Precision medicine and lymphoma

James A Heward*, Emil A Kumar, Koorosh Korfi, Jessica Okosun and Jude Fitzgibbon

Author Affiliations: Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ, United Kingdom.

*** Corresponding Author:** James Heward, Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ, United Kingdom; Tel: +44 (0)20 7882 8780; Email: j.a.heward@qmul.ac.uk.

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Abstract

Purpose of review

The treatment of the germinal center (GC) lymphomas, diffuse large B-cell (DLBCL) and follicular lymphoma (FL), has changed little beyond the introduction of immuno-chemotherapies. However, there exists a substantial group of patients within both diseases, for which improvements in care will involve appropriate tailoring of treatment.

Recent findings

DLBCL consists of two major subtypes with striking differences in their clinical outcomes paralleling their underlying genetic heterogeneity. Recent studies have seen advances in the stratification of GC lymphomas, through comprehensive profiling of 1001 DLBCLs alongside refinements in the identification of high-risk FL patients using m7-FLIPI and 23G models. A new wave of novel therapeutic agents is now undergoing clinical trials for GC lymphomas, with BCR and EZH2 inhibitors demonstrating preferential benefit in subgroups of patients. The emergence of cell-free DNA has raised the possibility of dynamic disease monitoring to potentially mitigate the complexity of spatial and temporal heterogeneity, whilst predicting tumor evolution in real-time.

Summary

Altogether knowledge of the genomic landscape of GC lymphomas is offering welcome opportunities in patient risk stratification and therapeutics. The challenge ahead is to establish how best to combine upfront or dynamic prognostication with precision therapies, whilst retaining practicality in clinical trials and the real-world setting.

Key words

Follicular lymphoma, diffuse large B-cell lymphoma, precision medicine, targeted therapies.

Introduction

The treatment of the two most common forms of germinal center (GC) non-Hodgkin lymphomas (NHL), diffuse large B-cell (DLBCL) and Follicular lymphoma (FL), was revolutionized by the addition of rituximab to chemotherapeutic regimens (R-CHOP). Over half of DLBCL patients are now curable by R-CHOP, whilst despite FL being considered an incurable disease, the majority of FL patients live for upwards of 15 years. Beyond these successes, prognosis remains poor for a third of relapsed-refractory DLBCL patients, and for a quarter of FL patients who are prone to early relapse and/or transformation to a more aggressive lymphoma (tFL). There is renewed optimism that the molecular characterization of these diseases will allow for the prediction of high-risk patients and the tailoring of treatments based on a patient's molecular profile. In this review, we will focus on the prognostic strategies to identify high-risk patients and the progress now being made in developing targeted therapies for these GC lymphomas.

The molecular landscape of GC lymphomas.

The introduction of next generation sequencing (NGS) sparked a dramatic increase in our understanding of the molecular events that drive GC lymphomas. There are now well over 200 recurring gene mutations described, although only a minority occur in greater than 5-10% of patients. The significance of gene translocations targeting *MYC*, *BCL2*, and *BCL6* are all well established, as is the recognition of widespread disruption of the epigenome, predominantly driven by somatic mutations within *KMT2D*, *CREBBP*, *EZH2* and linker histones [1-13]. DLBCL displays a greater degree of genetic heterogeneity than FL, and can be readily divided into at least two major

subtypes based on the “cell of origin” (COO): GC B cell (GCB)-like and activated B cell (ABC)-like DLBCL [14,15]. The ABC-DLBCL subtype has the poorer prognosis by far (40% 3-year overall survival (OS)) and is typified by constitutive NF-κB activation and signaling driven by somatic mutations in the B cell receptor (BCR) and NF-κB pathway genes (*CARD11*, *CD79B*, *MYD88*, and *TNFAIP3*) [16-20], whereas GCB-DLBCL has a better prognosis (80% 3-year OS) and more closely resembles FL. While there are specific gene mutations that are enriched in each group, the overall landscape is complex and contrary to earlier expectations, BCR and NF-κB pathways mutations also arise in GCB-DLBCL and FL, albeit at a lower frequency [2,11].

