Pathogenesis of follicular lymphoma: genetics to the microenvironment to clinical translation

Emil Kumar, Lucy Pickard and Jessica Okosun

Centre for Cancer Genomics and Computational Biology, Barts Cancer Institute, Queen Mary University of London, and Centre for Haematopo-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK

Summary

Follicular lymphoma (FL) represents a heterogeneous disease both clinically and biologically. The pathognomonic t(14;18) translocation can no longer be thought of as the primary genetic driver, with increasing recognition of the biological relevance of recurrent genetic alterations in epigenetic regulators that now feature as a pivotal hallmark of this lymphoma subtype. Furthermore, sequencing studies have provided a near complete catalogue of additional genetic aberrations. Longitudinal and spatial genetic studies add an additional layer to the biological heterogeneity, providing preliminary molecular insights into high-risk phenotypes such as early progressors and transformation, and also supporting evidence for the existence of persisting re-populating cells that act as lymphoma reservoirs and harbingers for FL recurrence. Simultaneously, understanding of the tumour microenvironmental cues promoting lymphomagenesis and disease progression continue to broaden. More recently, studies are beginning to unravel the convergence and co-operation between the genetics, epigenetics and microenvironment. There is a pressing need to marry biology with therapeutics, especially with the burgeoning treatment landscape in FL, to aid in optimising patient selection and guiding the ‘right drug to the right patient’.

Keywords: epigenetics, microenvironment, follicular lymphoma, immunotherapy.

Follicular lymphoma (FL) is the commonest indolent non-Hodgkin lymphoma (NHL), with an incidence of approximately three-four cases/100 000 per annum. There is variability in the clinical spectrum, spanning from patients with limited stage disease to those that follow a more aggressive clinical course demonstrating inferior outcomes. Significant strides have been made in recent years in our understanding of FL biology, with next generation sequencing (NGS) particularly pivotal in delineating the genomic determinants of the multistep pathogenesis, disease evolution and key disrupted oncogenic processes. Meanwhile, accumulating evidence of the contribution of non-genomic determinants, such as the tumour microenvironment (TME), to tumorigenesis has even more prominence given the swathe of modern immunotherapies entering the clinical arena. While the molecular characterisation of FL and the profiling of its TME have often occurred in parallel, an increasing portrait of the crucial crosstalk between FL cells and their microenvironment niche is emerging. In the present review, we provide recent updates in FL biology and highlight both the therapeutic opportunities afforded by these advances, and the key remaining questions.

(t14;18) is the founding lesion in FL

The t(14;18) (q32;q21) reciprocal translocation is considered the genetic hallmark and founding lesion in FL, occurring as an aberrance of RAG-mediated V(D)J rearrangement at the pre-B cell stage of development in the bone marrow (BM), and seen in 85–90% of FL cases. The resulting placement of the B-cell lymphoma 2 (BCL2) gene under the transcriptional control of the immunoglobulin heavy chain (IGH) locus leads to constitutive overexpression of the anti-apoptotic BCL2. However, the presence of t(14;18) alone is insufficient for lymphomagenesis, with as many as half of healthy adult individuals displaying detectable mono- or oligo-clonal t(14;18) B cells in peripheral blood, with the frequency rising with age, although the determinants of which cases subsequently progress to FL remain poorly understood.

These cells in healthy individuals are thought to represent a genuine pre-malignant pool of so-called ‘FL-like cells’ (FLLCs), displaying a germinal centre (GC)-experienced, immunoglobulin (Ig)D+/IgM+ cluster of differentiation (CD) 27+ memory B cell phenotype with the ability to persist and clonally expand. A higher frequency of circulating FLLCs appears predictive of risk of transformation to overt FL. It is postulated that FLLCs undergo repeated transits through
secondary lymphoid tissue GCs, exploiting the inherent genomic instability of the GC reaction to acquire additional genomic aberrations, while evading apoptotic negative selection via ectopic BCL2 expression. As evidence for the multistep pathogenesis of FL, and importance of the lymph node (LN) niche, in situ follicular neoplasia (ISFN), formerly termed follicular lymphoma in situ (FLIS), a BCL2+ premalignant entity with low level of progression to overt FL and most commonly discovered incidentally, bears a number of FL-associated epigenetic mutations.

Germinal centre biology hijacked in FL

The GC-centre origins of FL are underlined pathologically by its predominant follicular morphology, immunophenotypically by CD10+ BCL6; and mechanistically by evidence of ongoing class-switch recombination and somatic hypermutation (SHM).

While the proportion of small centrocytes to larger centroblasts underpins the histological grading system in FL, a crucial aberrant feature is loss of the usual morphological light zone (LZ)–dark zone (DZ) separation of these cell types. For decades, we have thought bulk FL tumours to predominantly resemble normal LZ B cells, given the observed similarities in gene expression profiles. However, a recent study examining the cells at single-cell resolution has shown that rather than being ‘frozen’ at a particular GC stage, FL cells fall within a distinct and dynamic continuum that lies outside the classic binary LZ/DZ transcriptional states.

