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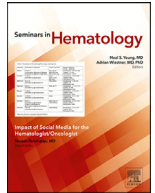
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DLBCL arising from indolent lymphomas: How are they different?

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ABSTRACT

Transformation to diffuse large B-cell lymphoma (DLBCL) is a recognized, but unpredictable, clinical inflection point in the natural history of indolent lymphomas. Large retrospective studies highlight a wide variability in the incidence of transformation across the indolent lymphomas and the adverse outcomes associated with transformed lymphomas. Opportunities to dissect the biology of transformed indolent lymphomas have arisen with evolving technologies and unique tissue collections enabling a growing appreciation, particularly, of their genetic basis, how they relate to the preceding indolent lymphomas and the comparative biology with *de novo* DLBCL. This review summarizes our current understanding of both the clinical and biological aspects of transformed lymphomas and the outstanding questions that remain.

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Introduction

Indolent B-cell lymphomas account for approximately 40–50% of non-Hodgkin's lymphomas (NHL) in the Western world and, by their nature, classically follow a gradually progressive clinical course. However, a well-recognized theme shared amongst indolent B-cell lymphomas is the variable propensity for transformation to an aggressive lymphoma. Risk of transformation occurs with any of the pathologically diverse indolent lymphoma entities including follicular lymphoma (FL), small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL), marginal zone lymphoma (MZL) and Waldenström's macroglobulinemia (WM), with FL and CLL the most extensively studied to date.

Across the indolent lymphomas, transformation should be suspected in patients with sudden clinical deterioration including new onset of B-symptoms, rapidly enlarging lymphadenopathy or laboratory investigations showing rising LDH and/or hypercalcemia. In most instances, SLL/CLL transforms to diffuse large B-cell lymphoma (DLBCL), in this setting referred to as Richter's syndrome (RS), or more rarely to Hodgkin's lymphoma. Similar to SLL/CLL,

MZL and WM commonly transform to DLBCL. There are a number of histologic entities that FL can transform to, the most common being DLBCL (referred to as tFL), but also to double or triple-hit high-grade lymphoma (High-grade B-cell lymphoma, not otherwise specified). Rarely, transformation of indolent lymphomas to atypical histologies has been documented, including lymphoblastic lymphoma, plasmablastic lymphoma and myeloid-associated malignancies such as histiocytic sarcoma and dendritic cell tumors [1–4]. Histological confirmation should be the gold standard approach to diagnosing transformation in these indolent entities (referred to as histological transformation, HT). Whilst PET-based imaging can guide the biopsy approach, it is insufficient on its own for diagnosing transformation in either FL or CLL [5–7]. Expert hematopathology opinion is important for confirming histological diagnoses of transformation and equally important in differentiating mimics that can lead to misdiagnoses, for example, CLL with proliferation centers from transformation [8].

Incidence of transformation

There is considerable variability in the reported incidence of indolent lymphoma transformation with limited epidemiological data that are mostly drawn from retrospective single institution or pooled multicenter studies, particularly for the rarer indolent B-NHL entities. Therefore, the true risk of transformation is likely

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still inadequately captured. Summarized in Table 1 are selected series detailing transformation incidence.

For FL, in the pre-rituximab era, there was a notion that HT was inevitable and an expected outcome for all FL patients in time, with a number of studies demonstrating a continuous risk of transformation of 2% to 3% per year for the first 15 years after diagnosis [9–11]. In this current therapeutic era, most studies suggest a reduction in risk to <10% at 10 years (ie <1% risk per year), indicating that contemporary FL treatment could be influencing the risk of transformation [12–16]. In the pan-European multi-institution ARISTOTLE study, rituximab usage appeared to reduce but not completely mitigate the risk of transformation [15]. In this study, the 10-year cumulative risk of HT as a first event in FL was 8% in those not receiving rituximab treatment compared with 5.9% in those that received rituximab at induction and, even lower for those patients who had rituximab both at induction and as maintenance (3.6%). Similarly, a recent population-based study from BC Cancer demonstrated that the induction chemotherapy backbone could impact later transformation risk. A significant proportion of patients who experienced early progression after induction treatment with rituximab-bendamustine had transformed FL (76%), compared to a lower risk in those treated with R-CHOP (40%), although the overall incidence of transformation in both groups were comparable. This interesting observation allows one hypothesize that certain therapies might have the potential to biologically select out or enrich for clones with a propensity to transform [17]. Across several studies, certain clinical parameters are demonstrable risk factors for transformation including baseline elevated LDH, high-risk FLIPI and poor performance status [11–16].

