efforts have largely focused on mobilizing HSPCs, followed by ex vivo clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 editing. The most common strategy involved downregulating BCL11A expression using CRISPR-Cas9 editing with a single guide RNA (sgRNA) that targets tissue-specific enhancers that regulate the expression of BCL11A in erythroid cells.[7]

In this study, the investigators aimed to increase the applicability and efficacy of this strategy by targeting different combinations of these regulatory elements using CRISPR-Cas9 editing and by simplifying the delivery of sgRNA and Cas9 enzyme through the use of an adenoviral vector in vivo. First, they compared the relative efficiency of editing the HBG-200 locus vs editing the *BCL11A* enhancer or the HBG-115 locus in reactivating HbF. Having identified sgRNAs for the HBG-200 locus that efficiently increased HbF expression, they used multiplex sgRNA that targeted different combinations of the *BCL11A* enhancer or the HBG-200 or HBG-115 loci in $CD34^+$ cells to systematically compare the relative merits of each approach. This demonstrated that combining *BCL11A* enhancer editing with either of the HBG loci resulted in an increased frequency of HbF^+ cells and total γ globin levels.

Because the ultimate goal of such technology is to establish long-lived progenitors, the investigators could show that this gene editing approach did not lead to a reduction in the clonogenic potential of edited HSPCs. It should also be noted that they did not see any clear evidence of an increase in off-target DNA damage, which is a recurring concern in this field. Furthermore, human HSPCs doubly edited at the *BCL11A* enhancer and HBG-115 were able to engraft in vivo and stably reconstitute human hematopoiesis in a recipient mouse.

Current gene therapy approaches require harvesting cells from patients, followed by ex vivo genome editing. The genetically modified cells are then transplanted back into patients using conditioning chemotherapy. Therefore, an in vivo CRISPR-Cas9 editing strategy has significant benefits. In this study, the team further optimized a nonintegrating adenoviral vector that can now contain 2 sgRNA. This was able to successfully edit primary $CD34^+$ HSPCS from thalassemic patients and resulted in an improvement in erythroid differentiation. In a murine model engrafted with human $CD34^+$ thalassemic cells, they were able to successfully reactivate HbF production by mobilizing stem cells into the peripheral circulation, followed by in vivo transduction with the dual sgRNA–containing adenoviral vector (see figure). Engraftment of human $CD45^+$ thalassemic cells in secondary transplantation assays suggests that editing of true hematopoietic stem cells was achieved.

Of course, a number of obstacles remain before this work can be translated into the clinic and impact patients. The investigators note that a particular caveat to their strategy is the immunogenicity of using adenoviral vectors, although this is likely to be ameliorated by rapidly developing viral vector technology. Nonetheless, the investigators should be congratulated on developing a potentially important strategy in improving HbF production in patients with severe defective hemoglobin phenotypes.

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REFERENCES

1. Psatha N, Georgakopoulou A, Li C, et al. Enhanced HbF reactivation by multiplex mutagenesis of thalassemic $CD34^+$cells in vitro and in vivo. *Blood*. 138(17): 1540-1553.
2. Taher AT, Musallam KM, Cappellini MD. β-Thalassemias. *N Engl J Med*. 2021;384(8): 727-743.
3. Martyn GE, Wienert B, Yang L, et al. Natural regulatory mutations elevate the fetal globin gene via disruption of BCL11A or ZBTB7A binding. *Nat Genet*. 2018;50(4): 498-503.
4. Masuda T, Wang X, Maeda M, et al. Transcription factors LRF and BCL11A independently repress expression of fetal hemoglobin. *Science*. 2016;351(6270): 285-289.
5. Bauer DE, Kamran SC, Lessard S, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science*. 2013;342(6155):253-257.
6. Canver MC, Smith EC, Sher F, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature*. 2015; 527(7577):192-197.
7. Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *N Engl J Med*. 2021;384(3):252-260.