The importance of ongoing B-cell receptor (BCR) signalling in FL B cells is evidenced by the retention of surface immunoglobulin (Ig) expression, and selective pressure against loss of the intact, untranslocated IGH allele in the majority of cases. One hypothesis regarding the significance of this Ig retention relates to the ~80% of cases in which SHM of IGV sequences results in novel amino acid motifs that permit post-translational addition of N-glycans at the resultant Ig antigen-binding sites. These glycan modifications on the B cells permit the binding of dendritic cell-specific intercellular adhesion molecule (ICAM) 3-grabbing non-integrin (DC-SIGN)-expressing macrophages, stimulating downstream signalling. N-glycosylation site alterations appear to be early, clonal and stable events, suggesting a role in early FL pathogenesis, although this has not yet been functionally demonstrated.

Mutational landscape of FL

Next generation sequencing studies have been pivotal, not only in providing a compendium of the genomic events that occur in addition to t(14;18), but also in identifying numerous candidate genetic drivers. While the molecular heterogeneity is considerable, the second genomic hallmark in FL is the high frequency of mutations affecting epigenetic regulation, together with other recurrently disrupted pathways including immune recognition [tumour necrosis factor ligand superfamily member 14 (TNFRSF14), cathepsin S (CTSS)], BCR- nuclear factor kappa B (NFkB) [caspase recruitment domain family member 11 (CARD11), TNF alpha-induced protein 3 (TNFAIP3), myeloid differentiation factor 88 (MYD88)], mammalian target of rapamycin (mTOR) signalling [Ras-related GTP-binding protein C (RRAGC), ATPase H+ transporting accessory protein 1 (ATP6V1B2), ATP6V1B2, SESTRIN1] and Janus kinase-signal transducers and activators of transcription (JAK-STAT) signalling (STAT6).

Epigenetic mutations are early and ubiquitous events in FL

Mutations in epigenetic regulators (epimutations) in FL are focussed particularly on histone post-translational modifications, including histone-lysine N-methyltransferase 2D (KMT2D), cAMP response element-binding protein binding protein (CREBBP), enhancer of zeste homologue 2 (EZH2), and ElA binding protein P300 (EP300) (Fig 1); in addition to linker histones [e.g. histone cluster 1 H1 family member E (HIST1H1E)] and regulators of chromatin structure [AT-rich interaction domain 1A (ARID1A)]. These typically occur as early, clonal and temporally stable events, implying a central role in disease initiation and maintenance, with most cases carrying multiple such hits. The sum of these loss-of-function (with the exception of EZH2) mutations is overall transcriptionally repressive.

Histone lysine methyltransferase KMT2D is the most commonly mutated gene in FL (70–80% of cases). Its loss leads to global reduction in histone H3, lysine 4 (H3K4) methylation marks, impacting on expression of genes involved in CD40, JAK-STAT, Toll-like receptor (TLR) and BCR signalling, while in vivo mouse models demonstrate that Kmt2d ablation in B cells leads to GC expansion and impaired terminal differentiation, and promotes lymphomagenesis. Histone acetyltransferases (HATs) CREBBP and EP300 are mutated in ~70% and ~15% of cases, respectively, CREBBP deposits activating histone acetylation marks [histone 3, lysine 18 (H3K18Ac), histone 3, lysine 27 (H3K27Ac)] at enhancers, seemingly antagonising BCL6-mediated transcriptional repression; while also acetylating non-histone targets including p53 and BCL6 itself. In mouse studies, Crebbp is a bona fide tumour suppressor, with its loss co-operating with BCL2 overexpression to drive lymphoma development, via immune evasion with major histocompatibility complex (MHC) Class II downregulation, impaired terminal differentiation and modulated CD40/BCR signalling. CREBBP and EP300 share a high degree of structural similarity, and despite regulating distinct GC transcriptional programmes, these two proteins indeed play an overlapping role in GC...
biology, with simultaneous deletion of Crebbp and Ep300 abrogating the GC reaction in vivo.  

The histone methyltransferase EZH2 forms the catalytic component of the polycomb repressive complex 2 (PRC2) and is responsible for laying repressive H3K27 methylation marks, with its specific upregulation in GC B cells required for GC formation. 

Approximately 20–25% of FL cases are subject to gain-of-function mutations, the majority centred on the hotspot Y641 residue within its catalytic SET domain, leading to globally increased repressive H3K27me3. Reminiscent of the other epigenetic insults, EZH2 mutations are associated with terminal differentiation block and MHC Class I/II downregulation. Notably, the increased H3K27me3 in EZH2-mutant cells are found to be enriched within the three-dimensional (3D) genome in specific structural chromatin subunits termed ‘topologically associated domains’ (TADs) that in turn modulate promoter interactions, switching off multiple tumour suppressor genes; thus, providing an additional layer as to how these epigenetic mutations regulate oncogenic gene expression.

