of changes: protection from apoptosis, mutations in chromatin-modifiers, and an ability to interact with lectin-expressing macrophages. These changes are common and persistent. Genetic/epigenetic analysis is providing important data but investigation of the post-translational landscape is the next challenge. We have one glimpse of its operation via the influence of added glycan on the B-cell receptor of FL. The consequential interaction with environmental lectins illustrates how post-translational modifications can be exploited by tumor cells, and could lead to new approaches to therapy.

Introduction

Follicular lymphoma (FL) is a paradigm of an indolent tumor where the steps from an early chromosomal translocation to a more developed clonal proliferation can be tracked. Understanding the biology involved in this process, whereby cells often have only limited additional genetic changes, but retain a complex and essential dependence on the microenvironment, is relevant for all B-cell malignancies and possibly for cancer in general. The theme of dependence of tumor cells on surrounding cells and how it offers opportunities for intervention, is a major part of the 2018 ASH Meeting on Lymphoma Biology.

In this review, the focus is on FL and how it arises and continues to occupy the germinal center (GC), a site hostile to normal B cells unless selected by antigen. We will concentrate on this setting, while recognizing that transformation into a more aggressive high grade lymphoma occurs in 2-3% of patients per year. Frustratingly, the factors involved in transformation remain unclear but appear not to be single genetic events but rather multiple hits within a varying molecular landscape.[1]

A striking feature of FL is the histological appearance which, at early stages, closely resembles that of a reactive lymph node (Figure 1). The distinction in FL lies mainly in a poorly defined mantle zone and a lack of the usual dark/light zone polarity. Gene expression analysis indicates a similarity between FL cells and light zone B cells.[2] However, further probing reveals significant changes which have occurred in the FL cells.

Histogenesis and pre-malignant lesion

The histogenesis of FL is closely linked to key events of normal B-cell development and differentiation. During early B-cell development in the bone marrow, progenitor B cells undergo V(D)J recombination processes to assemble immunoglobulin (Ig) heavy and light chain V region genes that encode the variable parts of antibody molecules.[3] This involves DNA double-strand breaks at specific recombination signal sequences (RSS), located at the ends of the rearranging V, D and J genes. First, in a pro-B cell an *IGHD* gene is joined to an *IGHJ* gene, and in the next step, an *IGHV* gene is recombined to the D_HJ_H joint.[3] B-cell precursors expressing a heavy chain gene as pre-B-cell receptor are pre-B cells. At the recombination sites, further diversity is generated by exonucleolytic removal of several bases and by addition of non-germline encoded bases, the N-nucleotides.[3] Once a functional heavy chain is

expressed, light chain gene rearrangments are performed to generate a mature B cell receptor (BCR).

In rare instances, mistakes happen during V(D)J recombination, and when the ends of the rearranging genes in one of the Ig loci are erroneously joined to DNA breaks in another chromosome, a reciprocal chromosomal translocation occurs.[4] Such a translocation is likely the first event in the pathogenesis of FL. About 90% of FL show a t(14;18)(q32;p21) in which the *BCL2* gene is joined to an *IGHJ* gene or a D_HJ_H joint in the IGH locus.[5,6] Specific characteristics of the translocations, in particular the presence of N-nucleotides at the joining sites of the two chomosomes and the location of the breakpoints in the IGH locus close to the RSS site of an *IGHD* or *IGHJ* gene strongly argue for misguided V(D)J recombination at the pro B cell stage as the mechanism.[4] As a consequence, the *BCL2* gene is brought under control of the enhancers of the IGH locus, causing constitutive expression of the anti-apoptotic BCL2 protein.[4]

BCL2 is not a strong proto-oncogene, and as naive B cells physiologically express BCL2, it seems that the presence of a t(14;18) IGH-*BCL2* translocation does not cause a major disturbance of mature naive B-cell physiology. It unfolds its pathogenetic function when a naive B cell is driven into a T cell-dependent immune response and becomes a GC B cell. In the dark zone of the GC, antigen-activated B cells undergo massive clonal expansion and activate the process of somatic hypermutation (SHM) to modify their *IGV* region genes.[3,7] As somatic mutations are largely random, only few cells will acquire affinity-increasing mutations and are positively selected by T follicular helper (TFH) cells in the light zone.[8] Most acquire disadvantagous mutations and are destined to die by apoptosis.[8,9] The apoptosis proneness of GC B cells is also a tolerance mechanism to prevent survival of autoreactive B cells.[10,11] Apparently apoptosis is the default pathway for GC B cells, and an important factor for this intrinsic program is that they downregulate the anti-apoptotic factor BCL2 (Figure 1).[9,12] Only in positively selected light zone GC B cells that undergo differentiation towards memory B cells or plasma cells is BCL2 expression reinduced.[12,13] If a B cell with an IGH-*BCL2* translocation is driven into a GC reaction, the normal apoptosis and selection process is disturbed, and such B cells have a survival advantage.[14] This then increases the risk for acquisition of further genetic lesions and ultimately may lead to the development of a FL in some of

such cells. As will be discussed in more detail below, these additional genetic lesions and pathogenetic events cause differentiation arrest at the stage of a GC B cell.

B cells with IGH-*BCL2* translocations are detectable also in healthy human adults. The frequency of such cells is about $1/10^5$ peripheral blood B cells, with wide variation between individuals.[15,16] The frequencies tend to rise with increasing age.[17] Initially, it was thought that these *BCL2* translocation-positive circulating cells are polyclonal naive B cells, stemming from numerous independent translocation events, and accumulating in this mature B cell compartment. However, later elegant studies revealed that t(14;18)-carrying cells in healthy adults are mostly present among $IgM^+IgD^+CD27^+$ memory B cells with somatically mutated *IGV* genes, and that the pool of such cells is mostly dominated by one or a few clones.[18,19] These clones often persist, and in a few instances it was shown that circulating clones present in peripheral blood several years before diagnosis of a FL already carried a number of genetic lesions in addition to the *BCL2* translocation.[20,21] Combined studies with human B cells and a murine model indicate that B cells constantly overexpressing BCL2 undergo several GC passages until they finally give rise to a FL (Figure 2).[22]

B cells carrying *IGH-BCL2* translocations can also be been detected in GC of ~2-3% of reactive lymph nodes from heathly adults.[23-25] Often, their numbers are rather small and they are scattered among normal GC B cells.[26] In other instances, typically several GC are dominated by BCL2-expressing GC B cells with the t(14;18) translocation. This has been designated as FL in situ (FLIS).[24,27] The cells express typical GC B cell markers, such as CD10, are monoclonal and show active hypermutation.[24,28] However, their proliferation rate is lower than that of normal GC, with an increased fraction of light zone B cells.[24] Progression of FLIS to FL is seen only in rare instances, but FLIS nevertheless seem to represent precursor lesions of FL and pre-malignant B cell clonal expansions, because in a few instances a clonal relationship between a FLIS and a subsequent FL was demonstrated, and FLIS frequently already carry genomic imbalances as further genetic lesions in addition to the t(14;18).[29,30]

The critical receptor of B cells: surface immunoglobulin (sIg)

A key point for FL is that retention of expression of sIg is mandatory, in spite of the loss of one allele by the t(14;18) translocation. This indicates that, like normal B cells, the receptor has an important function in growth/survival of FL cells. The known

functions of sIg are twofold: first, to provide a low level "tonic" signal essential for survival;[31] the exogenous drivers for normal B cells are not known but could involve low-affinity binding by environmental (auto-)antigens.[32] The second function is to mediate antigen-dependent responses in mature B cells. One distinction between "tonic" and antigen-dependent signaling, derived from studies of developing B cells in knock-out mice, appears to be a differential dependence of survival on the canonical NFκB pathway. This pathway regulates NFκB activation downstream of the BCR and appears essential for "tonic" signal-mediated survival but not for short-term survival of follicular B cells. However, the differential does not hold for long-term survival or functional fitness of the latter,[33] leaving no strict distinction between the two kinds of signal at this stage. This raises the question of what kind of signal might be involved in maintaining malignant B cells. There is no single answer to this since some tumors, such as CLL, do appear to be undergoing chronic antigen-mediated signaling, with consequent downregulation of sIg expression due to reversible endocytosis.[34] This is not apparent in FL, suggesting a different kind of exogenous stimulus.

