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

Marthe Minderman[1,2] and Steven T. Pals[1,2]

[1]Department of Pathology, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam and [2]Lymphoma and Myeloma Center Amsterdam – LYMMCARE, Amsterdam, the Netherlands.

E-mail: STEVEN T. PALS - s.t.pals@amsterdamumc.nl

doi:10.3324/haematol.2020.255448

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., germinal center B-cell-like (GCB) and activated B-cell-like (ABC) DLBCL.[1] The ongoing revolution in genomics has since shed light on the genetic landscape of these subtypes. Whereas ABC DLBCL is characterized by frequent mutations in nuclear factor-κB (NF-κB) pathway drivers and intermediates, including *TNFAIP3*, *MYD88*, *CARD11* and *CD79B*, as well as loss of cell cycle regulators *CDKN2A* and *CDKN2B*, GCB DLBCL carry frequent mutations in epigenetic modifiers, such as *CREBBP*, *KMT2D* and *EZH2*.[2,3] GCB 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 heterogenous in both subtypes with an unfavorable outcome in a substantial proportion of patients, also in individuals with GCB DLBCL.[1,4] 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 *Haematologica*, 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*).[5] 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 GCB 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.[3,6] While *BCL2* translocations and SNV were highly enriched in the GCB 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.[7,8] Preliminary findings from the phase II CAVALLI 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 and follicular lymphoma (FL) patients demonstrate that tazemetostat achieves favorable clinical responses in patients carrying activating *EZH2* mutations.[9] 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*.[10] 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.[11]

Two independent landmark studies published in 2018 by Schmitz *et al.*[12] and Chapuy *et al.*[13] 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.[14] 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.*[5] 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.*,[14]

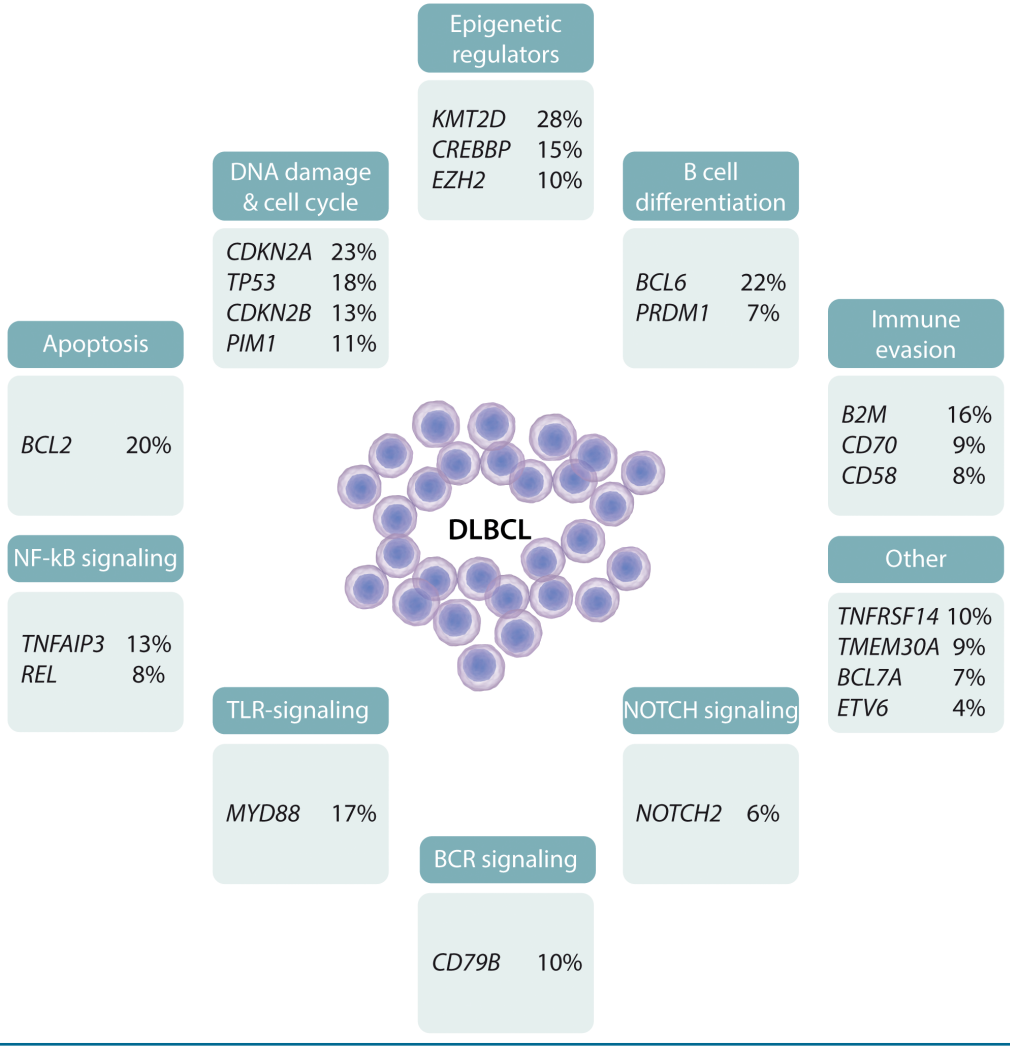

Figure 1. Prevalence of mutations in genes involved in various functional pathways in diffuse large B-cell lymphoma (DLBCL). Most frequently mutated genes as identified by Bolen *et al.*[5] in a cohort of 482 DLBCL patients arranged according to the affected pathways. NF-κB: nuclear factor kappa B; TLR: Toll-like receptor; BCR: B-cell receptor.