Identifying high-risk DLBCL patients

Overall, progress has been sluggish in discriminating high- from low-risk patients within the distinct COO entities with the exception of the “high-grade B cell lymphomas, with *MYC* and *BCL2* and/or *BCL6* rearrangements”, also known as double- and triple-hit lymphomas, and the “double-expressors” that highly express both BCL2 and MYC in the absence of rearrangements [21,22]. Both groups demonstrate an aggressive phenotype associated with poor outcomes and therefore are a high priority for clinical intervention. Nevertheless, we are likely to see an acceleration in improved prognostic models and refined patient subsets in the foreseeable future, as our understanding of the landscape of coding aberrations in DLBCL nears completion. A monumental study by Reddy and colleagues has set the pace with an extensive analysis of 1001 newly diagnosed DLBCL patients, integrating mutational profiling, gene expression analysis and clinical information, which has offered novel insights into combinatorial factors influencing outcome for near

enough the first time. While many of these observations will require validation, (for example *KLH14* mutations being predictive of high-risk ABC-DLBCL*,* and *EZH2* mutations predicting low-risk GCB-DLBCL), the size and depth of the data allowed the authors to devise an encouraging prognostic multivariate genomic risk model [11]. Such large collaborative studies will undoubtedly be the benchmark for future studies and provides a framework for developing prospective risk-adapted strategies for DLBCL.

A new era in FL stratification?

Molecular stratification of FL has lagged behind DLBCL, with the COO classification first described nearly 20 years ago [14]. Traditional attempts at risk stratification in the era of immunochemotherapy have been based on clinical parameters such as the Follicular Lymphoma International Prognostic Index (FLIPI) scores, the FLIPI and FLIPI-2, but are for the most part fairly ineffective tools for directing management decisions [23,24]. While molecular prognostication has long been opined as having more potential, it is only of late that we are seeing inroads into the development of tools capable of navigating the clinical heterogeneity within FL and able to predict high-risk patients. The earliest studies by the Lymphoma/Leukemia Molecular Profiling Project stratified FL based on the patterns of two gene signatures associated with T cell and myeloid cell infiltration [25]. However, we may well have turned the corner with the recent development of the m7-FLIPI, a clinico-genetic risk model that incorporates the clinical features of the FLIPI and the mutational status of 7 genes (*EP300*, *FOXO1 CREBBP*, *CARD11*, *MEF2B*, *ARID1A*, *EZH2*) [26], and an alternative prognostic strategy based on the expression of 23 genes (23G model)

from both the tumor and microenvironment [27]. It is likely that these models will continue to evolve as they are impacted by treatment changes, such as the move towards chemotherapy-free interventions, and we need to lay the groundwork for these changes by developing the infrastructure to enable head-to-head comparisons and reach a consensus on the level of discrimination needed to guide treatment, especially for an indolent disease such as FL where a high prognostic accuracy is necessary.

Upfront prognostication verses dynamic disease monitoring

All of the aforementioned clinical and molecular prognostic strategies are based on upfront testing and prognostication. However, sampling of a single site at diagnosis fails to account for the temporal [1,28-31] and spatial heterogeneity [32] observed in GC lymphomas. The assessment of cell-free DNA (cfDNA) from plasma samples offers the possibility of more effectively sampling the heterogeneity in GC lymphomas, and dynamically monitoring changes in prognostic markers and actionable targets in a minimally invasive manner. Whilst it has not been widely applied so far, early studies have suggested a number of possible applications including the ability to classify the COO in DLBCL patients and to predict transformation of FL [33-35]. In an era where there is a headlong shift away from excision biopsies, it is likely that non-invasive cfDNA-based diagnostics and prognostics will have a significant future role within GC lymphomas.