Interestingly, mutations in linker histone H1 proteins, seen in 30% of FL cases (especially HIST1H1E) also impact the 3D genome, as loss of H1c/H1e in murine GC cells resulted in focal chromatin relaxation, re-awakening the expression of repressed stem cell genes; thus, enhancing the fitness of GC B cells. 

In vivo mouse models have indicated that the loss of KMT2D or CREBBP at earlier stages of B-cell development impacts more markedly on the aberrant B-cell phenotype, hinting that epimutations may be required to prime B cells prior to GC entry. Meanwhile, a recent study demonstrated that missense CREBBP mutations within the catalytic histone acetyltransferase (HAT) domain lead to a more profound reduction in global H3K27Ac and MHC Class II downregulation in vitro, and poorer clinical outcomes following immunotherapy compared to truncating/loss of protein mutations. Together these findings highlight that both mutation timing and type are relevant to disease pathogenesis, and are likely important variables to consider in the observed clinical heterogeneity. Furthermore, while most FL cases carry multiple epigenetic lesions, the degree and nature of co-operation between them remains incompletely understood; and indeed the spectrum of genomic disruption to KMT2D and CREBBP points to a variable dosage effect that may in turn relate to the required combination of these and other (epi)genetic insults.

A convergence on metabolic reprogramming in FL

mTOR signalling acts as a master regulator of cell growth and metabolism. In healthy cells, a multi-subunit lysosomal super-complex is responsible for sensing amino acid availability and triggering mTORC1 pathway activation in response. Components within this super-complex are recurrently mutated in FL (RRAGC 17%, ATP6V1B2 11-3%, and ATP6AP1 9-9%); with these mutations found only rarely in other cancers, hinting at a specific dependence on this pathway in FL. RRAGC mutations are activating, strengthening

Fig 1. Mutations affecting histone-modifying genes are crucial in follicular lymphoma (FL) pathogenesis. Histone-modifying enzymes shown with their respective histone modifications and incidence of mutation. The sum of these mutations is transcriptionally repressive, with convergence on key downstream effects.
its interaction with the mTORC1 complex, and consequently permit ‘inappropriate’ mTORC1 signalling despite amino acid deprivation. Ragc-mutated mice accelerate lymphomagenesis in combination with Bcl2-overexpressing mice, producing tumours that are sensitive to pharmacological mTOR inhibition.40

Furthermore, the upstream negative mTORC1 regulator Sestrin1 is subject to copy number loss or epigenetic silencing in ~20% cases of FL, with Sestrin1 knock down also promoting lymphoma development.41 Sestrin1 itself is under transcriptional regulation by EzH2, with EzH2-mutant FL showing a lower expression of Sestrin1. In a cohort of primary FL samples, Rragc mutation, EzH2 mutation and Sestrin1 loss affected 47% cases, and demonstrated significant mutual exclusivity, suggesting a convergence on mTORC1 dysregulation by independent means in FL.41

The immune microenvironment is co-opted by genomic aberrations in FL

Several key examples have emerged to illustrate mechanisms by which genetic lesions corrupt the normal GC microenvironment to support lymphomagenesis and progression. Amongst the most notable is Herpes virus entry mediator (HVEM), which is normally expressed on both B and T cells and regulates both stimulatory and inhibitory T-cell immune responses through context-dependent bidirectional signalling pathways.42 Approximately 40% of FL cases harbour loss of HVEM, encoded by the TNFRSF14 gene, through mutation, deletion and/or copy neutral loss of heterozygosity.16,18,43 Its role in lymphomagenesis appears dependent on interaction with its inhibitory binding partner B- and T-lymphocyte attenuator (BTLA), expressed both on FL B cells themselves and on T-follicular helper (Tfh) cells, with the loss of this axis promoting unopposed BCR signalling, secretion of stroma-activating cytokines, increased Tfh recruitment and strengthened T-cell help.44,45 In a similar fashion, up to 20% of FL cases harbour gain-of-function mutations or amplifications in CTSS, a cysteine protease involved in MHC Class II-mediated antigen processing and presentation. Genetic aberrations in CTSS lead to increased recruitment and interaction with CD4+ Tfh cells, and extrusion of CD8+ T cells; thus, promoting a tumour-supportive microenvironment.21,46

Intriguingly, both Rragc40 and EzH247 mutations appear conversely to reduce dependence on Tfh help, pointing to a potential divergence in adaptive strategy, supported by the finding that Rragc and TNFRSF14 mutations display mutual exclusivity in one recently studied cohort.49

Disease progression occurs by predominant divergent clonal evolution from the putative common progenitor cell