Across studies in CLL, the incidence of transformation is estimated to range from 2% to 10% with an estimated 0.5% per year incidence in untreated CLL patients and a 1% annual incidence in CLL patients that have received prior CLL-directed therapy [18,19]. Recent analysis of CLL patients treated with chemotherapy or chemoimmunotherapy across German CLL study group trials identified an incidence of transformation of just above 3% [20]. While initial reports of novel targeted agent therapies in CLL suggested a high transformation rate [21], this has reassuringly not been observed in subsequent analyses and frontline targeted agent trials. In recent studies of CLL patients treated with novel targeted agent therapies, a rate of transformation of approximately 4% has been observed, similar to historical data [22,23].

Within MZL, there are 3 subtypes classified on the basis of the site of lymphoma involvement, comprising extranodal MZL (EMZL) of mucosa-associated lymphoid tissue (MALT), nodal MZL (NMZL) and splenic MZL (SMZL) [24]. Across all MZL subtypes, the risk of transformation is estimated at 1% per year, although there is a broad range of estimates across studies. In a retrospective series by Conconi and colleagues, HT was observed in 5% of SMZLs, 4% of EMZLs and 3% of NMZLs suggesting a similar incidence across all MZL subtypes [25]. More recent series highlight a much heightened risk in SMZL and NMZL patients, compared to EMZL [26,27]. Risk factors including failure to achieve complete response (CR) after initial treatment, raised LDH and greater than four nodal sites at the time of MZL diagnoses have been significantly associated with increased risk of HT [25–27].

For WM, a rarer lymphoma subtype, the data on incidence of HT is relatively sparse but collectively suggests that the rate is much lower than the other indolent lymphoma entities. Castillo and colleagues reported a 5-, 10-, 15-yr cumulative incidence of HT at 1%, 2.4% and 3.8% in a cohort that comprised both treatment-naïve and treatment-experienced patients [28]. Extra-nodal involvement, particularly central nervous system (CNS) involvement, frequently occurs at transformation (incidence ranging between 13% and 18%) [29].

Table 1
Summary of studies on transformation incidence.

Histology	Study cohort	No. of patients	Treatment type	Type of transformed lymphoma	Median follow up	Risk/frequency
tFL	Montoto et al., [9]	325	Mixed, no rituximab-treated	DLBCL	15 y	28% at 10 y
tFL	Link et al., [11]	631	Mixed including rituximab-treated	Aggressive lymphoma	5 y	10.7% at 5 y
tFL	Wagner-Johnston et al., [12]	2652	Mixed including rituximab-treated	Aggressive lymphoma	6.8 y	10.9% at 6 y
tFL	Sarkozy et al., [13]	1017	Mixed including rituximab-treated	DLBCL/BL	6.1 y	4% at 6 y
tFL	Alonso-Alvarez et al., [14]	1734	Mixed including rituximab-treated	DLBCL/BL/FL3b/BCLU	6.2 y	8% at 10 y
tFL	Federico et al., [15]	8116	Mixed including rituximab-treated	Aggressive lymphoma	7.25 y	7.7% at 10 y
tFL	Casulo et al., [16]	441	Mixed including rituximab-treated	Aggressive lymphoma	6 y	7% at 5 y
RS	Byrd et al., [22]	533	Novel agent treated CLL	RS	3.4 y	3.8 v 4.9 in acla/ibru treated
RS	Parikh et al., [19]	1641	Newly diagnosed CLL	RS	4 y	2-3%
RS	Al-Sawaf et al., [20]	2975	Chemotherapy trial treated CLL	RS	4.4 y	3%
RS	Tsimberidou et al., [96]	3986		RS		3.7%
RS	Seymour et al., [23]	389		RS		3.2 v 3.6 in VenR vs BR
tMZL	Aldenuccio et al., [26]	453	VenR or BR treated CLL	DLBCL	4.9 y	6.6% at 5 y 8.4% at 10 y
tMZL	Conconi et al., [25]	340	Mixed	DLBCL	4.8 y	5% at 10 y
tMZL	Kalashnikov et al., [27]	1454	Mixed	DLBCL	5.4 y	4.7% at 10 y
tWM	Castillo et al., [28]	1466	Mixed	DLBCL	7 y	1% at 5 y 2.4% at 10 y
tWM	Zanwar et al., [83]	1147	Mixed	DLBCL	9.1 y	2.4% 5 y 4.7% at 10 y

Across these indolent lymphoma entities, there is increasing use of novel therapies including immunotherapies, in earlier lines of therapies and it is unclear if the risk of transformation is or can be further reduced with such approaches.