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LYMPHOID NEOPLASIA

Comment on Chiodin et al, page 1570

Sugar-modified B-cell receptors in DLBCL

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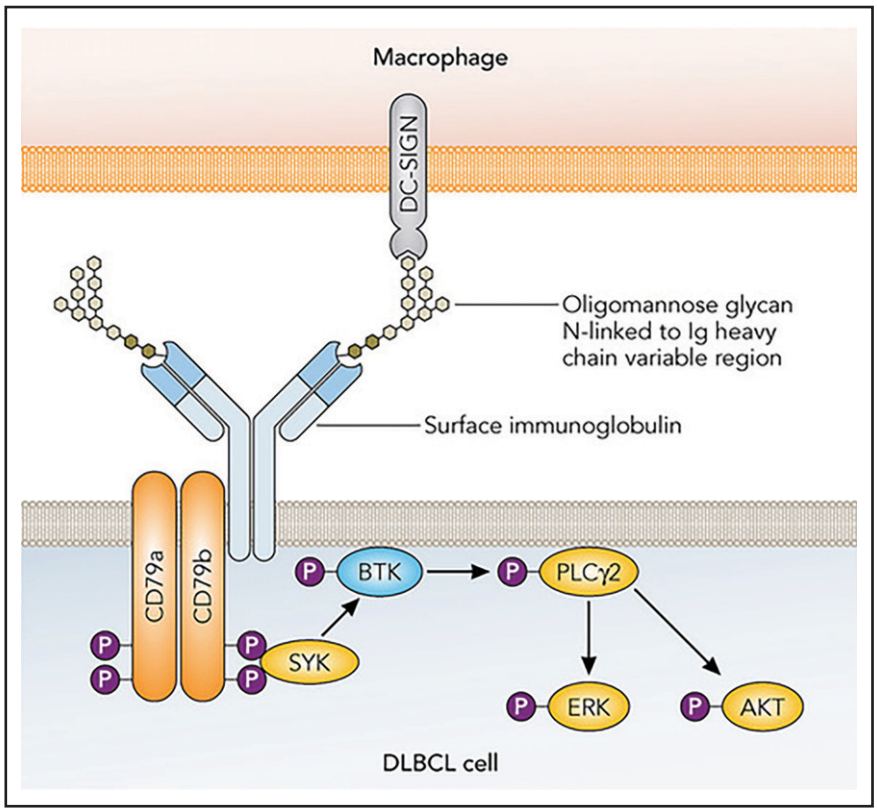
In this issue of *Blood*, Chiodin et al[1] show that diffuse large B-cell lymphomas (DLBCLs) of a particular subgroup frequently acquire *N*-glycosylation sites in their B-cell receptor (BCR) genes and carry oligomannose-type glycans that promote BCR signaling, most likely by binding to lectins in the lymphoma microenvironment.

Most human B-cell lymphomas express a BCR.[2] This expression is not trivial, as many lymphomas have chromosomal translocations that destroy immunoglobulin loci. Moreover, most lymphomas derive from germinal center B (GCB) cells that have undergone somatic hypermutation, which can result in destructive mutations in IGV region genes. Thus, immunoglobulin locus–involving translocations are tolerated only when the nonexpressed immunoglobulin alleles are targeted, and destructive somatic mutations are stringently counterselected in B-cell lymphomas and their precursor cells.[2] Therefore, even malignant B cells are still dependent on BCR expression.[2] For several types of B-cell lymphomas, not only the presence of a BCR and the tonic BCR signaling deriving from it are essential for the lymphoma cells, but the BCRs of these lymphomas recognize autoantigens that provide sustained triggering of the BCR.[3] In some instances, foreign antigens may



Model of lectin-mediated BCR signaling in DLBCL. Somatic mutations in the IGV region genes of DLBCL (mostly in the complementarity-determining regions) generate motifs for the addition of *N*-linked glycans. The BCRs carry unusual oligomannose glycans. These can be bound by DC-SIGN and perhaps additional lectins that are expressed by macrophages in the lymphoma microenvironment. Binding of DC-SIGN to the modified BCRs causes signaling that involves phosphorylation of SYK and factors farther downstream, finally leading to activation of ERK and AKT, which have prosurvival effects on the lymphoma cells. Modified from Strout et al.[10] Professional illustration by Patrick Lane, ScEYEnce Studios.

also provide a trigger for the lymphoma (and/or its precursor cell) BCR.[3]

Nearly 20 years ago, Stevenson and colleagues recognized a unique form of BCR triggering in lymphomas: most follicular lymphomas have acquired somatic mutations in their rearranged IGV genes that create *N*-glycosylation sites (ie, the amino acid motif asparagine-X-serine/threonine, where X is any amino acid except proline).[4] Follicular lymphomas indeed have unusual oligomannose-type glycans on these sites.[5] Such glycans can bind to lectins (sugar-binding molecules). There is now evidence that the lectin DC-SIGN on macrophages and dendritic cells in the microenvironment of follicular lymphomas causes BCR signaling in the lymphoma cells, thereby contributing to lymphoma cell survival and, perhaps, proliferation.[5] This phenomenon is lymphoma specific, as normal B cells very rarely have such modifications.[4]