The protracted and frequently relapsing natural history of FL has provided the opportunity to decipher the clonal dynamics of disease evolution. Longitudinal or sequential disease episodes from the same individual are typically clonally related, with shared (14;18) breakpoints and SHM patterns.48,49 NGS studies have corroborated earlier genomic efforts in showing that most longitudinal biopsies genetically evolve in a branched rather than linear manner, and particularly by a divergent mode of clonal evolution whereby there are genomic aberrations that are unique to each disease episode, but importantly there are shared events (Fig 2A). This has allowed the field to hypothesise that every disease episode originates from an ancestral common progenitor cell (CPC). This population is postulated to harbour the ‘trunk’ of core mutations, persisting between disease episodes and demonstrates the capacity to evade treatment. Further evidence for the existence of this CPC lies in the rare cases of donor-derived FL, whereby haematopoietic stem cell transplant (HSCT) donors and recipients develop clonally related FL or histological transformation (HT) events, even several years post-transplant (Fig 2B).49,50

The eradication of the CPC holds promise of reduction in relapse or transformation events, and indeed disease cure. Hence, the precise phenotype, niche and therapeutic vulnerabilities of this putative population represent an area of intense research interest. The core of the mutations harboured within the CPC are those involving epigenetic regulators including KMT2D and CREBBP in addition to t(14;18), highlighting these as early disease events.16 Furthermore, rare cases of FL transformation to a clonally related B-acute lymphoblastic leukaemia51 or histiocytic sarcoma52 where the t(14;18) is shared, suggests the precursor population could de-differentiate to immature lymphoid, or trans-differentiate to non-lymphoid entities, respectively; and thus, suggest that the CPC has phenotypic plasticity.

Besides longitudinal genetic heterogeneity, another added layer of complexity is our understanding of the existence of spatial genetic heterogeneity within a single patient at different disease sites,53 posing challenges for precision medicine approaches. Circulating tumour DNA (ctDNA), fragments of tumour-derived DNA present in a patient’s peripheral blood, can be monitored and may provide one route to achieving a more robust reconstruction of a patient’s molecular heterogeneity in space and time,54 although further correlative studies are required in this area.

Genomic drivers of early progression and histological transformation (HT)

Progression within 24 months of initial immunochemotherapy occurs in about 10–20% of patients and is associated with poor outcomes in FL, with only 50% of such patients alive at 5 years.55 Similarly, HT to diffuse large B-cell lymphoma (DLBCL) confers inferior outcomes, especially early transformation.56 Attention has rightly turned to identifying the biological mediators of these high-risk cohorts of FL patients. Both of these entities display greater genomic
complexity and increase in specific copy number changes. Comprehensive studies in this area are still lacking and somewhat hindered by inconsistent repeat biopsy practices to distinguish indolent relapse from HT, but are required to build sample repositories of sufficient size for a thorough biological characterisation.

Kridel et al. demonstrated that these two high-risk entities are likely to occur via distinct genetic mechanisms. First, they described 10 genes [including tumour protein p53 (TP53), KMT2C, β2 microglobulin (B2M) and MYD88] enriched in the diagnostic samples of patients who experienced early indolent relapse. Next, using high-resolution mutation detection, they tracked the clonal dynamics of progression, with HT clones typically undetectable in the diagnostic biopsy, while early indolent progression was characterised by the expansion of pre-existing, therapy-resistant clones. Both of these events genetically evolve from the ancestral CPC population. Early study indicates this contrasting clonal evolution may be used to predict HT from serial ctDNA samples, although further refinement and validation is required.

HT can occur at different points during a patient’s clinical journey, with the genomic complexity, mutational burden, and activation-induced cytidine deaminase (AID)-driven aberrant SHM (aSHM) increased at transformation. Recurrent genomic events particularly affect DNA damage response and cell cycle regulation, with two-thirds of HT biopsies showing biallelic loss of CDKN2A/B and/or TP53, and dysregulation of (MYC) through translocation, amplification and/or aSHM in 40% of cases. Other aberrations enriched at HT include NF-kB activation via REL amplification, deletion/mutation of TNFAIP3 and MYD88 mutations; and additional immune evasion strategies through disruption to human leucocyte antigen (HLA) Class I components (particularly B2M) and CD58. The majority of HT cases are classified as germinal centre B cell (GCB)-subtype DLBCL by gene expression as might be expected, but interestingly, ~20% of cases are classified as activated B cell (ABC)-subtype, with this group enriched for t(14;18) negativity. Altogether, these studies emphasise the different genetic events. but also the lack of a unique molecular signature for HT.

**Composition and role of the FL TME**

Follicular lymphoma cells exist immersed within a TME milieu of non-malignant immune, stromal and extracellular components, and are critically dependent on the bidirectional crosstalk between itself and its TME (Fig 3). There are numerous supporting lines of evidence for the crucial role of the microenvironment. First, FL B cells reside within specific niches and assume a spatial architecture with

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Fig 2. Evidence for the common progenitor cell (CPC). (A) Genomic profile of serial follicular lymphoma (FL) biopsies from an illustrative case demonstrating the clonal evolution pattern. Mutations shared between disease episodes allow inference of the genetic identity of the inferred CPC, while mutations unique to each disease episode are consistent with divergent clonal evolution from the CPC. (B) Rare cases of donor-derived FL, whereby donor and recipient develop clonally related FL many years after a haematopoietic stem cell transplant (HSCT) underline the ability of the CPC to lay dormant with long latency periods.
neighbouring follicular dendritic cells (FDCs) and Tfh cells that is reminiscent of reactive GCs. Second, FL B cells are unable to grow in vitro in the absence of microenvironmental signals and are notoriously difficult to engraft in immunocompromised mice. The seminal work by Dave et al.,61 further demonstrated that the composition of the microenvironment has a substantial impact on patient outcomes.