Biology of indolent B-cell lymphoma transformation

Across indolent histologies, evolution towards transformation is thought to occur as a result of the acquisition of additional genetic and epigenetic alterations and complex microenvironment interactions that facilitate immune evasion. While next-generation sequencing and immuno-profiling have transformed the modern understanding of DLBCL and indolent lymphomas and their associated microenvironments, the comprehensive study of transformed lymphomas has proved more challenging. This is due in part to a need to pair samples (both preceding indolent and subsequent transformed entities) for comprehensive comparative analyses, technical challenges such as sample admixture and lymphoma purity, and the difficulty of achieving *in vivo* modelling that faithfully recapitulates the biology and natural history of transformed lymphomas. Despite these challenges, scientific study of transformation has illuminated many aspects of biology in recent years.

FL transformation

The genomic landscape of tFL

Genome-wide studies based on high-resolution single nucleotide polymorphism array, whole exome (WES) and whole genome sequencing (WGS) have provided a detailed description of the genetic events acquired with transformation. Overall, the tFL genome carries a significantly higher mutational burden (including copy number aberrations (CNA), coding and non-coding mutations and structural rearrangements) than the preceding FL specimen pointing to an increased genomic complexity acquired or selected in the transformed genome [30] that appears independent of the time interval between the 2 longitudinal samples [31]. Among genetic lesions evolving and enriched specifically in tFL, deletions of chromosome 1p, 6q, gains in chr 2, *FAS* mutations, biallelic alterations of *TP53* (15%–30%) and *CDKN2A/2B* (20%–30%) genes, alterations affecting immune surveillance genes (*CD58* and *B2M* and *HLA-I*, 20%–25%) and regulators of NF- κ B signaling have consistently been described as contributors to the transformation process [32–35]. Genetic lesions affecting *MYC* through translocations, CNA or point mutations are commonly found in 25% of transformed cases, compared to indolent FL where *MYC* translocations are rarely detected. All studies consistently demonstrate dysregulation of *MYC* as a feature of HT [31]. The majority of paired FL and tFL tumors share the *BCL2* translocation in line with the early origin of the translocation during early B cell rearrangement in the bone marrow. *BCL6* translocations tend to be more abundant in FL samples undergoing HT (25% vs 14%), as measured in recent WGS studies [36]. Another distinctive feature acquired by tFL cells is the higher incidence of aberrant somatic hypermutation in recognized target genes (reaching > 85% of cases) and distributed in coding and non-coding sequences, including *PIM1*, *MYC*, *CIITA* and *SOCS1* [33]. Overall, there is no compelling evidence for a unifying genetic mechanism promoting transformation, as a subset of tFL abnormalities are also shared with indolent FL, and it is currently not known whether some of the mutations present at diagnosis may influence HT.

Clonal evolution and early seeding

Seminal studies conducted on sequential tumor biopsies taken at diagnosis and at histological transformation or relapses have