It was recognized in the first description of acquired *N*-glycosylation sites (AGSs) in lymphomas that a fraction of DLBCLs also had IGV gene mutations creating AGSs.[4] However, it was not clear whether *N*-glycosylation of the IGV domains was linked to particular subsets of DLBCL, and whether they had functional consequences. DLBCL is a heterogenous type of lymphoma. In an early subdivision based on gene expression patterns, DLBCLs were subdivided into activated B-cell like-DLBCL (ABC-DLBCL), GCB-DLBCL, and a third group of unclassified cases.[6] More recently, DLBCLs have been grouped based on their pattern of frequently mutated genes.[7-9]

Chiodin et al studied the occurrence of AGSs in more than 300 cases of DLBCL. AGSs were much more frequent in GCB-DLBCL than in ABC-DLBCL (60% and 13%, respectively), and the sites were mainly located in the antigen-binding areas of the V domains, which are the complementarity-determining regions. In the genetically defined subgroups of DLBCL, cases with AGSs in the antigen-binding sites were highly enriched in the EZB-type of DLBCL, with 65% of such DLBCLs carrying the mutations. The EZB-type of DLBCL is characterized by *BCL2* translocations and mutations in genes encoding epigenetic regulators.[8] Importantly, further analyses showed that BCRs with AGSs in the antigen-binding sites were consistently mannosylated, and that DC-SIGN bound specifically to these surface immunoglobulins. Furthermore, exposure of primary DLBCL samples or cell lines carrying AGSs caused a moderate BCR signaling, as evidenced by the phosphorylation of SYK, extracellular signal-regulated kinase (ERK), and AKT. Thus, these modified BCRs are competent to signal after DC-SIGN binding (see figure). The sugar modification of the BCR may be of clinical relevance, because when focusing on DLBCL without a *MYC* translocation (which itself has a strong prognostic impact), DLBCL with AGSs in the complementarity determining regions of their BCRs showed a significantly faster clinical progression, although this finding needs validation in an independent cohort .

The findings presented by Chiodin and colleagues have important implications. The observation that the AGSs are highly enriched among EZB-type DLBCLs demonstrates a further similarity of this type of DLBCL to follicular lymphomas. These 2 types of lymphomas share many transcriptomic features and a similar pattern of mutated proto-oncogenes and tumor suppressor genes, indicating that they are closely related.[9] EZB-type DLBCL may sometimes present as a transformation from an (unrecognized) follicular lymphoma. The findings presented herein that both carry AGSs further supports a close relationship and similar pathogenesis of these entities. Earlier, there was a focus on BCR signaling in ABC-DLBCL, because these lymphomas have strong constitutive BCR signaling causing NF-κB activity, termed chronic active signaling.[6] Besides mutations in BCR pathway components, frequent autoreactivity of the BCR of ABC-DLBCLs most likely contributes to chronic active BCR signaling in ABC-DLBCL.[6] Importantly, ABC-DLBCL depend on this activity, because inhibition of this pathway is toxic for these lymphoma cells.[6] In the earlier studies on BCR dependency of DLBCL, GCB-DLBCL was felt to depend much less, if at all, on BCR signaling.[6] The present study by Chiodin and colleagues modifies this picture. BCR signaling is of

relevance for GCB-DLBCL (in particular, EZB-DLBCL), at least during early stages of lymphoma development, when the lymphoma cells or their precursors obtain the AGSs through somatic hypermutation. The signaling is distinct in ABC-DLBCL vs GCB-DLBCL and causes moderate AKT pathway activation in GCB-DLBCL, which is a hallmark of tonic BCR signaling. Considering the known moderate toxic effect of BCR pathway inhibition in GCB-DLBCL, it remains unclear how effective inhibition of, for example, DC-SIGN binding to the AGS of DLBCL would be. Finally, Chiodin et al have broadened the picture of human B-cell lymphomas in which the BCR is involved in lymphoma pathogenesis. Besides several lymphoma types with BCRs specific for autoantigens or foreign antigens,[3] follicular lymphomas, and GCB-DLBCL represent a situation in which the BCRs are modified so that they can receive chronic stimulation by switching from stimulation through the original cognate antigen to a lectin-mediated stimulation of sugar-modified BCR (see figure).