Ibrutinib preferentially benefits ABC-DLBCL patients

Running in tandem with efforts at exploiting genetic data for defining risk, access to genetic profiles are also pointing the way towards novel therapies. Ibrutinib, which inhibits the BTK enzyme responsible for propagating pro-survival signals from the BCR, which is constitutively active in ABC-DLBCLs (Figure 1) [36], has proved to be revolutionary in the treatment of chronic lymphocytic leukemia [37], and accumulating data suggest that it may be efficacious in poor-risk, BCR/NF-κB-dependent ABC-DLBCL patients. In a Phase I/II trial of 80 patients, single agent ibrutinib was well tolerated and had an overall response rate (ORR) of 37% in ABC-DLBCL, compared to only 5% in GCB-DLBCL patients [36], and it is encouraging that all non-GCB patients achieved complete remission in a Phase I study examining ibrutinib in combination with rituximab-based chemotherapy [38]. Following the success of these trials, ibrutinib is being tested in several phase III trials for ABC-DLBCL, that includes combination with R-CHOP for newly diagnosed ABC-DLBCL patients (NCT01855750), and in relapsed/refractory ABC-DLBCL patients undergoing stem cell transplant NCT02443077).

In FL, single agent ibrutinib was shown to only have modest activity [39], despite evidence that BCR signaling has a role in FL pathogenesis [40-44]. Combination studies with rituximab have improved on the single-agent outcomes [45] but it seems sensible that upfront selection should be a feature of future trials, with evidence that *MYD88* mutations are associated with a positive outcome in DLBCL [36], while *CARD11* mutations appear to influence ibrutinib resistance in FL [39].

EZH2 inhibitors may selectively benefit *EZH2*-mutant patients

The recent recognition of gain-of-function mutations in *EZH2* demonstrates the speed by which discoveries relating to the molecular pathogenesis of a disease are translatable into potential clinical benefit. EZH2 is a histone methyltransferase that acts as the catalytic subunit of the polycomb repressor complex 2 and catalyzes repressive mono-, di- and tri-methylation of the histone 3 lysine 27 (H3K27) residue. In GC lymphomas, its important role in regulating the normal GC reaction [46] is subverted by heterozygous mutations most commonly altering tyrosine 646 (Y646) within the catalytic SET domain [47]. These gain-of-function mutations alter the catalytic activity of the mutant EZH2 enzyme so that it preferentially catalyzes the conversion of H3K27me1 into the strongly repressive H3K27me2/3 marks, whilst the wild-type protein continues to deposit H3K27me1 [48].

Selective EZH2 inhibitors have been developed by Epizyme (EPZ6438, tazemetostat [49]), GlaxoSmithKline (GSK126 [50]) and Constellation Pharmaceuticals (CPI-1205 [51]), with pre-clinical data indicating that these compounds are generally more active in mutant cell lines and able to re-activate genes repressed or silenced by mutant EZH2 [49-51]. Phase I/II clinical trials have now been launched for all of these compounds to examine their efficacy for NHL with recent interim data suggesting that tazemetostat is efficacious for *EZH2*-mutated FL patients (92% ORR in mutant vs 26% in wild-type) and to a lesser extent in DLBCL (29% ORR in mutant vs 15% in wild-type) [52]. Altogether, these results are encouraging and highlight the attractions of precision medicine although given that *EZH2* mutations have been provisionally linked with better outcomes in GC lymphomas [26,53], EZH2 inhibitors will need to

demonstrate significant benefit over the current standard of care to justify their use as a targeted therapy in this sub-group of patients.