been instrumental in reconstructing temporal evolutionary pathways underlying FL tumor evolution and defining the likely sequence of acquisition of genetic events over time. Earlier studies assessing the *BCL2* translocation and immunoglobulin rearrangements have proven that most tFL cases are clonally related to the FL at diagnosis, carrying the same *BCL2/IGH* breakpoint signature and the same Ig VDJ clonotypic rearrangement [35,37,38]. This is in contrast with other indolent lymphomas like CLL where as high as 20% of RS transformations are unrelated to CLL clone at diagnosis [24]. Characterization of the subclonal architecture and dynamics of the mutations between sequential FL and tFL biopsies through genetic analysis clearly established that tFLs do not evolve purely from a direct evolution of the dominant FL clone at diagnosis. Rather, it emerges from a "divergent" or "branched" evolution from a common precursor clone, or CPC, carrying a subset of early precursor mutations shared by the FL and tFL tumor, that later clonally diverges by distinct additional alterations that are private to the dominant FL or t-FL clone [32–34]. By contrast, the evolutionary trajectory that accompanies CLL to RS is predominantly linear wherein the majority of CLL genetic alterations are maintained in RS with the acquisition of additional lesions at the time of transformation [39]. Because FL CPCs are considered as the root of every episode of relapse or transformation, a better characterization of the putative population represents a major challenge for the field [40]. Phylogenetic analysis depicting the genetic evolution from FL and tFL confirms that, besides the t [14,18] translocation, early initiation events include mutations in *CREBBP*, *KMT2D* and to a lesser extent *EZH2* histone modifying enzymes as well as acquisition of BCR N-glycosylation sites [32–34,37,41,42]. This is consistent with these alterations being acquired early during FL genesis, likely present in a less evolved CPC clone propagating FL at diagnosis [43] and seeding HT through divergent evolution. To date, the phenotypic, molecular, and spatial attributes of FL CPCs are still unknown, as these cells have never been directly isolated due to the lack of discriminative markers to purify them. Most recently, a unique collection of paired prediagnostic blood and tumor biopsies of FL patients collected from 6 months up to 12 years before diagnosis permitted the mapping of the genomic landscape of FL CPCs using a sensitive deep sequencing approach. This study provides direct evidence for recurrent genetic aberrations preceding FL diagnosis [44], supporting previous inferential studies, and demonstrates that the combination of *BCL2* translocations with *CREBBP* mutations, especially affecting the lysine acetyl transferase domain (KAT) are likely harbored within committed lesions of FL CPCs [45]. Despite the lack of comprehensive clinical follow-up data, particularly HT, in the screening cohorts, it is tempting to speculate that strategies targeting the residual CPCs in FL may help to prevent disease recurrence and perhaps HT. Therapies targeting these early lesions (*CREBBP* and *KMT2D*) have been pre-clinically evaluated [46,47].

Genetic prediction of FL HT

Identifying patients at risk of FL transformation remains an area of unmet need. As HT emerges from the expansion of mutated clones that are very rare or undetectable in diagnostic samples with current detection technologies, there have been attempts to identify predictors of HT based on the inherent genetic features present in the diagnosis sample. In contrast to *de novo* DLBCL where genetic subtypes at diagnoses are predictive of clinical outcome, genetic subtyping in FL remains in its infancy. A recent study using targeted sequencing to genotype > 500 FL patients revealed 3 subtypes: one associated with a high burden of aberrant somatic hypermutation, a second with frequent *STAT6* and *CREBBP* mutations, and a third group enriched for *KMT2D* mutations without the features of the other groups [48]. None of these three subgroups

were predictive of HT, highlighting the potential limitation of panel sequencing in capturing the considerable genetic heterogeneity of FL.

More recently, comprehensive characterization using WGS of 423 diagnostic biopsies from FL (who later transformed or not), transformed FL and *de novo* DLBCL cases led to the discovery of two genetic FL subtypes: constrained FL (cFL) vs DLBCL-like FL (dFL) with significantly different risks of transformation. The constrained FL (cFL) cohort was associated with a reduced risk of transformation and genetically harbored a lower mutational burden, less aSHM but an enrichment of *CREBBP* KAT missense mutations and mutations in genes involved in mTORC1 signaling (*RRAGC*, *ATP6V1B2*, *ATP6AP1*). The other group, dFL, had a much higher risk of HT and was associated with more frequent aSHM and a mutational landscape with similarities to DLBCL including *GNA13* mutations, *MYC* translocations and *CREBBP* nonsense mutations. Hereby, for the first time, a machine learning-based genetic classifier demonstrates the potential to predict increased future risk of HT from the initial diagnosis [36]. *CREBBP* mutation status alone appeared a strong dFL to cFL discriminator and validation of these subgroups in additional cohorts will define its clinical applicability.