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REFERENCES

1. Chiodin G, Allen JD, Bryant DJ, et al. Insertion of atypical glycans into the tumor antigen-binding site identifies DLBCLs with distinct origin and behavior. *Blood*. 2021;138(17):1570-1582.
2. Küppers R. Mechanisms of B-cell lymphoma pathogenesis. *Nat Rev Cancer*. 2005;5(4):251-262.
3. Thurner L, Hartmann S, Neumann F, et al. Role of specific B-cell receptor antigens in lymphomagenesis. *Front Oncol*. 2020;10:604685.
4. Zhu D, McCarthy H, Ottensmeier CH, Johnson P, Hamblin TJ, Stevenson FK. Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. *Blood*. 2002;99(7):2562-2568.
5. Küppers R, Stevenson FK. Critical influences on the pathogenesis of follicular lymphoma. *Blood*. 2018;131(21):2297-2306.
6. Young RM, Phelan JD, Wilson WH, Staudt LM. Pathogenic B-cell receptor signaling in lymphoid malignancies: new insights to improve treatment. *Immunol Rev*. 2019;291(1):190-213.
7. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes [published correction appears in *Nat Med*. 2018;24(8):1292]. *Nat Med*. 2018;24(5):679-690.
8. 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.
9. 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.
10. Strout MP. Sugar-coated signaling in follicular lymphoma. *Blood*. 2015;126(16):1871-1872.

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Comment on Alameda et al, page 1583

Why is amyloidosis not multiple myeloma?

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In this issue of *Blood*, Alameda et al provide a transcriptomic atlas of successive stages of normal plasma cell development. They show that plasma cells from patients with light-chain amyloidosis (AL) most closely resemble secondary lymphoid organ plasma cells (SLO-PCs), whereas multiple myeloma (MM) cells are closer to peripheral blood plasma cells (PB-PCs) and newborn bone marrow plasma cells (BM-PCs).[1]

AL and MM share a common causal root, namely, the presence of clonal malignant plasma cells in the bone marrow, but the behavior of these cells differ, leading to profoundly different clinical consequences. In the first case, the clonal plasma cells do not accumulate but induce the deposition of light chains in various organs as amyloid fibrils. The deposition of amyloid fibrils is independent of the tumor burden, which is true for all monoclonal gammopathies of clinical significance.[2] In the case of MM, clonal plasma cells accumulate in the bone marrow, leading to anemia and bone destruction, among other harmful effects. So far, attempts to explain why these differences occur have not been very successful. t(11;14) is more common in AL compared with MM (~60% vs 20%). However, it is also seen in plasma cell leukemia, making 3 strikingly different disorders with the same translocation. This important finding prompted the evaluation of venetoclax for the treatment of AL.[3] The same Navarra team and others have recently shown that the genomic landscape of AL is characterized by the same heterogeneity as that of MM. None of the few differences observed in terms of mutations and copy number abnormalities were sufficient to explain the clinical presentation of amyloidosis, suggesting that the explanation may not lie in the biology of the tumor plasma cell.[4,5] A return to physiology of plasma cell development was clearly warranted .

In this issue of *Blood*, Alameda et al performed RNA sequencing of SLO-PCs, PB-PCs, and BM-PCs from healthy adults and observed 13 transcriptional programs (TPs) during the migration and transition of plasma cells through SLO, PB, and BM. This atlas is a wealth of information for researchers exploring the physiology of long-lived immunoglobulin-producing plasma cells, including unprecedented data on the ectoenzyme CD39 as a discriminatory biomarker of newborn BM-PCs.

The origin of monoclonal gammopathies is an old and never resolved debate: is it circulating postgerminal center B cells or long-lived BM-PCs? By comparing these physiological TPs, the authors show that tumor PCs of 46 MM, 37 AL, and 6 monoclonal gammopathies of undetermined significance express signatures from SLO-PCs, PB-PCs, newborn BM-PCs, and long-lived BM-PCs. However, a predominant TP from SLO-PCs was expressed in AL, and from both PB-PCs and newborn BM-PCs in MM (see figure). What about at the cellular level? With Wang's recent