Historically, identification and enumeration of the TME components have mainly relied on morphological assessment, immunohistochemistry (IHC) and flow cytometry. Evaluation of the TME and its association with clinical outcomes using IHC has at times led to contradictory prognostic relevance. This, in part, has been due to studies in small, heterogeneously treated cohorts of patients, inter-operator variability in IHC interpretation and an under-appreciation for the overall complexity in the balance and spatial localisation of the immune cells within the TME. Use of higher resolution techniques, including spatial immune analyses and the use of time-of-flight mass cytometry (CyTOF) have and will continue to add immeasurably to our understanding of the TME.

**Tumour-infiltrating T cells**

The most prominent finding is the increase in CD4+ T-cell subsets, such as Tfh cells, regulatory T cells (Treg) and follicular regulatory T cells (Tfr) compared to normal LNs.62,63

Tfh cells are a specialised subset of CD4+ T cells that have a key role in normal GC development and function. Tfh cells prime B cells, leading to initiation of GC and extra-follicular antibody responses and are essential for affinity maturation and the maintenance of humoral memory. Tfh cells notably express the transcription factor BCL6 and cell surface markers, CXC chemokine receptor type 5 (CXCR5), programmed cell death protein 1 (PD-1) and inducible T-cell co-stimulatory (ICOS). In FL, Tfh display specific gene expression and cytokine profiles,64,65 with overexpression of interleukin 2 and 4 (IL-2 and IL-4), mediating STAT6 signalling, contributing to proliferation of FL and preventing apoptosis. IL-4 also triggers chemokine C-X-C motif chemokine ligand 12 (CXCL12) secretion from stromal cells that increases recruitment and migration of FL cells, potentially contributing to disease dissemination.66

Fig 3. Cells of the tumour microenvironment (TME) and follicular lymphoma (FL) cells engage in reciprocal cross talk. FL cells express transmembrane receptor cluster of differentiation 40 (CD40), while its ligand CD40 is expressed on T-follicular helper cell (Tfh) cells, which also secrete cytokines such as interleukin 4 (IL-4) and IL-21, favouring growth and survival of FL cells. Chemokines, C-X-C motif chemokine ligand 12 (CXCL12) and CXCL13, secreted by stromal subsets bind to CXC chemokine receptor type 5 (CXCR5) on Tfh and FL cells. B-cell activating factor (BAFF) is also produced by follicular dendritic cells (FDC), and binds to its receptor BAFFR on FL cells. The acquisition of N-glycosylation sites within the variable region of immunoglobulin triggers antigen-independent B-cell receptor (BCR) activation and survival signals by interacting with dendritic cell-specific ICAM 3-grabbing non-integrin (DC-SIGN)-expressing tumour-associated macrophages (TAM). Programmed cell death 1 ligand 1 (PD-L1) is expressed by macrophages, whilst PD-L1/PD-L2 expression on FL cells remains unclear. MHC, major histocompatibility complex; TCR, T-cell receptor; ICOS, inducible T-cell co-stimulatory; PD-1, programmed death-1; FRC, fibroblastic reticular cell.
Not only is there a skew in T-cell proportions in the FL microenvironment, but immune suppressive signalling pathways are hijacked, inducing T-cell exhaustion and tolerance, and thereby facilitating tumoral immune evasion. PD-1 is expressed on both dysfunctional CD4⁺ and CD8⁺ T lymphocytes as well as on functional Tfh. In FL, a subset of CD10⁺ PD-1⁺ co-expressing Tfh have increased capacity to secrete IL-4, IL-21 and tumour necrosis factor α (TNFα), which in turn sustain malignant B-cell growth.⁶⁷ This Tfh subset can also provide signals to recruit Tregs through CC chemokine ligand 22 (CCL22), decreasing the anti-tumour response, and subverting the TME in favour of the malignant cells.

Regulatory T cells, identified by the expression of CD25 and forkhead box P3 (FOXP3), are an immune-suppressive population of CD4⁺ T cells that, under homeostatic conditions, are critical in maintaining peripheral immune tolerance.⁶⁸ FL LNs contain a higher proportion of Tregs relative to normal LNs. These FL Tregs possess enhanced suppressive capacity through upregulation of immune checkpoint molecules including glucocorticoid-induced TNF receptor (GITR), T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) and ICOS.⁶⁹⁻⁷¹ Notably, the T-cell receptor (TCR) repertoire of FL Tregs is much more restricted compared to reactive LNs, suggesting that FL Tregs are highly clonal,⁷² likely influencing the anti-tumour response of these typically heterogeneous FL tumours.