A further limitation to predicting HT from initial diagnosis is the recognized intra-patient heterogeneity observed in spatially-distinct FL-involved sites, for example, between lymph nodes (LN) and bone marrow (BM), as this might contribute to the difficulty in tracking rare mutations associated with transformation risk at diagnosis [49,50]. Although multi-site sampling could capture this layer of heterogeneity, they are rarely performed in clinical practice. Dynamic monitoring of circulating tumor DNA (ctDNA) with sensitive analytical methods, such as CAPP-seq or PhasED-seq, have proven to be very informative in various lymphoma subtypes for outcome prediction [51,52]. The use of a minimally invasive approach to detect rare or low-frequency variants predictive of HT at an earlier clinical time point would be particularly advantageous. This was illustrated in a study by Scherer and colleagues [51] showing that in a FL patient who transformed 9 months later, ctDNA profiling at the time of diagnosis identified unique genetic mutations specific to the transformed biopsy that were not detected within the initial tissue biopsy, indicating that the transformed clone was already pre-existing at diagnosis and can be captured by ctDNA monitoring. It also suggests that ctDNA provides a better overall representation of the genetic alterations across multiple anatomic tumor niches. With a single case and an *a priori* knowledge of the genomic landscape of the tFL biopsy, further work is required to fully understand the utility of ctDNA tools in HT prediction.

FL functional heterogeneity and link with HT

Single-cell transcriptomic analyses of FL LN biopsies have revealed a high degree of inter-patient tumor heterogeneity including the functional plasticity of FL B cells within a given patient and the distinct tumor-infiltrating T cell composition [53–55]. Prior studies showed FL cells to diverge from the dynamically regulated GC transcriptional programs that operate during the immune response [53], and are instead engaged in a dynamic continuum of states ranging from GC-like (associated with cell cycle programs) to memory-like (associated with quiescence and dissemination programs) states that are conserved across patients [56]. Similar B cell phenotypic diversity was observed in a larger study of almost 150 FL patients studied by mass cytometry. Interestingly, in this study the memory B cell-like subtype of FL was associated with increased risk of transformation [57]. To date, single-cell RNA-seq studies have been conducted in small population-based cohorts and with limited clinical follow-up. The impact of FL func-

tional plasticity and of the identified TME subtypes in promoting HT remains therefore largely unexplored. Ongoing investigations using integrative scRNA-seq and scDNA-seq of paired FL-tFL samples have begun to document the phenotypic and genotypic co-evolution of malignant B cells during transformation and the shifting of the TME landscape, with an 'exhausted' CD8 T cell signature emerging during transformation [58]. The implementation of high-throughput single cell analysis on larger FL cohorts followed for subsequent HT will become a major asset in identifying rare initiating CPCs likely to be at the origin of HT as well as identifying biomarkers of future HT that already pre-exist at diagnosis.

Richter's transformation of CLL

The genomic landscape of RS

In RS, retrospective studies have identified numerous genetic and biologic risk factors for transformation, including unmutated-IgHV, *NOTCH1* mutation, *TP53* loss, BCR stereotype subset #8, and complex karyotype [39,60–63]. In early genetic studies of RS, sequencing of the immunoglobulin gene locus demonstrated that RS could arise as clonally related to the antecedent CLL, thus true transformed CLL, or as a clonally unrelated large cell lymphoma [64], with the latter displaying a lower prevalence of *TP53* disruption and longer overall survival, not dissimilar to DLBCL [60]. Recent studies into RS genetics have now confirmed the presence of clonal-related and clonal-unrelated RS [65]. Thus, although commonly accepted nomenclature will define large cell lymphoma arising in a background of CLL as RS, a subset of these cases do not represent transformed CLL and instead represent a separate B cell malignancy. Recent RS investigation has highlighted increasing mutation rate, numerous copy number alterations, and genome disorder (including chromothripsis, kataegis, complex rearrangements and whole genome doubling) in the clonal evolution of CLL to RS [65–67]. These studies have confirmed high rates of *TP53* and *NOTCH1* alterations in CLL giving rise to RS along with frequent *CDKN2A/B* loss at transformation, and highlighted increasing *MYC* dysregulation, either by *MGA* loss or *MYC* amplification, as a central pathway as CLL evolves towards RS. At transformation, new genetic alterations were detected across core pathways of epigenetics, inflammation, immune evasion, DNA damage response, NF- κ B signaling and cell cycle with novel recurrent driver alterations not highlighted by prior CLL sequencing studies including *IRF2BP2*, *EZH2*, *CCND3*, *B2M*, and *DNMT3A* [65]. Through study of mutational signatures, RS displayed mutational signatures that reflected prior therapies, reactive oxygen species, DNA mismatch repair, and even a novel mutational process (SBS-RT), in addition to signatures detected across CLL [65–67].

