Towards genomic-based prognostication and precision therapy for diffuse large B-cell lymphoma

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The development of precision medicine for diffuse large B-cell lymphoma (DLBCL) is complicated by its great clinical and molecular heterogeneity. Twenty years ago, two distinct cell-of-origin (COO) subtypes were identified based on distinct gene expression profiles that reflect different stages of B-cell development, i.e., germin center B-cell-like (GC) and activated B-cell-like (ABC) DLBCL. The ongoing revolution in genomics has shed light on the genetic landscape of these subtypes. While ABC DLBCL is characterized by frequent mutations in nuclear factor-κB (NF-κB) pathway modifiers, such as CREBBP, KMT2D, and EZH2, GC DLBCL patients have a more favorable prognosis and a better clinical response to standard R-CHOP immunochemotherapy than those with ABC DLBCL. Nevertheless, the clinical outcome is heterogeneous in both subtypes with an unfavorable outcome in a substantial proportion of patients, also in individuals with GCB DLBCL. Currently, the impact of somatic mutations and other genomic aberrations on the clinical outcome and therapy response is still not completely understood and there is a high need for targeted precision therapy.

In this issue of Haematologia, Bolen et al. report the frequency and prognostic impact of genomic alterations in 499 untreated DLBCL patients enrolled in the GOYA study (clinicaltrials.gov identifier: NCT01287741). Using a well-validated targeted next-generation sequencing (NGS) approach, the authors demonstrate that only alterations of the BCL2 gene, translocations as well as single nucleotide variants (SNV), were significantly associated with reduced progression-free survival (PFS), independent of other molecular or clinical factors, including COO or the International Prognostic Index (IPI). In line with previous studies, BCL2 was the most frequently mutated gene in GC B DLBCL and there was a strong correlation between a BCL2 translocation and presence of BCL2 mutations, which presumably are a consequence of aberrant somatic hypermutation. While BCL2 translocations and SNV were highly enriched in the GC subtype, BCL2 gene amplifications were more frequently detected in the ABC subtype. The findings suggest that a select subset of DLBCL patients may likely benefit from pharmacological inhibition of BCL2, as an addition to standard immunochemotherapy. The highly selective BCL2 inhibitor venetoclax strongly improved PFS in chronic lymphocytic leukemia (CLL) patients and is currently under investigation for DLBCL. Preliminary findings from the phase II CAVALI trial indicate that addition of venetoclax to R-CHOP therapy can improve therapy outcome in patients with BCL2-positive lymphomas. The largest benefit was observed for BCL2-translocated and double-hit lymphomas, suggesting that the addition of venetoclax may be of particular value for these therapy-resistant and aggressive subgroups.

Although the other genetic aberrations identified by Bolen et al. did not offer prognostic value over well-established risk factors, such as COO and IPI, they nevertheless provide biological insights that are crucial for the design of precision therapies (Figure 1). For example, in line with previous studies, the authors demonstrate that a large fraction of GCB DLBCL (16%) carry gain-of-function mutations in the transcriptional repressor EZH2, implying that these patients could benefit from treatment with EZH2 inhibitors, such as tazemetostat. Indeed, preliminary results from a phase II clinical trial in 165 DLBCL patients carrying activating EZH2 mutations. Likewise, the presence of inactivating mutations in the transcriptional activators KMT2D, CREBBP, EP300 and MEF2B implies that a subset of patients could benefit from treatment with histone deacetylase (HDAC) inhibitors. A phase II clinical trial evaluating the safety and therapeutic benefits of pan-HDAC inhibitor panobinostat showed durable responses in 11 out of 40 DLBCL patients, an effect that may be associated with mutations in transcriptional activator MEF2B. Activating mutations in MEF2B are present in approximately 10% of DLBCL and FL patients and contribute to lymphomagenesis by enhancing the transcription of the BCL6 oncogene.