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.[15] 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.[16-19] 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.[20] These observations suggest that (extranodal) lymphomas belonging to the MCD subtype could also be targeted by inhibition of BCR signaling. Indeed, a phase Ib study in a panel of 18 primary central nervous system lymhpomas (PCNSL) demonstrated that ibrutinib monotherapy reduced tumor mass in 94% of patients.[21] Additionally, a second phase I clinical trial showed clinical responses to ibrutinib in 10 out of 13 PCNSL patients, including five complete responses.[22] Collectively, these suggest that patients with other primary 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.*[5] 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

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.

Funding
Funded by a grant from Lymph&Co.

References

1. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403(6769):503-511.
2. Pasqualucci L, Trifonov V, Fabbri G, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet. 2011;43(9):830-837.
3. Lohr JG, Stojanov P, Lawrence MS, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci U S A. 2012;109(10):3879-3884.
4. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(25):1937-1947.
5. Bolen CR, Klanova M, Trneny M, et al. Prognostic impact of somatic mutations in diffuse large B-cell lymphoma and relationship to cell-of-origin: data from the phase III GOYA study. Haematologica. 2020;105(9):2298-2307.
6. Schuetz JM, Johnson NA, Morin RD, et al. BCL2 mutations in diffuse large B-cell lymphoma. Leukemia. 2012;26(6):1383-1390.
7. Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. N Engl J Med. 2018;378(12):1107-1120.
8. Morschhauser F, Feugier P, Flinn IW, et al. Venetoclax plus rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) improves outcomes in BCL2-positive first-line diffuse large B-cell lymphoma (DLBCL): first safety, efficacy and biomarker analyses from the phase II CAVALLI study. Blood. 2018;132 (Supplement 1):782.
9. Morschhauser F, Salles G, McKay P, et al. Interim Report from a Phase 2 Multicenter Study of Tazemetostat, an EZH2 Inhibitor: Clinical Activity and Favorable Safety in Patients with Relapsed or Refractory B-Cell Non-Hodgkin Lymphoma. Clin Lymphoma Myeloma Leuk. 2017;17(Suppl 2):S380-381.
10. Assouline SE, Nielsen TH, Yu S, et al. Phase 2 study of panobinostat with or without rituximab in relapsed diffuse large B-cell lymphoma. Blood. 2016;128(2):185-194.
11. Ying CY, Dominguez-Sola D, Fabi M, et al. MEF2B mutations lead to deregulated expression of the oncogene BCL6 in diffuse large B cell lymphoma. Nat Immunol. 2013;14(10):1084-1092.
12. Schmitz R, Wright GW, Huang DW, et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. N Engl J Med. 2018;378 (15):1396-1407.
13. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med. 2018;24(5):679-690.
14. Wright GW, Huang DW, Phelan JD, et al. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. Cancer Cell. 2020;37 (4):551-568.e14.
15. Vermaat JS, Somers SF, de Wreede LC, et al. MYD88 mutations identify a molecular subgroup of diffuse large B-cell lymphoma with an unfavorable prognosis. Haematologica. 2020;105(2):424-434.
16. Kraan W, Horlings HM, van Keimpema M, et al. High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. Blood Cancer J. 2013;3(9):e139.
17. Yonese I, Takase H, Yoshimori M, et al. CD79B mutations in primary vitreoretinal lymphoma: Diagnostic and prognostic potential. Eur J Haematol. 2019;102(2):191-196.
18. Schrader AMR, Jansen PM, Willemze R, et al. High prevalence of MYD88 and CD79B mutations in intravascular large B-cell lymphoma. Blood. 2018;131(18):2086-2089.
19. Kraan W, van Keimpema M, Horlings HM, et al. High prevalence of oncogenic MYD88 and CD79B mutations in primary testicular diffuse large B-cell lymphoma. Leukemia. 2014;28(3):719-720.
20. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015;21(8):922-926.
21. Lionakis MS, Dunleavy K, Roschewski M, et al. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma. Cancer Cell. 2017;31(6):833-843.e5.
22. Grommes C, Pastore A, Palaskas N, et al. Ibrutinib Unmasks Critical Role of Bruton Tyrosine Kinase in Primary CNS Lymphoma. Cancer Discov. 2017;7(9):1018-1029.

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

Armando Tripodi

IRCCS Ca' Granda Maggiore Hospital Foundation, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milano, Italy

E-mail: ARMANDO TRIPODI - armando.tripodi@unimi.it

doi:10.3324/haematol.2020.253047

The article by van Paridon *et al.*[1] 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[2] 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.[3-5] 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