Genetic diversity was observed within RS, with subtypes emerging after non-negative matrix factorization clustering that were further supported by transcriptome analysis. This included 3 *TP53*-altered subtypes with high fraction of the genome altered due to abundant CNA and two subtypes with intact *TP53* and improved overall prognosis, namely *NOTCH1*-Tri12 RS and *SF3B1*-*EGR2* mutated RS. Among the *TP53*-altered subgroups was WGD-RS, which contained the majority of transformed M-CLL cases [65].

CLL functional heterogeneity and link to RS

Analysis of paired RS and CLL transcriptomes, encompassing both bulk and single-cell approaches, has highlighted a core group of transcriptional pathways altered in transformation, including *MYC* signaling, cell cycle, DNA damage and oxidative phosphorylation [65,66], the latter of which was demonstrated functionally [66]. While RS clinically seems to retain some BCR-dependence [68], a comparative decrease in BCR signaling com-

pared to CLL cells was identified across recently examined transcriptional datasets [65,66,69]. Cell state changes in transformation were accompanied by dramatic changes in chromatin, as assessed by ATAC-seq and H3K27 acetylation, converging on impacted pathways of oxidative phosphorylation, cell cycle, mTOR, MYC and interferon regulatory factor (IRF) signaling, which had not been previously identified in large-scale CLL epigenome analysis [66]. In comparison of CLL and RS methylation patterns, increased global hypomethylation was identified as a hallmark of RS, with differentially methylated regions in RS mapping to NOTCH and Wnt signaling and immune evasion pathways [70].

Evolution and early seeding

As sequencing technologies advance, the understanding of clonal evolution and timing of RS evolution has been examined. Ultra-deep UMI-based sequencing recently highlighted early RS clonal genetic changes at a very low frequency in a subset of 9 RS patients, even as many as 19 years prior to RS [66]. This finding was subsequently verified by single-cell DNA sequencing of these RS clonal mutations and further supported by copy number alterations and expression states in single-cell transcriptomic data from the same patients [66]. In data presented at ASH, xenografting early CLL samples from these individuals resulted in a similar genetic transformation in immunodeficient mice [71]. Study of ctDNA samples from transformed patients also identified a few patients with genetic changes of RS present in plasma, and absent from circulating CLL disease, prior to clinical diagnosis by ultra-low pass whole genome sequencing [59]. In parallel, novel technologies are increasingly facilitating analysis of the complex immune microenvironment of transformation of RS and allowing comparison to that of CLL. Through immunohistochemistry approaches, RS has been shown to have an increase in FOXP3+ T regulatory cells and CD163+ macrophages as well as PD-1+ RS cells. In the upcoming years, it is likely that we will better understand the complex immune evolution that facilitates and constrains transformation.

Modeling of RS

In parallel to new human biologic investigations, murine models of disease have evolved in recent years, with generation of both new RS xenograft and genetically engineered mouse models [72]. A CRISPR-Cas9 editing strategy has now successfully modelled CLL development and natural evolution to DLBCL RS, with selection of *Trp53*, *Mga*, and *Chd2* mutations in the RS phase, and shared genetic and functional similarity to human RS [73]. The murine RS further implicates the MYC/PI3K axis in transformation, linked to reduced PTEN, and provides an experimental roadmap for mouse engineering to model clonal evolution to transformation. In studies utilizing the Eμ-TCL1 CLL murine model, the functional roles of *Trp53* and *Atm* loss [70] CDKN2A/B loss [74], and PRMT5 [75] and MYC overexpression [76] have been highlighted in promoting transformation. The Eμ-TCL1 model, which represents an aggressive CLL biology, displays altered apoptotic priming and reduced venetoclax susceptibility compared to CLL patient-derived cells, which may impact identification of therapeutic vulnerabilities of RS that arises on this backbone [77]. In parallel, xenograft-based approaches are increasingly being developed to test and identify new potential therapeutic dependencies of RS and thus serving as valuable preclinical models [78,79].