Two independent landmark studies published in 2018 by Schmitz et al. and Chapuy et al. have assessed the occurrence of somatic mutations, copy number alterations (CNA) and structural variants (SV) in a large cohort of DLBCL patients. Building on these observations, Wright et al. recently developed the ‘LymphGen algorithm’, which calculates the probability that a given tumor belongs to one of seven subtypes, based on its genetic features. The identified genetic subtypes are associated with differential responses to immunochemotherapy and, moreover, provide ample opportunity for the design of novel targeted (combination) treatments. In accordance with these and other previous studies, the study of Bolen et al. reports that B-cell receptor (BCR) complex component CD79B and Toll-like receptor (TLR) adaptor protein MYD88 are among the most frequently mutated genes in ABC DLBCL. These mutations define the ‘MCD’ cluster of Wright et al.
comprising tumors characterized by a high prevalence and co-occurrence of MYD88 and CD79B mutations that are almost exclusively classified as ABC DLBCL and have an unfavorable prognosis. Intriguingly, this genetic subgroup is linked to primary extranodal lymphomas, including lymphomas arising in the central nervous system (CNS), ocular vitreo-retina and testis, all considered ‘immune-privileged’ sites as they tolerate allografts and permit only selective entrance of immune cells. Importantly, Wilson et al. established that ABC DLBCL harboring mutations in CD79B, particularly those with concurrent MYD88 mutations, were highly responsive to treatment with ibrutinib, a selective Bruton’s tyrosine kinase (BTK) inhibitor. These observations suggest that (extranodal) lymphomas belonging to the MCD subtype, such as primary testicular or vitreoretinal lymphoma, might also benefit from treatment with BCR pathway inhibitors.

In conclusion, the study by Bolen et al. fully confirms the previously described genetic heterogeneity and complexity of DLBCL. As a consequence of this complexity, well-established prognostic classifiers, such as COO and IPI, can only partially account for the differential responses to R-CHOP (and related) immunochemotherapy. The identification of alterations of the BCL2 gene as the only genetic abnormalities significantly associated with reduced PFS points towards targeting BCL2 as a rational addition to standard immunochemotherapy. Although not of (independent) prognostic value in the context of standard immunochemotherapy, genetic abnormalities defining potentially druggable targets/pathways were

Figure 1. Prevalence of mutations in genes involved in various functional pathways in diffuse large B-cell lymphoma (DLBCL). Most frequently mutated genes as identified by Bolen et al. in a cohort of 482 DLBCL patients arranged according to the affected pathways. NF-κB: nuclear factor kappa B; TLR: Toll-like receptor; BCR: B-cell receptor.
The article by van Paridon et al. published in this issue of Haematologica on results of thrombin generation (TG) in cardiovascular disease and mortality, stemming from the Gutenberg Health Study, provides an opportunity to comment on TG as a global laboratory procedure to investigate hypo- and hyper-coagulability.

TG as a laboratory test was developed in the early 1950s by McFarlane and Biggs and was based on the activation of coagulation in whole blood or plasma by triggers such as tissue factor or cephaline and calcium chloride. The amount of thrombin generated over time was titrated by sampling the mixture at different time points into a fibrinogen solution and the resultant clotting times interpolated from a dose-response calibration curve to derive thrombin concentrations. Years later, Hemker et al. made substantial changes. The fibrinogen solution was replaced by a chromogenic substrate specific for thrombin, test plasma was defibrinated prior to testing and computer software was developed to derive the parameters stemming from the TG curve. These changes made

identified in a large proportion of the tumors across the distinct COO subtypes (Figure 1). Applying the publicly available LymphGen algorithm on the GOYA dataset could help classify patients into well-defined molecularly and clinically distinct subgroups. These newly characterized subsets can identify patients with an unfavorable prognosis and may guide the development of new precision therapies for these aggressive lymphomas.

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Thrombin generation: a global coagulation procedure to investigate hypo- and hyper-coagulability
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