Since each FL case has a distinct receptor, and the original (foreign) antigen has probably long disappeared, some other ligand, likely a tissue-resident factor, might be operative. Autoantigen could be involved and, as for normal B cells, Ig rescued from a proportion of FL cells (~26%) can bind to certain autoantigens.[35,36] However, the spectrum of autoreactivity appears variable and is less common than for other B-cell tumors. To set the question in context, we will first consider the nature of the sIg in FL cells and then describe the distinctive feature which overcomes heterogeneity among FL Igs by conferring a novel universal ability to bind to a local autoligand.

Surface Ig generation in FL

The sIg of normal B cells has the clear function of antigen recognition via the Ig variable regions (IGHV and IGLV), which are assembled as described above and further modified by the process of SHM. The rather risky interference with DNA allows generation of a wide range of sequences able to cope with the vast array of invading pathogens. The price paid is generation of B-cell malignancies. These carry the Ig of the parental normal B cell, which indicates the point of differentiation reached prior to transformation. In the case of FL, that is the GC, as evidenced by the accumulation of ***IGV*** somatic mutations at levels which, although widely varying

between cases, are comparable to the similarly variable levels in normal GC B cells.[37] Another feature shared with normal GC B cells is expression of activation-induced cytidine deaminase (AID), an essential requirement for SHM and isotype switch. AID is detectable in ~25% of FL cells and accompanies the ongoing SHM which leads to intraclonal variation in *IGV* sequences.[38] Although SHM evidently continues post-transformation, it may be limited and does not appear to lead to an overall accumulation of mutations, but rather to dynamic emergence of different subclones over time.[39,40]

About 40-50% of FL have undergone isotype switching and express IgG.[41,42] Curiously, most IgM^+ FL show class-switch recombination on the translocated allele and *IGHC* deletions downstream of IgM on the expressed IGH allele.[42] This indicates selection to retain IgM expression in IgM^+ FL, and links these cells to the t(14;18)-carrying B cells in healthy individuals, which are mostly IgM^+ memory B cells (see above). Retention of IgM expression, at least in the early phase of FL development, might be advantagous for the (pre-)malignant cells, as there is indication that IgM^+ memory B cells have a higher propensity to reenter GC than class-switched memory B cells.[43,44] However, during the pathogenetic process this preference is apparently frequently lost, perhaps due to the particular combination of transforming events acquired by a FL clone, and many cases switch to IgG expression.

The *IGHV* gene repertoire used by normal B cells is well documented and biased usage by B-cell tumors is an indicator of a drive on parental B cells, and possibly on tumor cells, by a superantigen and/or autoantigen.[45] This is evident in some B-cell tumors such as CLL and some mucosa-associated lymphomas, but does not appear to be the case in FL. Classical superantigens, often expressed by pathogens and some autoantigens, are therefore apparently not driving FL cells.

Modification of sIg in FL cells by glycan addition

During SHM, replacement mutations tend to concentrate in the complementarity-determining regions (CDRs).[46] Amino acid sequence motifs consisting of Asn-X-Ser/Thr can arise, where X can be almost any amino acid except Pro. The motifs are cues for addition in the endoplasmic reticulum (ER) of a dolichol-linked oligosaccharide chain to the Asn residue, a process known as N-glycosylation. Although germline-encoded motifs are present in the constant regions of normal Ig and in a few *IGV* sequences, motifs introduced into the IGV regions during SHM are

rare in normal memory B cells, in mutated CLL or in myeloma. However, they are present in almost all cases of FL where they accumulate in the antigen-binding sites. They are found in most (90%) sIgM$^+$ cases but there are slightly fewer (73.5%) in IgG$^+$ cases.[47] Motifs are also present in a subset of diffuse large B cell lymphomas,[48] and cell line studies indicate that, as might be anticipated, it is the GC B cell-like subset which has the higher frequency.[49,50]

We have described a further curiosity in FL which is that, after addition of the so-called "high mannose" glycan in the ER, the usual further processing of the oligosaccharides which occurs as the Ig passes through the Golgi stacks, does not take place for the IGV glycan.[51] Normal Ig has germline-encoded glycan addition sites in the constant region and these are processed by addition of further sugars, trimming and addition of terminal sugars such as fucose and sialic acid before reaching the cell surface.[52] This also occurs for the constant region sites in the FL Ig.[51] However, the introduced sites in the IGV regions appear to be protected from further processing so that the glycan remains highly mannosylated.[53] The result of this is expression of unusual mannosylated glycan in the antigen binding site of almost all cases of FL.

Microenvironmental interactions

In some ways the mannoses resemble those expressed by pathogens and we have found that FL sIg binds to the lectin DC-SIGN in a similar way.[54] DC-SIGN also induces tumor-specific downstream signaling in FL cells.[53,54] The lectin is expressed by macrophages in FL tissue, possibly upregulated by IL-4 produced by local TFH cells,[55] indicating a potential interactive loop operating between FL cells and macrophages in the tissue. The association between macrophage infiltration and prognosis has been evident in several studies,[56,57] although this broke down when anti-CD20 was part of the therapy, possibly because antibody-mediated attack requires macrophages.[58] However, macrophages are clearly present in FL tissue sites, often sited close to proliferating tumor cells (Figure **3**). Interestingly, binding of DC-SIGN to FL cells does not induce endocytosis, possibly explaining the maintenance of relatively high expression of sIg in FL, and offering a persistent signaling mechanism for tumor survival/proliferation.[54] We envisage the binding between the FL cell and the macrophage expressing tetrameric DC-SIGN to involve a persistent low-level signal, potentially important for survival (Figure 4).

Obviously the microenvironment of FL includes not only macrophages, but many interacting cell types, including TFH cells and follicular dendritic cells. These have been extensively reviewed,[59] and we have added the novel interaction between sIg and DC-SIGN-expressing tumor-associated IL-4-polarized macrophages to the mix (Figure **5**).

Clinical relevance of modified sIg

Two distinctive early features of the majority of FL cases are: the t(14;18) translocation and mutations in one or more of the chromatin modifying genes, with changes in *KMT2D (MLL2)* being the most frequent, but those in *CREBBP* apparently enriched in the earliest inferable progenitor (see below).[60] We can now add the glycan modification of sIg evident in 74-90% of cases to define a common three-component setting for FL which then can accumulate further genetic change (Table 1). Interestingly, in one available case, the glycan addition motifs were also detected at the stage of FLIS, consistent with the timing of SHM.[61] While the correlative data indicate the importance of the glycan addition for the life-style of FL cells, functional proof is difficult to obtain. One reason for this is the lack of a mouse model. In our view any attempt to model indolent human lymphomas is challenging, but in the case of the mannosylated Ig-DC-SIGN interaction there is the added problem that expression patterns and function of lectins in mice are different from human subjects.[62] A possible step toward solving this is the availability of a transgenic mouse model expressing human DC-SIGN.[62]

The triad of foundational changes including t(14;18) translocation, mutations in chromatin modifying genes and the mannosylation of the sIg binding sites could be used to assist diagnosis and, since they are clonal markers, for monitoring. For therapy, the apparently essential microenvironmental interaction with macrophage lectins could be blocked by mannose-specific antibodies, possibly derived from those developed for HIV,[63] or by glycans in various formats such as galactomannans,[64] star glycans[65] or mannose-expressing nanoparticles.[66]

Genetic lesions

With the development of novel sequencing technologies, a multitude of recurrent somatic mutations were identified that provided exciting new insights into the molecular pathogenesis of FL (Table 2).[67-78] The products of several of the most

frequently mutated genes, including *KMT2D* (*MLL2*), *CREBBP*, *EP300*, *EZH2* and histone H1 variants, modify histones and hence play a role in epigenetic regulation (Table 2). *KMT2D* encodes a histone methyltransferase of H3K4 that is affected by inactivating mutations in 60-90% of FL.[67,71,76] Mouse studied revealed that KMT2D is indeed a tumor suppressor, and that its functional impairment causes an expansion of GC B cells and promotes lymphomagenesis.[79,80] CREBBP and EP300 are histone acetyltransferases that are mutated in 60-70% and 10-20% of FL, respectively.[71,75] The mutations are inactivating, but mostly affect only one allele, suggesting a haploinsufficiency. Importantly, these genes not only modify histones and thereby have genome-wide effects on gene regulation, but they also acetylate non-histone proteins. Among the non-histone targets in B cells are TP53 and BCL6, two key transcription factors for GC B cell physiology.[75] TP53 is normally activated by acetylation and BCL6 is inactivated. Hence, the impaired function of CREBBP and EP300 in GC B cells causes an inhibition of TP53-mediated apoptosis and leads to enhanced BCL6 function in the mutated GC B cells.[75] EZH2 functions as a histone methyltransferase that methylates H3K27. *EZH2* is monoallelically mutated at particular positions causing a gain-of-function in 20-30% of FL.[70,81] It is the combined function of wildtype and mutated EZH2 that causes an increased di- and trimethylation of H3K27.[81] Mutations were also identified in various members of the linker histone H1 family, together affecting 30-40% of cases.[68,69] The linker histone functions in nucleosome spacing and gene regulation, but the specific consequences of the mutations in *HIST1H* genes remain to be clarified. Chromatin structure of FL is additionally affected by mutations in two genes that function in nucleosome remodeling, namely *ARID1A* and *BCL7A*, which are mutated in about 10-15 and 5-15% of FL, respectively.[68,69]