A recently identified specialised subset of FOXP3⁺ CXCR5⁺ Tfr reside primarily in the GC, share phenotypic characteristics between Tfh and classical Tregs, and can potently suppress Tfh cells and the overall GC reaction.⁷³ In FL, the interconnection and careful balance in the Tfh–Tfr ratios is thought to partly shape its biology, although the mechanisms that dictate this Tfh–Tfr dichotomy remain to be elucidated. There is likely to be a great deal of plasticity and range of T-cell subsets present within and potentially unique to the FL niche. Notably, Yang et al.⁶³ recently demonstrated using CyTOF® that the FL niche is enriched for ‘prematurely aged’ T cells that lack the co-stimulatory molecules CD27 and CD28, and increased numbers of CD27⁻/CD28⁻ T cells were associated with inferior clinical outcomes.

Cytotoxic CD8⁺ T lymphocytes (CTLs) are an essential component of anti-tumour immunity. In FL, increased CD8⁺ T cells are associated with a better outcome independent of clinical prognostic factors.⁷⁴⁻⁷⁵ Using confocal microscopy, Laurent et al.⁷⁵ showed an infiltrate of CTLs containing granulysyme B lytic granules in the FL inter-follicular spaces. These CTLs form synapse-like structures with FL B cells and apoptotic cells, suggesting an in situ cytotoxic function. Persistent antigen stimulation results in dysfunctional, exhausted CD8⁺ T cells with progressive loss of effector function (cytokine production and killing function), and increased expression of multiple inhibitory receptors including PD-1, lymphocyte-activation gene 3 (LAG3), T-cell immunoglobulin domain and mucin domain 3 (TIM3) and TIGIT.⁷⁶⁻⁷⁷ Although CTL enrichment has been associated with better outcomes, FL B cells can subvert the CTL anti-tumour response leading to T-cell exhaustion and recruitment of Tregs that inhibit the activity of infiltrating CTLs, which together promote immune escape. Exhausted T cells likely comprise an umbrella of heterogeneous populations with unique differentiation and functional states. A greater understanding of these populations, the mechanisms that promote exhaustion and how to revert them will have crucial implications for the success of existing and potential immunotherapies.

**Tumour-associated macrophages**

Macrophages are a critical part of the innate immune system phagocytosing pathogens and apoptotic cells, but also are efficient antigen-presentation cells. Historically, macrophages were classically described as polarising into either a classical M1 phenotype or an activated M2 phenotype, depending on the stimulatory signals received; lipopolysaccharide (LPS) and interferon γ for M1, and IL-4 and IL-13 for M2. In a cancer context, tumour-associated macrophages (TAMs) are thought to be more M2-like in phenotype. However, this M1/M2 binary classification framework incompletely captures the heterogeneous, dynamic and cellular plasticity of macrophages in vivo.⁷⁹

Dave et al.⁶¹ demonstrated that FL cases enriched in genes mainly expressed in macrophages and FDCs were associated with an inferior outcome. Currently, there remains the challenge of defining the exact prognostic relevance of TAMs in FL, especially when IHC is used. Typically, TAMs are identified using CD68 and/or CD163 protein expression. In FL, increased numbers of CD68⁺ TAMs have been linked with adverse outcomes in those treated with chemotherapy, although this effect is abrogated by the addition of rituximab.⁸⁰⁻⁸¹ On the other hand, defining macrophages by CD163⁺ expression gives a much stronger staining by IHC, and low numbers of intra-tumoral CD163⁺ macrophages are associated with early treatment failure and inferior prognosis.⁸⁰⁻⁸¹ However, its prognostic relevance appears again to vary according to treatment, suggesting that specific chemotherapy and/or immunotherapy components can modulate the TME composition.

Tumour-associated macrophages contribute directly to malignant cell growth through BCR, B-cell activating factor (BAFF) and IL-15 signalling.⁸² Conversely, antibody-dependent cellular phagocytosis (ADCP) is also mediated by macrophages; and plays a key role in rituximab-induced tumour cell clearance alongside natural killer (NK) cell antibody-dependent cellular toxicity (ADCT).⁸³ Signal-regulatory protein α (SIRPα), expressed on macrophages, is a key member of the ‘do not eat me’ signalling pathway, and suppresses both phagocytic and inflammatory function. Variable expression of SIRPα in FL biopsies delineates subsets of TAMs, with CD14⁺ SIRPαhigh macrophages associated with inferior clinical outcomes.⁸⁴ Notably, CD47 is abundantly expressed
on B cells and interacts with SIRPα, inhibiting macrophage-mediated anti-tumour surveillance.85