Transformation of MZL and WM

Unlike FL and CLL, there have been limited studies evaluating the biology of transformed MZL and WM. Transformed MZL is usually characterized by DLBCL of nongerminal centre B-cell

type cell-of-origin, sometimes double expressor (dual expression of MYC and BCL2). A recent study by Grau and colleagues [80] investigated a cohort of 41 transformed SMZL patients, where samples were evaluable from both the indolent and transformed time points in half the cohort and the remainder just from the diagnostic or transformation time points. Compared to indolent SMZL, transformation was associated with higher genomic complexity, *TNFAIP3* and *TP53* alterations, and 9p21 (*CDKN2A/2B*) losses. The paired analyses demonstrated that nearly all transformed biopsies were clonally related to the preceding indolent SMZL, with divergent evolution from a common altered precursor cell. Notably, *KLF2* mutations and complex karyotypes were associated with a shorter OS after transformation. A case series in transformed NMZLs identified deletions of chr20q12 as a frequent genetic event [81].

In terms of studies focused on transformed WM biology, Jimenez and colleagues [82] did not find a common recurrent pattern of mutations at WM diagnoses or transformation, although only 4 patients were evaluated in this series, with all cases being *MYD88* mutated. Several studies demonstrate that *MYD88*-wild type WM confers a higher risk of transformation [83,84], in one study, a 5-fold higher risk of transformation compared to L265P *MYD88*-mutated WM. *MYD88*-wild type WM have a characteristically different genetic profile compared to *MYD88*-mutated WM [85], including higher incidence of mutations in genes involved with NF-κB signaling (*TBL1XR1*, *MALT1*, *BCL10*, *NFKBIZ*), epigenomic dysregulation (*KMT2D*, *KMT2C*, and *KDM6A*) or those that impair DNA damage repair (*TP53* and *ATM*). Many of these genetic profiles are reminiscent of mutation profiles characteristic of the recently identified molecular subgroups in DLBCL [86–88].

Beyond these studies, it is unknown if there are specific non-genetic drivers of transformation for these indolent NHL entities. There is a strong predisposition and association of chronic antigenic stimulation from viral, bacterial and/or autoantigens contributing to both MZL and WM pathogenesis, for example *Helicobacter pylori* and Hepatitis C infection in MZLs [24,89]. However, it is unclear the impact of infections on the risk and evolution towards transformation.

While these collective studies have advanced knowledge of drivers, risk factors and origins of transformation and highlighted some common themes, such as the shared likely role of *TP53*-aberrations, epigenetic alterations, *CDKN2A/B* loss and immune evasion pathways, further work is certainly needed to define transformation-triggering events as well as identify mechanisms by which transformed clones evade immune recognition as they evolve from indolent lymphoma. In addition, mechanistic work is needed to better elucidate therapeutic vulnerabilities that can be exploited by novel therapeutics to improve outcomes in transformed lymphomas. The promise of rapidly evolving single-cell technologies, spatial profiling and better model systems provides promise for major scientific advances in transformation in the upcoming decade.

Biology of transformed lymphomas compared to *de novo* DLBCL

The availability of large well-annotated DLBCL datasets has now facilitated comparative analysis with transformed datasets [86,87]. Through non-negative matrix factor clustering of genetic alterations across 97 RS and 304 DLBCL cases, the majority of RS was found to cluster separately from DLBCL, with closest similarity to *TP53*-aberrant Cluster 2 DLBCL [65]. In contrast, clonally unrelated RS cases clustered with DLBCL molecular subtypes. In parallel, Broséus et al. [69], found that both methylation and transcriptional signatures distinguished RS and DLBCL, with RS displaying a pattern of distinct global hypomethylation, a CLL epigenetic imprint, and comparative upregulation in NOTCH1, PI3K and BCR pathways. The identified methylation and transcriptomic signatures

could separate clonally related RS and clonally unrelated RS, similar to genetic study. Based on this work, a subset of ABC-like DLBCL samples similar to RS was identified, enriched for unclassified and N1 classes of DLBCL, and associated with CD5⁺ staining and poor prognosis. The recently reported high prevalence of *NOTCH1* and *IRF2BP2* mutations [66] in RS further hints of potential shared genetic features between RS and the N1-DLBCL subtype. Overall, these studies highlight that clonally related RS, or transformed CLL, is a distinct entity with unique molecular features that associate with adverse prognosis. On the other hand, clonally unrelated RS, which represents expansion of an independent malignant B cell clone, shares biologic features with *de novo* DLBCL.