Three transcription factors are affected by recurrent somatic mutations in FL, i.e. MEF2B (10-20% of cases), STAT6 (10-15%), and FOXO1 (5-10%) (Table 2).[71,77,82,83] The mutations in *MEF2B* lead to its enhanced activity and as a consequence mediate enforced activity of BCL6.[83] Mutations in *FOXO1* lead to a gain-of-function and cause its nuclear retention and consequently enforced activity.[84] FOXO1 is critical to maintain the dark zone program in GC B cells and cooperates with BCL6, the master regulator of the GC B cell program.[85,86] Mutations in *STAT6* occur in hot spot motifs in its DNA-binding domain and hyperactivate IL4/JAK/STAT6

signaling.[77] IL4 levels are increased in the microenvironment of FL, so that here microenvironmental and a genetic factor cooperate in FL pathogenesis.

TNFRSF14 is affected by genetic lesions in about 30-40% of adult FL, and is even more frequently mutated in pediatric-type FL.[87,88] TNFRSF14 is a member of the tumor necrosis factor receptor family, that can have both stimulatory and inhibitory functions. In a FL mouse model, loss of *TNFRSF14* (*HVEM*) leads to cell-autonomous activation of B cells by preventing inhibitory TNFRSF14-BTLA interactions with B cells, and inducing a tumor-supportive microenvironment.[89] Recurrent mutations in *RRAGC*, *ATP6V1B2*, and *ATP6AP1* sustain mTORC1 signaling, which plays a major role in regulating cellular metabolism and growth.[68,73] A further mechanism for sustained mTORC1 activity even under nutrient deprivation conditions is mediated by deletions and/or epigenetic inactivation of *SESTRIN1*, which physiologically causes cell growth inhibition under genotoxic stress.[74]

Taken together, FL show a wide variety of genetic lesions, in particular in factors involved in epigenetic and transcriptional regulation, microenvironmental interactions, and signaling. Importantly, several of the most frequently affected genes (EZH2, CREBBP, KMT2D, MEF2B) play major roles in sustaining the GC B cell program. Hence, these genetic lesions cause a "freezing" of the FL cells at the proliferative GC stage of differentiation and prevent the lymphoma cells from differentiating into resting memory or plasma cells.

Epigenetic dysregulation

As several of the recurrently mutated genes in FL affect epigenetic regulators, epigenetic dysregulation seems to be a hallmark of FL pathogenesis. Globally, FL show a hypomethylation of their DNA, but specific regions are hypermethylated in comparison to normal GC B cells.[90] The DNA methylation pattern of FL overall is more similar to the one of light zone GC B cells than to dark zone B cells, in line with the idea that FL are frozen at the light zone differentiation stage, which is also indicated from gene expression profiling studies.[2,90,91] The finding that EZH2 target genes are enriched among hypermethylated genes in FL fits to the frequent finding of EZH2-activating mutations in FL, as discussed earlier. Whereas the epigenetic profile of super enhancers that mediate B cell identity are retained in FL cells, some aspects of B cell functions that appear not to be essential or even detrimental for FL cells are epigenetically silenced, including gene sets regulating apoptosis and cell cycle

checkpoints.[91] Moreover, FL cells also acquired epigenetic features of other cell lineages that are frequently found in various types of cancers, indicating that they control genes involved in cellular transformation.[91] Detailed epigenetic profiling also indicated existence of two subsets of FL, one with a GC-specific profile, and another subgroup which has acquired some features of plasmablasts.[91] The relevance of this finding needs further investigation.

Conclusions

In several of its key pathogenetic characteristics, FL can serve as a paradigm for lymphomagenesis. The presence of t(14;18)-carrying B cells in healthy individuals and the fact that the translocations happen in pro/pre-B cells exemplify two hallmarks of the multistep transformation process in human cancers. One is that the transfomation process frequently involves pre-malignant cells with first genetic lesions that may persist in the body for years or even decades before in some individuals they give rise to fully malignant tumor clones. The other hallmark is that initial genetic lesions may occur at an earlier differentiation stage than the one from which the malignant clone derives. Transforming events may even occur in haematopoietic stem cells in several types of tumor from mature lymphocytes.[92-94] A further hallmark of FL is that many of the frequent genetic lesions identified contribute to stabilizing the GC differentiation program and in particular the activity of BCL6 as the master regulator of the program. This keeps the cells in a proliferative state and prevents differentiation into resting memory B or plasma cells. The contribution of mutations affecting epigenetic regulators to this process highlights the important role of epigenetic dysregulation in lymphomagenesis, that has similarly emerged also in other types of lymphomas.[71,75] FL is also a prototype of a B cell lymphoma that depends on BCR expression and on microenvironmental interactions in addition to the genetic lesions.

A key pathogenetic mechanism that appears specific for FL is that the lymphoma cells have acquired a peculiar way for constitutive BCR triggering by presenting mannosylated glycans on the IGV regions, which bind to receptors on macrophages. This unique modification of the BCR must occur during SHM in the GC and is found in the early stage of lymphoma development, FLIS. In FL, sites for mannosylation are retained during ongoing SHM[95] and presumably confer a growth/survival advantage on emergent GC-located clones, which can then

accumulate additional (epi)genomic modifications. BCR stimulation remains essential but has become independent from the presence of foreign antigens that may initially have triggered proliferation of the GC B cell precursors of the lymphoma. This post-translational modification appears vital for the life style of FL and draws our attention to a new level of tumor-cell opportunism which may offer possibilities for intervention.

Acknowledgements

We thank Sylvia Hartmann, Martin-Leo Hansmann (both Senckenberg Center for Pathology, University of Frankfurt/Main, Germany) and Maurilio Ponzoni (IRCCS San Raffaele Scientific Institute, Milan, Italy) for providing histological pictures of FL. We thank Dr. D Taylor for help with figures and Lynsey Block for administrative support. We also acknowledge support of our own studies by the German Ministry of Science and Education (BMBF) in the framework of the ICGC MMML-Seq (01KU1002A-J) project, by the Deutsche Krebshilfe, the Dr. Werner Jackstädt-Stiftung, Cancer Research UK, the NIHR Experimental Cancer Medicine Centre, University of Southampton, Bloodwise and the Gilead UK & Ireland Oncology Fellowship Programme 2016.

Authorship contributions

FKS and RK wrote the manuscript.

Conflict of interest Disclosures

The authors declare no competing financial interests.

References

1. Kridel R, Sehn LH, Gascoyne RD. Can histologic transformation of follicular lymphoma be predicted and prevented? *Blood*. 2017;130(3):258-266.
2. Victora GD, Dominguez-Sola D, Holmes AB, Deroubaix S, Dalla-Favera R, Nussenzweig MC. Identification of human germinal center light and dark zone cells and their relationship to human B-cell lymphomas. *Blood*. 2012;120(11):2240-2248.
3. Rajewsky K. Clonal selection and learning in the antibody system. *Nature*. 1996;381(6585):751-758.
4. Küppers R, Dalla-Favera R. Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene*. 2001;20(40):5580-5594.
5. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. *Science*. 1985;228(4706):1440-1443.
6. Yunis JJ, Frizzera G, Oken MM, McKenna J, Theologides A, Arnesen M. Multiple recurrent genomic defects in follicular lymphoma. A possible model for cancer. *N Engl J Med*. 1987;316(2):79-84.
7. Küppers R, Zhao M, Hansmann ML, Rajewsky K. Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. *EMBO J*. 1993;12(13):4955-4967.
8. Victora GD, Nussenzweig MC. Germinal centers. *Annu Rev Immunol*. 2012;30:429-457.
9. Liu YJ, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennan IC. Mechanism of antigen-driven selection in germinal centres. *Nature*. 1989;342(6252):929-931.
10. Han S, Zheng B, Dal Porto J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. IV. Affinity-dependent, antigen-driven B cell apoptosis in germinal centers as a mechanism for maintaining self-tolerance. *J Exp Med*. 1995;182(6):1635-1644.
11. Pulendran B, Kannourakis G, Nouri S, Smith KG, Nossal GJ. Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature*. 1995;375(6529):331-334.
12. Liu YJ, Mason DY, Johnson GD, et al. Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. *Eur J Immunol*. 1991;21(8):1905-1910.