Stromal cells

Whilst the emphasis within the TME has mostly resided within the immune components, non-immune components such as endothelial cells, fibroblasts and mesenchymal stromal cells (MSCs) contribute to the aberrant ecosystem. Advanced stage disease with BM infiltration occurs in 70% of patients with FL at diagnosis and is characterised by ectopic differentiation of lymphoid-like stromal cells and local enrichment of CD4+ T cells.86 Differences in cell composition and organisation exist between the BM and LN niches. Prior studies focus on stromal cells within the BM, with limited understanding of these populations within the LN niche. In FL, BM MSCs are recruited to the tumour and incorporated into the stroma, becoming activated with increased secretion of pro-tumoral cytokines including CXCL13 and CXCL12 that promote B-cell homing, retention and activation.87 MSCs overexpress CCL2 and IL-8, which supports the recruitment of monocytes to the TME and triggers differentiation into pro-angiogenic and anti-inflammatory TAM-like macrophages.88 FL MSCs additionally have the ability to differentiate into fibroblastic reticular cells and FDCs, the supporting cast necessary for FL B cells to infiltrate the BM. MSCs isolated from FL-involved BM support the growth of malignant cells more efficiently than MSCs from healthy donors.88

There is a growing appreciation of the relevance of cancer-associated fibroblasts (CAFs) as a strong influencer in tumour growth and therapy responses in solid tumours. FL CAFs display some overlapping features with lymphoid stromal cells, yet exhibit specific phenotypic and gene expression features.82 FL CAFs in LN and BM overexpress CXCL12, triggered by crosstalk with IL-4high FL Tfh cells that in turn leads to FL B-cell activation and adhesion to stromal cells, a process that can be antagonised by Bruton tyrosine kinase and phosphatidylinositol-3 kinase inhibitors.86 There remains an incomplete understanding of the origins, spectrum and heterogeneity of the stromal niche and detailed ex vivo analyses of purified FL stromal components will further clarify the crosstalk with malignant B cells and how this might be therapeutically exploited.

How does the TME promote FL pathogenesis?

The role of the TME is twofold; first, promoting a tumour-conducive ecosystem to support the growth and survival of the tumour B cells and second, the tumour cells can modulate the immune cells, enabling evasion of the host immune surveillance. In summary, this is achieved through:

1. Intrinsic genetic features of the tumours that dictate and shape the TME. As seen earlier, aberrations in genes such as CREBBP and B2M impact the antigen presentation machinery thereby allowing the tumour cells to hide away from the immune system, whilst mutations in genes such as EZH2, TNFRSF14 and RRAGC can reprogramme the crosstalk between the tumour B cells and the microenvironment by modulating the composition of the TME.

2. A reduction in anti-tumoral immune populations to drive immune escape whilst enriching in a milieu of immune suppressive cells including Tregs and myeloid-derived suppressor cells.

3. An increase in the network of dysfunctional and exhausted T-cell populations within the TME.

Despite a greater appreciation of the key players within the microenvironmental ecosystem, there remain a number of questions. A better understanding is required of the spectrum of the dysfunctional and exhausted T cells within the FL TME, how they develop, how they are sustained over time and if they can be reversed. Longitudinal analyses of the TME composition at diagnosis, in the context of residual disease and at relapse might be important to define which populations wax and wane during the disease course. Importantly, comparative analyses between extremes of clinical phenotypes, such as patients on watch and wait who rarely undergo spontaneous regressions versus those with progressive disease, may provide further insight into the identity of the cell niches that are tumour destructive versus tumour protective. The role of the TME in the selection of specific tumour subclones within different spatially separated niches, and how this reflects in intra- and inter-patient disease heterogeneity are still lacking.

Harnessing biology to novel therapeutics

With our recent tsunami of knowledge, are we in a position to leverage our understanding of the genetic and non-genetic drivers of FL into targeted therapeutic approaches?

Despite the central role of t(14;18) in FL pathogenesis, Venetoclax, a selective BCL2 inhibitor has proved disappointing in FL in the relapsed-refractory (R/R) setting, as a monotherapy and even in combination with treatment backbones known to be active in FL.89,90 This lies in stark contrast to the exquisite sensitivity seen in chronic lymphocytic leukaemia (CLL), where nearly all patients have increased BCL2 expression. The modest responses of Venetoclax in FL are somewhat perplexing and might be explained by a combination of heterogeneity in BCL2 expression, reliance on other anti-apoptotic BCL2 members like myeloid cell leukaemia-1 (MCL-1) or BCL-XL and the importance of parallel genetic and microenvironmental mechanisms that render the established tumour less dependent on BCL2 expression. In contrast, EZH2 inhibition with the oral selective small molecule, Tazemetostat, recently United States Food and Drug Administration (FDA) approved for the treatment of R/R FL, can be considered a paradigm for a precision approach in
FL. In a Phase II trial in patients with R/R FL, a markedly higher overall response rate (ORR) of 69% was observed in the EZH2-mutated patients compared to 35% in EZH2-wild type patients. Loss-of-function mutations in the epigenetic regulators, CREBBP and KMT2D, are inherently more challenging to reverse. However, promising pre-clinical data evaluating histone deacetylase 3 (HDAC3) inhibition in CREBBP-mutated lymphoma and lysine demethylase 5 (KDM5) inhibition in KMT2D-mutated lymphoma lend support to the potential of inhibiting the opposing or antagonistic partners of these disrupted epigenetic regulators as a means of restoring the balance of global histone acetylation or methylation respectively. Taking a contrasting approach, a dependence of CREBBP-mutated lymphoma cells on residual EP300 function represents a synthetic lethality relationship that has been shown to be exploited in vitro via dual CREBBP/EP300 inhibition. As such, the coming years will define if any of these approaches demonstrate suitable efficacy in early clinical studies to be translated into the clinical arena.