Revisiting mTOR inhibitors in the light of recent developments

It is also possible that armed with new insights into the genetic basis of GC lymphomas, we can revisit historical clinical studies and explore opportunities for precision treatments. Inhibition of the mTOR (mammalian Target Of Rapamycin) pathway, previously evaluated in FL, serves as a notable example with two phase II trials of second generation mTOR inhibitors, temsirolimus and everolimus, demonstrating promising results in multiply relapsed cohorts (ORR 53.8% [54] and ORR 38% [55], respectively). These trials however were performed before the discovery of unique mutations of the *RRAGC* and the V-ATPase *ATP6V1B2* and *ATP6AP1* genes in around 30% of FL patients, enforcing mTORC1 activation [4,,56,57]. With the spectrum of different molecular lesions that we are now aware of in GC lymphomas, there may be opportunities to re-examine previously trialed agents, to determine whether events such as mTOR pathway mutations are predictive of response. Equally this also reaffirms the need and value of rigorously collecting biopsy material as part of clinical trials for later correlative studies.

New strategies to reverse the early loss of CREBBP in precursor cells

Given the frequency and recognition of *CREBBP* mutations as one of the earliest events in GC lymphomas [8-11, 58-60], and their widespread role in promoting GC-lymphoma development [4,58-62], the successful targeting of these lesions would offer an exciting new therapy with the potential to eradicate disease-propagating

cells. Indeed, phylogenetic analysis has revealed that overt FL, and subsequent relapses of the disease, are likely to develop from long-lived pre-malignant cells known as common progenitor cells (CPCs), which are believed to be t(14;18)-positive and typically contain mutations within the histone regulatory genes *CREBBP* and *KMT2D* [1,3-5,63-66]. There is a persuasive argument to suppose that inhibition of the histone deacetylase (HDAC) enzymes that normally oppose CREBBP by removing acetylation marks could mitigate the deep-rooted loss of histone acetyltransferases CREBBP and EP300. Overall, whilst pan-HDAC inhibitors have demonstrated efficacy in GC lymphomas [67-69], the occurrence of significant toxicities, alongside the absence of a biomarker and an unclear mechanism of action, have limited progress beyond phase II trials. Their fortunes may well change in the future, with recent studies exploring the relationship between *CREBBP* mutations and HDAC isoforms indicating that HDAC3 opposes the activity of CREBBP at enhancers and hyper-represses these enhancers following the loss of *CREBBP,* resulting in an increased dependency on HDAC3 for survival [58]. Targeting the HDAC3 isoform thus offers a potential therapeutic strategy with the promise of re-activating CREBBP-regulated genes whilst minimizing toxicity associated with pan-HDAC inhibitors.

Conclusion

Our understanding of the biology of the GC lymphomas has increased dramatically with the introduction of NGS, and our ability to parallel clinical and molecular heterogeneity. There are signs that we are on the edge of a new precision era for GC lymphomas. Several new upfront prognostic strategies have been published for FL, and the seminal study by Reddy et al examining 1001 DLBCL patients has highlighted

the need for similar large scale, multi-institutional collaborative studies across all lymphomas, including FL. Furthermore, cfDNA assessment have raised the possibility of undertaking dynamic disease monitoring accounting for spatial and temporal heterogeneity and forecasting the trajectory of the tumor's evolution in real-time.

The identification of therapeutics targeted towards specific molecular lesions has also seen significant advances in GC lymphomas, including the observations of selective benefit for ibrutinib in ABC-DLBCL and tazemetostat in EZH2 mutant FL. The challenge now is to establish how best to combine upfront prognostication with dynamic monitoring, whilst retaining practicality, to ensure that patients receive the best possible treatment.

Key points

- Specific sub-groups of high-risk DLBCL patients within the COO entities are now recognized.
- Notable advances made in identifying high-risk FL with the development of novel molecular prognostication tools.
- Ibrutinib and tazemetostat are showing preferential benefit in specific populations of GC lymphoma patients.

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Conflicts of interest

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Figure legend

Figure 1. Actionable mutations in GC lymphomas. Diagram indicating key recurring and potentially targetable mutations. Highlighted therapeutics include ibrutinib for ABC-DLBCL [36] and EZH2 inhibitors for *EZH2*-mutant FL patients [49-52].

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