13. Merville P, Dechanet J, Desmouliere A, et al. Bcl-2+ tonsillar plasma cells are rescued from apoptosis by bone marrow fibroblasts. *J Exp Med*. 1996;183(1):227-236.
14. Smith KG, Light A, O'Reilly LA, Ang SM, Strasser A, Tarlinton D. bcl-2 transgene expression inhibits apoptosis in the germinal center and reveals differences in the selection of memory B cells and bone marrow antibody-forming cells. *J Exp Med*. 2000;191(3):475-484.
15. Limpens J, Stad R, Vos C, et al. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. *Blood*. 1995;85(9):2528-2536.
16. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J. Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. *J Clin Oncol*. 2001;19(2):420-424.
17. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. *Proc Natl Acad Sci USA*. 1994;91(19):8910-8914.
18. Hirt C, Dolken G, Janz S, Rabkin CS. Distribution of t(14;18)-positive, putative lymphoma precursor cells among B-cell subsets in healthy individuals. *Br J Haematol*. 2007;138(3):349-353.
19. Roulland S, Navarro JM, Grenot P, et al. Follicular lymphoma-like B cells in healthy individuals: a novel intermediate step in early lymphomagenesis. *J Exp Med*. 2006;203(11):2425-2431.
20. Roulland S, Lebailly P, Lecluse Y, Heutte N, Nadel B, Gauduchon P. Long-term clonal persistence and evolution of t(14;18)-bearing B cells in healthy individuals. *Leukemia*. 2006;20(1):158-162.
21. Weigert O, Kopp N, Lane AA, et al. Molecular ontogeny of donor-derived follicular lymphomas occurring after hematopoietic cell transplantation. *Cancer Discov*. 2012;2(1):47-55.
22. Sungalee S, Mamessier E, Morgado E, et al. Germinal center reentries of BCL2-overexpressing B cells drive follicular lymphoma progression. *J Clin Invest*. 2014;124(12):5337-5351.
23. Bermudez G, Gonzalez de Villambrosia S, Martinez-Lopez A, et al. Incidental and isolated follicular lymphoma in situ and mantle cell lymphoma in situ lack clinical significance. *Am J Surg Pathol*. 2016;40(7):943-949.

24. Cong P, Raffeld M, Teruya-Feldstein J, Sorbara L, Pittaluga S, Jaffe ES. In situ localization of follicular lymphoma: description and analysis by laser capture microdissection. *Blood*. 2002;99(9):3376-3382.
25. Henopp T, Quintanilla-Martinez L, Fend F, Adam P. Prevalence of follicular lymphoma in situ in consecutively analysed reactive lymph nodes. *Histopathology*. 2011;59(1):139-142.
26. Tellier J, Menard C, Roulland S, et al. Human t(14;18)positive germinal center B cells: a new step in follicular lymphoma pathogenesis? *Blood*. 2014;123(22):3462-3465.
27. Mamessier E, Broussais-Guillaumot F, Chetaille B, et al. Nature and importance of follicular lymphoma precursors. *Haematologica*. 2014;99(5):802-810.
28. Kosmidis P, Bonzheim I, Dufke C, et al. Next generation sequencing of the clonal IGH rearrangement detects ongoing mutations and interfollicular trafficking in in situ follicular neoplasia. *PLoS One*. 2017;12(6):e0178503.
29. Mamessier E, Song JY, Eberle FC, et al. Early lesions of follicular lymphoma: a genetic perspective. *Haematologica*. 2014;99(3):481-488.
30. Schmidt J, Salaverria I, Haake A, et al. Increasing genomic and epigenomic complexity in the clonal evolution from in situ to manifest t(14;18)-positive follicular lymphoma. *Leukemia*. 2014;28(5):1103-1112.
31. Lam KP, Kühn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell*. 1997;90(6):1073-1083.
32. Otipoby KL, Waisman A, Derudder E, Srinivasan L, Franklin A, Rajewsky K. The B-cell antigen receptor integrates adaptive and innate immune signals. *Proc Natl Acad Sci USA*. 2015;112(39):12145-12150.
33. Derudder E, Herzog S, Labi V, et al. Canonical NF-kappaB signaling is uniquely required for the long-term persistence of functional mature B cells. *Proc Natl Acad Sci USA*. 2016;113(18):5065-5070.
34. Mockridge CI, Potter KN, Wheatley I, Neville LA, Packham G, Stevenson FK. Reversible anergy of sIgM-mediated signaling in the two subsets of CLL defined by VH-gene mutational status. *Blood*. 2007;109(10):4424-4431.
35. Meffre E, Wardemann H. B-cell tolerance checkpoints in health and autoimmunity. *Curr Opin Immunol*. 2008;20(6):632-638.

36. Sachen KL, Strohman MJ, Singletary J, et al. Self-antigen recognition by follicular lymphoma B-cell receptors. *Blood*. 2012;120(20):4182-4190.
37. Klein U, Goossens T, Fischer M, et al. Somatic hypermutation in normal and transformed human B cells. *Immunol Rev*. 1998;162:261-280.
38. Smit LA, Bende RJ, Aten J, Guikema JE, Aarts WM, van Noesel CJ. Expression of activation-induced cytidine deaminase is confined to B-cell non-Hodgkin's lymphomas of germinal-center phenotype. *Cancer Res*. 2003;63(14):3894-3898.
39. Aarts WM, Bende RJ, Bossenbroek JG, Pals ST, van Noesel CJ. Variable heavy-chain gene analysis of follicular lymphomas: subclone selection rather than clonal evolution over time. *Blood*. 2001;98(1):238-240.
40. Carlotti E, Wrench D, Rosignoli G, et al. High throughput sequencing analysis of the immunoglobulin heavy chain gene from flow-sorted B cell sub-populations define the dynamics of follicular lymphoma clonal evolution. *PLoS One*. 2015;10(9):e0134833.
41. Scherer F, Navarrete MA, Bertinetti-Lapatki C, Boehm J, Schmitt-Graeff A, Veelken H. Isotype-switched follicular lymphoma displays dissociation between activation-induced cytidine deaminase expression and somatic hypermutation. *Leuk Lymphoma*. 2016;57(1):151-160.
42. Vaandrager JW, Schuuring E, Kluin-Nelemans HC, Dyer MJ, Raap AK, Kluin PM. DNA fiber fluorescence in situ hybridization analysis of immunoglobulin class switching in B-cell neoplasia: aberrant CH gene rearrangements in follicle center-cell lymphoma. *Blood*. 1998;92(8):2871-2878.
43. Seifert M, Przekopowitz M, Taudien S, et al. Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions. *Proc Natl Acad Sci USA*. 2015;112(6):E546-555.
44. Dogan I, Bertocci B, Vilmont V, et al. Multiple layers of B cell memory with different effector functions. *Nat Immunol*. 2009;10(12):1292-1299.
45. Zouali M. Exploitation of host signaling pathways by B cell superantigens--potential strategies for developing targeted therapies in systemic autoimmunity. *Ann N Y Acad Sci*. 2007;1095:342-354.
46. Dörner T, Foster SJ, Brezinschek HP, Lipsky PE. Analysis of the targeting of the hypermutational machinery and the impact of subsequent selection on the

distribution of nucleotide changes in human VHDJH rearrangements. *Immunol Rev*. 1998;162:161-171.