De novo DLBCL has conventionally been dichotomized into binary groups based on cell-of-origin: germinal center (GCB) and activated B-cell (ABC) type. Using GEP-based classification demonstrates that about 80% of tFL are GCB-type as might be expected, however, a not insignificant proportion of tFLs are classified as ABC-type. These ABC-type tFLs typically originate from BCL2-negative FLs [90], altogether suggesting that tFL might be on the biological spectrum between FL and *de novo* DLBCL. The intriguing genetic overlap between DLBCL genetic subtype EZB/C3 and indolent FL led several groups propose the hypothesis that both entities could arise from the same common precursor and *de novo* DLBCL might represent an occult indolent FL that has transformed [91,92]. Of interest, in the most recent molecular taxonomy of DLBCL, C1/BN2 and NOTCH2 molecular DLBCL clusters are postulated to be of extra-follicular/marginal zone origin with genetic profiles closely related to MZLs [93,94]. As genetic profiling increases, the molecular features of transformed lymphoma will be increasingly better understood and integrated within the framework of their relatedness to *de novo* DLBCL molecular subtypes.

Outcomes

The clinical trajectory associated with transformation to an aggressive lymphoma is characterized by markedly worse outcomes compared to its indolent counterpart. Transformed lymphomas remain a major clinical challenge for a number of reasons including: the absence of consensus therapeutic approaches to managing these patients, patients with transformation are commonly excluded from clinical trials and the therapeutic options are considerably narrower compared to options for the relapsed indolent entity.

Retrospective studies have long exhibited dismal outcomes for patients with CLL transformed to RS, with median overall survival (OS) of less than 1 year despite modern therapeutic advances [18]. Recently, new studies into the genetics and biology of RS have highlighted how RS largely differs from DLBCL, while demonstrating that the subset of clonally unrelated RS (14%–20% of RS) is biologically similar to DLBCL [65,69], which explains the relatively improved prognosis observed in this clonally unrelated RS subset.

Historically, the prognosis for FL patients who develop transformation was very poor with a reported median survival post-transformation of just 1 to 2 years [9,10]. Whilst contemporary 5yr post-transformation survival has improved, in the region of 40% to 50% [13,15], FL transformation remains a major cause of morbidity and mortality in FL patients, accounting for up to a third of lymphoma-related deaths [95].

The timing of transformation events following the indolent histology influences outcome. Early FL transformation, particularly within the first 2 years from the indolent event or prior to therapy, is associated with inferior outcomes compared to later transformation events [11,15]. Similarly, early transformation within 1 year of diagnosis of MZL was associated with 4-year OS of 43% compared with later HT with a 4-year OS of 81% [26].

Whilst comparative analyses are limited, the outcomes of transformed lymphomas do appear markedly worse than *de novo* DLBCL, perhaps attributed to a number of factors including different biologies and the influence of prior therapies in selecting out more aggressive disease clones.

Outstanding questions

At present, many questions remain about the biology of transformation, including the identity of the cell of origin and sequence of cell-intrinsic events, genetic and epigenetic, that cooperate to result in transformation. Furthermore, discoveries of founding transformation events that precede diagnosis raise questions about what causes emergence of transformation, and whether it is malignant cell intrinsic or due to an alteration in the surrounding immune microenvironment. A better understanding of whether the heterogeneity of the niche (eg, lymph node vs bone marrow) influences the selection of the precursor transformation clone could be informative for developing strategies to prevent or intercept transformation events. From an evolutionary perspective, there is little understanding whether this emergence is deterministic or stochastic in nature or perhaps both – are some patients with indolent lymphomas predestined to transform from the outset or is it a series of adverse events that culminate into eventual transformation. As the therapeutic landscape continues to evolve, additional questions emerge surrounding the impact of newer therapy approaches in promoting, or constraining, clonal evolution to transformation. While these open areas of discovery remain similar across indolent lymphomas, and while common hallmark lesions of transformation are seen, it remains to be uncovered exactly how transformation biology may be similar or shared across different indolent histologies given the differing cells of origin. Looking forward, future research in the upcoming decade is likely to provide further biological evidence of how transformation differs from DLBCL or indeed, if many presumed *de novo* DLBCLs perhaps arise from an unrecognized occult indolent lymphoma.

Conclusion

In conclusion, histologic transformation of indolent lymphoma to aggressive lymphoma, most commonly DLBCL, represents a well-recognized event. While work in the past decade has shed light on many of the genetic and biological changes of transformation, future investigations are needed to refine therapy approaches to improve patient outcomes. In addition, this work promises to provide further evidence on how transformation may, or may not, differ from DLBCL.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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