For decades, it has been evident that FL is a lymphoma subtype that is exquisitely sensitive to immunotherapeutic strategies from monoclonal antibodies to HSCT. We are now entering into a new era of cancer immunotherapies that have direct impact on the TME as well as the tumours itself. Lenalidomide, an oral immunomodulatory drug, enhances anti-tumour immunity through a plethora of actions including enhanced immune synapse formation with T and NK cells, antibody-dependent cellular cytotoxicity (ADCC), skew towards specific T-cell subsets like CTLs and increase in anti-inflammatory cytokines. The combination of rituximab and lenalidomide in the first-line setting of FL showed similar efficacy compared to standard rituximab chemotherapy and is approved in the R/R FL setting. Immune checkpoint inhibitors (ICI) that target PD-1 (e.g. nivolumab and pembrolizumab) are highly effective in classical Hodgkin lymphoma (cHL), which exhibit frequent copy-number gains of programmed death-ligand 1 (PD-L1) and PD-L2 on chromosome 9p24. Despite the rich infiltrate of PD1+ T cells in the FL TME, there was limited activity of nivolumab monotherapy in a Phase II study in R/R FL, with only a 4% ORR and no correlation with PD-L1 or PD-L1 expression.

In solid tumours, a range of predictive biomarkers of response and resistance including PD-L1 expression, mutational burden and degree of intratumoral T-cell infiltration, are recognised; however, the utility of these biomarkers has yet to be validated in lymphomas and requires further exploration. Notably, by examining the peripheral immune profiles in ICI-treated patients with cHL, Cader et al. observed that better responses were linked to patients with more clonally diverse CD4+ T cells and an increased abundance of activated NK cells. Clearly, the mechanism of action of ICIs is more complex than simply blocking the immune checkpoint axis, and may differ in different tumour types. From the macrophage axis, the CD47-SIRPα interaction provides a macrophage immune checkpoint pathway, and HuSF9-G4, an antibody targeting CD47, overcomes the inhibitory effects on macrophage phagocytosis, enhancing ADCP. In combination with rituximab, HuSF9-G4 induced an ORR of 71% and a complete response of 43% in seven patients with R/R FL. Another emerging immunotherapeutic strategy is CD20-CD3 bispecific antibodies (BSAbs) that dual target the tumour B cells and engages T cells. An example, Mosunztuzumab, has shown very promising activity in R/R FL and there are now numerous agents in this class being explored in early phase studies. Chimeric antigen receptor (CAR)-T cells that have been genetically engineered to recognise specific tumour-associated antigens, such as CD19, together with the incorporation of co-stimulatory T-cell signalling domains, such as CD28 and/or 4-1BB are being evaluated across the breadth of B-cell lymphomas. CD19 CAR-T therapy is already approved for R/R transformed FL, and there are initial signs of impressive response rates in patients with R/R FL and longer follow-up will inform as to the durability of these responses. Notwithstanding, there are important toxicities associated with both CAR-T and BS Abs therapies to consider, including cytokine release syndrome and neurological toxicities. It is without doubt that immunotherapies will become one of the central components of treatment strategies, particularly in the R/R setting.

At present, the majority of both tumour- and immune-directed therapies do not work in every patient with FL. Detailed studies to ascertain the predictive molecular and cellular determinants that drive responses and resistance are needed to improve patient selection and sequencing of these therapies. We must also be mindful of the interconnected ‘yin and yang’ nature of both the tumour-intrinsic and immune-driven characteristics of FL tumours, and the likely need to synergistically target both of these vulnerabilities to ultimately present the best precision approach.

**Conclusion**

Despite the now well-defined landscape of genomic lesions in FL, key questions remain, including the characteristics and residing niche of the CPC, the nature of the interaction between different epigenetic lesions, and the extent to which HT is pre-determined or predictable. The TME plays a crucial role in the development and evolution of the disease through a network of tumour–TME crosstalk of considerable complexity, with links to the underlying genetics increasingly appreciated. Genomic insights are giving rise to novel therapeutic avenues, although the optimal timing and combination of these approaches will require further investigation. In contrast, the emergence of immune-targeted therapies has largely outpaced our understanding of immune microenvironmental dynamics in FL to date, and thus, a higher resolution portrait of the composition and spatial heterogeneity of the TME may help identify beneficiaries of the various agents, as well as uncover novel targets and combinations.
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