47. Cha SC, Qin H, Kannan S, et al. Nonstereotyped lymphoma B cell receptors recognize vimentin as a shared autoantigen. *J Immunol*. 2013;190(9):4887-4898.
48. Zhu D, McCarthy H, Ottensmeier CH, Johnson P, Hamblin TJ, Stevenson FK. Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. *Blood*. 2002;99(7):2562-2568.
49. Young RM, Wu T, Schmitz R, et al. Survival of human lymphoma cells requires B-cell receptor engagement by self-antigens. *Proc Natl Acad Sci USA*. 2015;112(44):13447-13454.
50. Havranek O, Xu J, Kohrer S, et al. Tonic B-cell receptor signaling in diffuse large B-cell lymphoma. *Blood*. 2017;130(8):995-1006.
51. Radcliffe CM, Arnold JN, Suter DM, et al. Human follicular lymphoma cells contain oligomannose glycans in the antigen-binding site of the B-cell receptor. *J Biol Chem*. 2007;282(10):7405-7415.
52. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. *Cell*. 2006;126(5):855-867.
53. Coelho V, Krysov S, Ghaemmaghami AM, et al. Glycosylation of surface Ig creates a functional bridge between human follicular lymphoma and microenvironmental lectins. *Proc Natl Acad Sci USA*. 2010;107(43):18587-18592.
54. Linley A, Krysov S, Ponzoni M, Johnson PW, Packham G, Stevenson FK. Lectin binding to surface Ig variable regions provides a universal persistent activating signal for follicular lymphoma cells. *Blood*. 2015;126(16):1902-1910.
55. Amin R, Mourcin F, Uhel F, et al. DC-SIGN-expressing macrophages trigger activation of mannosylated IgM B-cell receptor in follicular lymphoma. *Blood*. 2015;126(16):1911-1920.
56. Dave SS, Wright G, Tan B, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med*. 2004;351(21):2159-2169.

57. Relander T, Johnson NA, Farinha P, Connors JM, Sehn LH, Gascoyne RD. Prognostic factors in follicular lymphoma. *J Clin Oncol*. 2010;28(17):2902-2913.
58. Stevenson FK, Stevenson GT. Follicular lymphoma and the immune system: from pathogenesis to antibody therapy. *Blood*. 2012;119(16):3659-3667.
59. Ame-Thomas P, Tarte K. The yin and the yang of follicular lymphoma cell niches: role of microenvironment heterogeneity and plasticity. *Semin Cancer Biol*. 2014;24:23-32.
60. Green MR, Kihira S, Liu CL, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. *Proc Natl Acad Sci USA*. 2015;112(10):E1116-1125.
61. Mamessier E, Drevet C, Broussais-Guillaumot F, et al. Contiguous follicular lymphoma and follicular lymphoma in situ harboring N-glycosylated sites. *Haematologica*. 2015;100(4):e155-157.
62. Schaefer M, Reiling N, Fessler C, et al. Decreased pathology and prolonged survival of human DC-SIGN transgenic mice during mycobacterial infection. *J Immunol*. 2008;180(10):6836-6845.
63. Behrens AJ, Crispin M. Structural principles controlling HIV envelope glycosylation. *Curr Opin Struct Biol*. 2017;44:125-133.
64. Demotte N, Bigirimana R, Wieers G, et al. A short treatment with galactomannan GM-CT-01 corrects the functions of freshly isolated human tumor-infiltrating lymphocytes. *Clin Cancer Res*. 2014;20(7):1823-1833.
65. Zhang Q, Collins J, Anastasaki A, et al. Sequence-controlled multi-block glycopolymers to inhibit DC-SIGN-gp120 binding. *Angew Chem Int Ed Engl*. 2013;52(16):4435-4439.
66. Hockl PF, Wolosiuk A, Perez-Saez JM, et al. Glyco-nano-oncology: Novel therapeutic opportunities by combining small and sweet. *Pharmacol Res*. 2016;109:45-54.
67. Green MR, Gentles AJ, Nair RV, et al. Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. *Blood*. 2013;121(9):1604-1611.
68. Krysiak K, Gomez F, White BS, et al. Recurrent somatic mutations affecting B-cell receptor signaling pathway genes in follicular lymphoma. *Blood*. 2017;129(4):473-483.

69. Li H, Kaminski MS, Li Y, et al. Mutations in linker histone genes HIST1H1 B, C, D, and E; OCT2 (POU2F2); IRF8; and ARID1A underlying the pathogenesis of follicular lymphoma. *Blood*. 2014;123(10):1487-1498.
70. Morin RD, Johnson NA, Severson TM, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*. 2010;42(2):181-185.
71. Morin RD, Mendez-Lago M, Mungall AJ, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*. 2011;476(7360):298-303.
72. Okosun J, Bodor C, Wang J, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. *Nat Genet*. 2014;46(2):176-181.
73. Okosun J, Wolfson RL, Wang J, et al. Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. *Nat Genet*. 2016;48(2):183-188.
74. Oricchio E, Katanayeva N, Donaldson MC, et al. Genetic and epigenetic inactivation of SESTRIN1 controls mTORC1 and response to EZH2 inhibition in follicular lymphoma. *Sci Transl Med*. 2017;9(396).
75. Pasqualucci L, Dominguez-Sola D, Chiarenza A, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature*. 2011;471(7337):189-195.
76. Pasqualucci L, Khiabanian H, Fangazio M, et al. Genetics of follicular lymphoma transformation. *Cell Rep*. 2014;6(1):130-140.
77. Yildiz M, Li H, Bernard D, et al. Activating STAT6 mutations in follicular lymphoma. *Blood*. 2015;125(4):668-679.
78. Weigert O, Weinstock DM. The promises and challenges of using gene mutations for patient stratification in follicular lymphoma. *Blood*. 2017;130(13):1491-1498.
79. Ortega-Molina A, Boss IW, Canela A, et al. The histone lysine methyltransferase KMT2D sustains a gene expression program that represses B cell lymphoma development. *Nat Med*. 2015;21(10):1199-1208.
80. Zhang J, Dominguez-Sola D, Hussein S, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. *Nat Med*. 2015;21(10):1190-1198.
81. Sneeringer CJ, Scott MP, Kuntz KW, et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on

histone H3 (H3K27) in human B-cell lymphomas. *Proc Natl Acad Sci USA*. 2010;107(49):20980-20985.

82. Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol*. 2015;16(9):1111-1122.
83. Ying CY, Dominguez-Sola D, Fabi M, et al. MEF2B mutations lead to deregulated expression of the oncogene BCL6 in diffuse large B cell lymphoma. *Nat Immunol*. 2013;14(10):1084-1092.
84. Trinh DL, Scott DW, Morin RD, et al. Analysis of FOXO1 mutations in diffuse large B-cell lymphoma. *Blood*. 2013;121(18):3666-3674.
85. Dominguez-Sola D, Kung J, Holmes AB, et al. The FOXO1 transcription factor instructs the germinal center dark zone program. *Immunity*. 2015;43(6):1064-1074.
86. Sander S, Chu VT, Yasuda T, et al. PI3 kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. *Immunity*. 2015;43(6):1075-1086.
87. Launay E, Pangault C, Bertrand P, et al. High rate of TNFRSF14 gene alterations related to 1p36 region in de novo follicular lymphoma and impact on prognosis. *Leukemia*. 2012;26(3):559-562.
88. Schmidt J, Gong S, Marafioti T, et al. Genome-wide analysis of pediatric-type follicular lymphoma reveals low genetic complexity and recurrent alterations of TNFRSF14 gene. *Blood*. 2016;128(8):1101-1111.
89. Boice M, Salloum D, Mourcin F, et al. Loss of the HVEM tumor suppressor in lymphoma and restoration by modified CAR-T cells. *Cell*. 2016;167(2):405-418 e413.
90. Kretzmer H, Bernhart SH, Wang W, et al. DNA methylome analysis in Burkitt and follicular lymphomas identifies differentially methylated regions linked to somatic mutation and transcriptional control. *Nat Genet*. 2015;47(11):1316-1325.
91. Koues OI, Kowalewski RA, Chang LW, et al. Enhancer sequence variants and transcription-factor deregulation synergize to construct pathogenic regulatory circuits in B-cell lymphoma. *Immunity*. 2015;42(1):186-198.

92. Chung SS, Kim E, Park JH, et al. Hematopoietic stem cell origin of BRAFV600E mutations in hairy cell leukemia. *Sci Transl Med*. 2014;6(238):238ra271.
93. Damm F, Mylonas E, Cosson A, et al. Acquired initiating mutations in early hematopoietic cells of CLL patients. *Cancer Discov*. 2014;4(9):1088-1101.
94. Quivoron C, Couronne L, Della Valle V, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell*. 2011;20(1):25-38.
95. McCann KJ, Ottensmeier CH, Callard A, et al. Remarkable selective glycosylation of the immunoglobulin variable region in follicular lymphoma. *Mol Immunol*. 2008;45(6):1567-1572.

Legends to Figures

Figure 1. Histological appearance of FL

A) Haemalaun and eosin staining of a FL grade 1 (40x magnification). Note the numerous follicles of the FL which lost the typical dark zone/light zone demarcation and are surrounded by thin mantle zones. B) Ki-67 staining of a FL, revealing the typical low fraction of proliferating cells in a FL follicle (100x magnification). C) BCL2 staining of a FL, showing strong BCL2 staining of the lymphoma cells (100x magnification). The center of the FL follicle is marked with a "*", the thin mantle zone surrounding the follicle with "#". D) BCL2 staining of a reactive lymph node with BCL2-positive mantle zone B cells and BCL2-negative GC B cells (100x magnification). For the BCL2 staining a mouse-anti-human monoclonal antibody (clone 124, DAKO Agilent, Hamburg, Germany) was used, and for the Ki-67 staining a mouse-anti-human monoclonal antibody (clone MIB-1, DAKO). Stainings were kindly provided by S. Hartmann and M.-L. Hansmann.

Figure 2. Scenario for FL pathogenesis

During early B cell development, in rare instances pro B cells acquire a t(14;18) IGH-*BCL2* translocation as a mistake of IGH recombination. This leads to constitutive expression of BCL2. t(14:18)-bearing B cells can develop further into mature naive B cells and may enter a GC reaction upon antigenic stimulation. In the GC, BCL2 is normally downregulated to promote apoptosis proneness of GC B cells. However, t(14;18)-carrying GC B cells have a survival advantage, may clonally expand and become memory B cells. t(14;18)-positive B cells in the peripheral blood are mainly found among IgM memory B cells. Such cells may undergo repeated GC reactions and thereby acquire further genetic lesions. In some reactive lymph nodes, GC dominated by monoclonal $BCL2^+$ GC B cells can be found. These are called FL in situ (FLIS) and the B cell clones can be considered as pre-malignant as they often carry besides the t(14;18) further genetic lesions. From such structures FL can develop after additional gene mutations occurred. Furthermore, mutations promoting N glycosylation of aminoacids in the variable regions of the BCR have been detected in FLIS,[28,61] so that chronic antigenic stimulation as an additional pathogenetic factor occurring through BCR stimulation by lectins on stromal cells can occur already in FLIS (and perhaps even earlier, as the mutations causing N glycosylation may well occur in early GC passages, when SHM is highly active). Nearly half of cases of FL

express IgG, so that at some stage during FL pathogenesis, a considerable fraction of cases have undergone class-switch recombination (CSR).

Figure 3. Macrophages are located close to proliferating FL cells. Macrophage marker CD68 (PG-M1) (red) and proliferation marker Ki-67 (MIB-1) (brown) show co-localisation of macrophages and proliferating FL cells.

Figure 4. **Interaction between DC-SIGN expressed by macrophages and mannosylated surface Ig on the surface of FL cells.** A diagrammatic view of a cell-cell interaction between a polarized macrophage expressing naturally tetrameric DC-SIGN and a FL cell carrying mannosylated mortifs in the IGV region. Data *in vitro* show that DC-SIGN engagement leads to phosphorylation of intracellular kinases in FL cells.[54]

Figure 5. Influences of the cellular microenvironment on FL cells. Potential interactions between FL cells and local follicular dendritic cells (FDC) and T-follicular helper cells (TFH) are indicated. High levels of IL-4 are likely to polarize the tumor-associated macrophages (TAM) to M2 type with upregulated DC-SIGN expression and subsequent engagement of mannosylated sIg on FL cells.

Table 1. Genetic changes found in earliest inferable progenitor of FL

	Genetic change	Approx. frequency in FL
1	Translocation of *BCL2*	80-90%
2	Mutations in one or more chromatin modifier genes	95%
3	Acquisition of N-glycosylation sites in IGV region of BCR	75-90%

Table 2. Genetic lesions in FL

Functional category	Gene	Approx. freq. of mutated cases [a]	Proposed functional censequences	Selected references
Apoptosis	*BCL2*	80-90%[b]	Rescue from apoptosis in the GC	5,6
Histone modification	*KMT2D* (*MLL2*)	60-90%	Reduced H3K4 methylation, Promotion of GC B cell proliferation	67,71,76
	CREBBP	60-70%	Reduced histone acetylation, enhances BCL6 function, impaired TP53 function	71,75
	EP300	10-20%	Reduced histone acetylation	71,75
	EZH2	20-30%	Increased bi- an trimethylation of H3K27, reduced expression of target genes	70,81
	HIST1H1B-E and other *HIST1* members	30-40%	Unclear	68,69
Nucleosome remodeling	*ARID1A*	10-15%	Alteration of chromatin remodeling, specific consequences unclear	69,82
	BCL7A	ca. 10%	Alteration of chromatin remodeling, specific consequences unclear	68
Signaling	*CARD11*	10-15%	Activation of NFκB signaling	68,82
	RRAGC	15-20%	mTORC1 activation, promotes cellular metabolism and growth	68,73
	GNA13	ca. 10%	Inactivating mutations promote B-cell growth and lymphoma cell dissemination	71
	TNFRSF14	30-40%	Loss of function mutations may prevent inhibitory	87,88

			HVEM signaling	
	SESTRIN1	ca. 20%[c]	Inactivating mutations promote mTOR activity	[74]
Transcription factors	*MEF2B*	10-20%	Enforced activity of BCL6	[82,83]
	FOXO1	ca. 10%	Mutations cause nuclear retention, maintains dark zone B cell program, cooperates with BCL6	[82,84]
	STAT6	10-15%	Hyperactivation of JAK/STAT signaling	[77]

[a]The Table is restricted to genetic lesions which are estimated to occur in at least 10% of cases of FL.

[b]Frequency of IGH-*BCL2* translocations

[c]Homozygous or heterozygous deletions at Chr6q21 which involve the *SESTRIN1* gene.

This Table has been adapted from a Table presented in a prior review.[78]

Figure 1

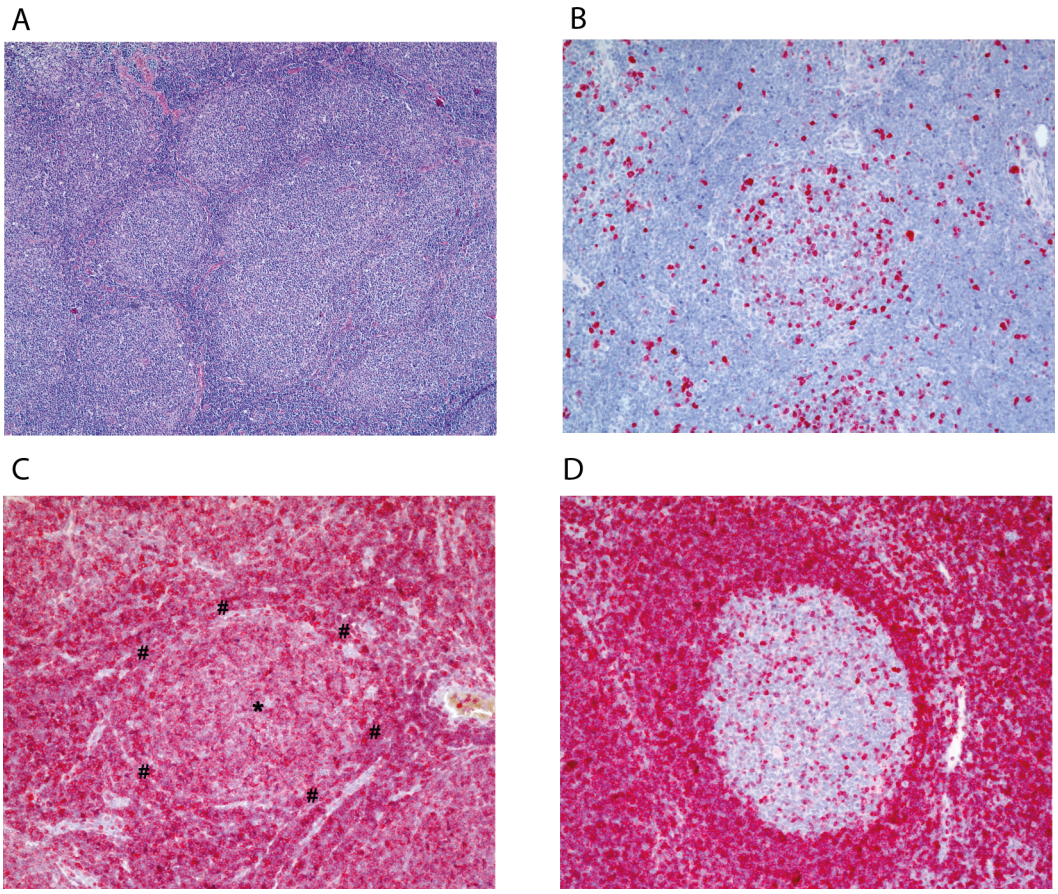


Figure 2

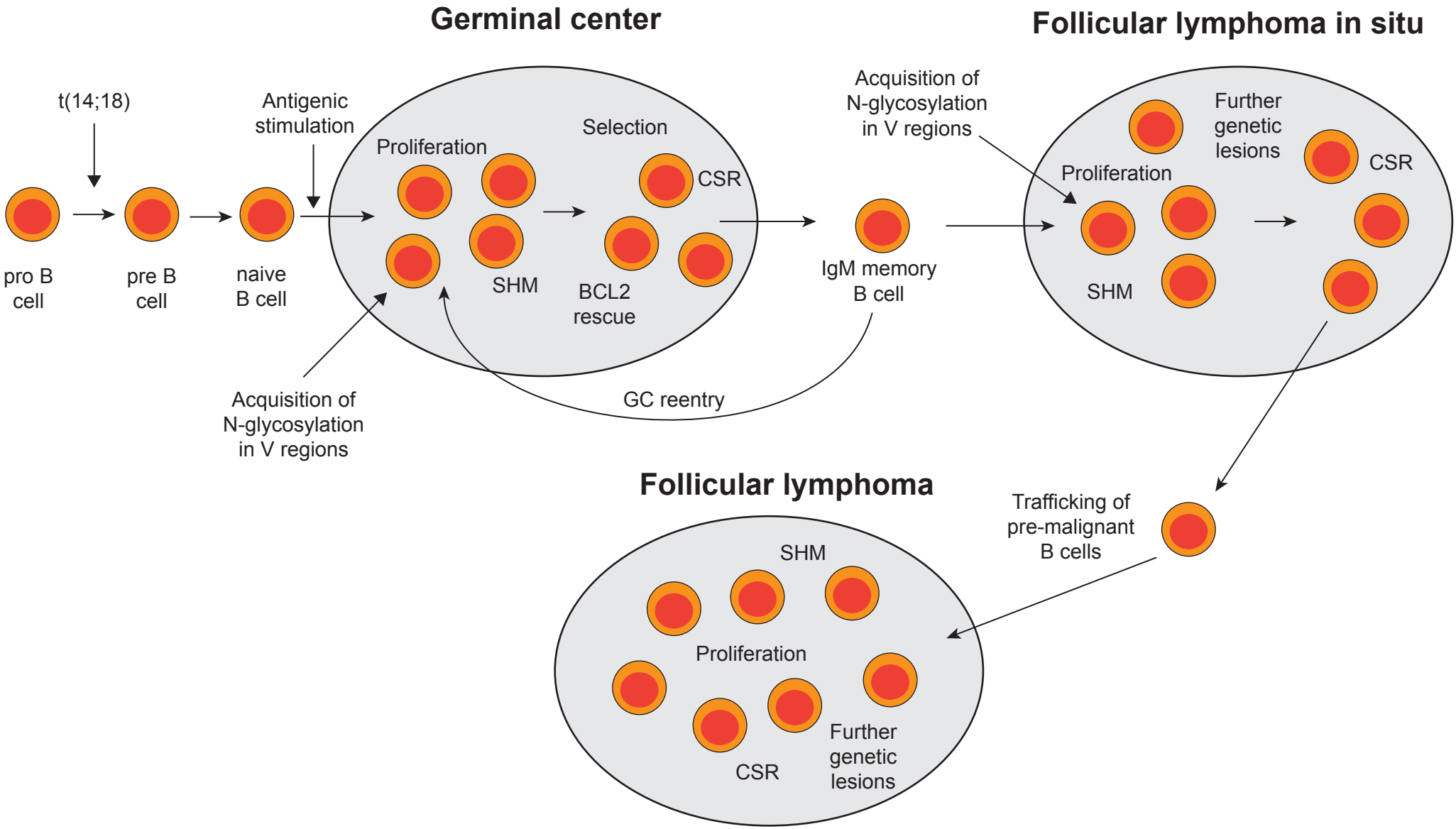

Germinal center
Follicular lymphoma in situ
t(14;18)
Antigenic stimulation
Proliferation
Selection
CSR
SHM
BCL2 rescue
pro B cell
pre B cell
naive B cell
Acquisition of N-glycosylation in V regions
IgM memory B cell
Acquisition of N-glycosylation in V regions
Proliferation
Further genetic lesions
CSR
SHM
GC reentry
Follicular lymphoma
Trafficking of pre-malignant B cells
SHM
Proliferation
CSR
Further genetic lesions

Figure 3

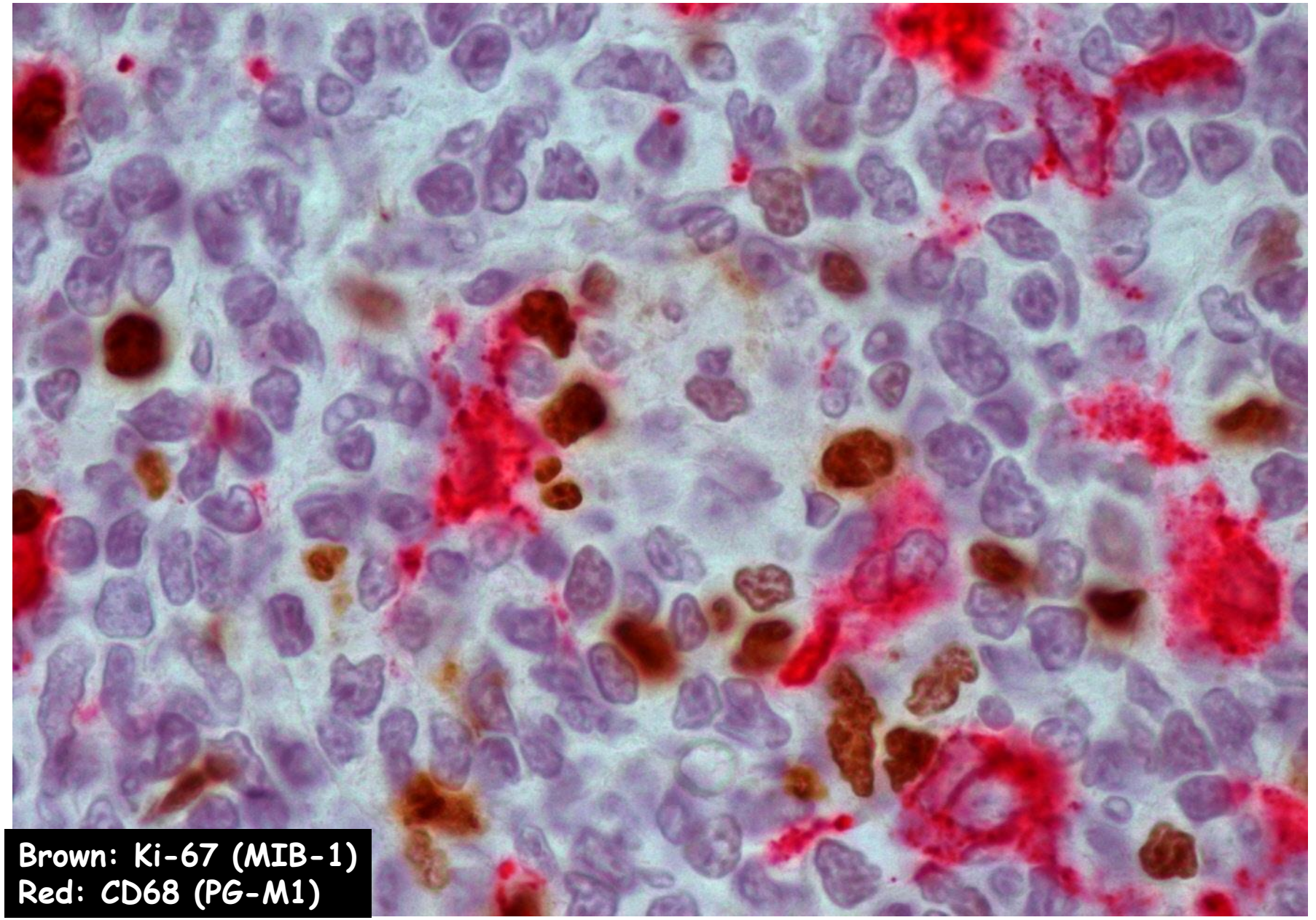


Figure 4

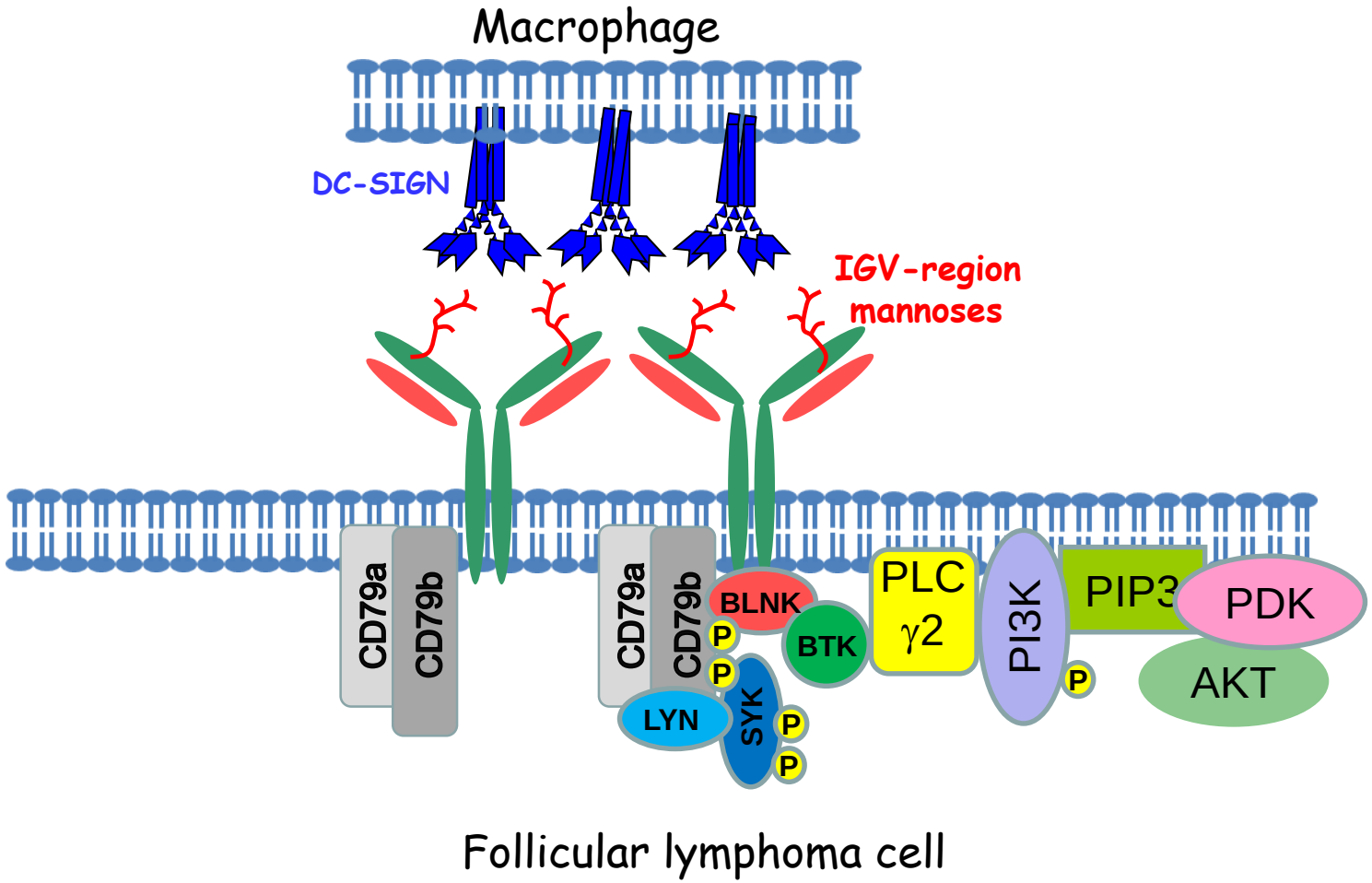
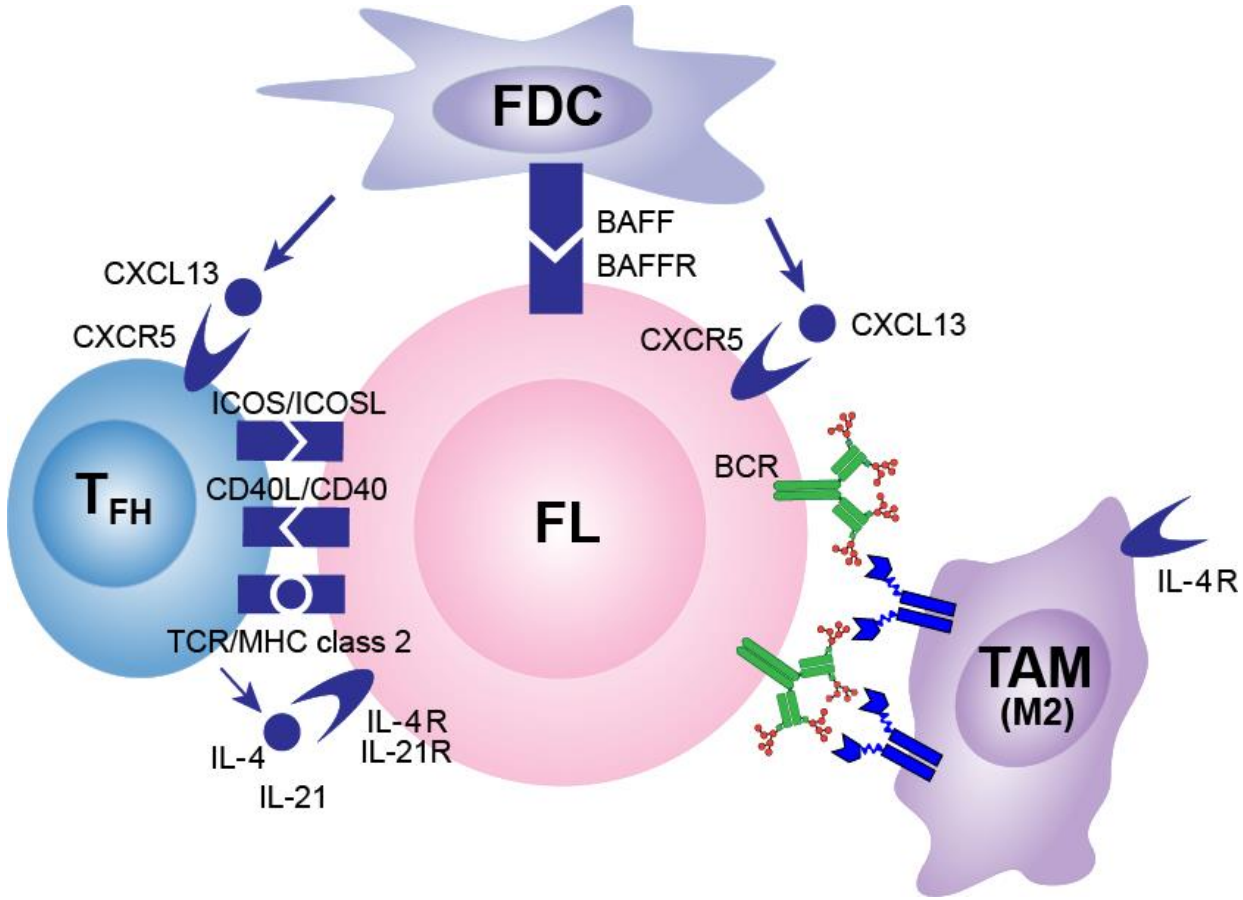

Macrophage
DC-SIGN
IGV-region
mannoses
CD79a
CD79b
CD79a
CD79b
BLNK
BTK
PLC
γ2
PI3K
PIP3
PDK
AKT
P
P
P
LYN
SYK
P
P
Follicular lymphoma cell

Fig 5


FDC
BAFF
BAFFR
CXCL13
CXCR5
CXCR5
CXCL13
ICOS/ICOSL
T_{FH}
CD40L/CD40
FL
BCR
IL-4R
TAM
(M2)
TCR/MHC class 2
IL-4R
IL-21R
IL-4
IL-21

Prepublished online April 17, 2018;
doi:10.1182/blood-2017-11-764365

Critical influences on the pathogenesis of follicular lymphoma

Ralf Küppers and Freda K. Stevenson