Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

How does BAHD1 find its target genes? Unlike GATA1, BAHD1 does not contain a DNA-binding domain, so it cannot recognize its target genes via specific DNA elements. Instead, it binds to a repressive chromatin modification, namely triple methylation of the twenty-seventh lysine residue of histone subunit H3 (H3K27me3).[5] This epigenetic mark is deposited by the polycomb repressor complex.[6] To investigate the interplay of FBXO11-BADH1 and epigenetic modifiers, the investigators performed a second CRISPR screen in FBXO11 KO cells, this time targeting genes that encode epigenetic modifiers. This screen revealed that inactivation of several genes of the polycomb repressor system partially rescues the erythroid differentiation defect in a manner similar to that seen for BAHD1 inactivation. Furthermore, the authors demonstrated protein-protein interactions between the polycomb factor EZH2 and BAHD1. Collectively, their work provides significant new insight into how the transition from progenitors to terminal erythroid differentiation is regulated (see figure).

As with any original research, new questions arise. Because transcription levels of some genes in FBXO11 KO cells are not fully restored upon BAHD1 suppression, FBXO11 likely targets additional regulators for degradation. Identification and functional analysis of these factors would further increase our understanding of the molecular control of erythropoiesis. Follow-up studies might identify the interplay of FBXO11 and repressive complexes, other than polycomb, that facilitate the transition from progenitor cells to terminally differentiated red cells. Another question raised by the Xu et al study is how FBXO11 is activated, especially because the decline in BAHD1 protein during erythroid maturation does not coincide with rising FBXO11 levels. Future studies are warranted to test the authors' hypothesis that a posttranslational modification of BAHD1 (eg, phosphorylation[7]) enables FBXO11-mediated ubiquitination. The role of BAHD1 in DNA methylation[8] may also be part of BAHD1-mediated repression of erythroid genes; global demethylation of DNA occurs during terminal erythroid differentiation.[9]

Some of the other BAHD1-interacting proteins that are not studied in the article by Xu et al provide additional avenues for further investigation. For instance, the histone methyl transferases EHMT1 and EHMT2 repress fetal hemoglobin in adult red cells.[10] Repurposing the FBXO11-BAHD1 axis to modulate EHMT1 and EHMT2 activity may reactivate fetal hemoglobin expression, thus providing a strategy to ameliorate the symptoms of patients with β-hemoglobinopathies. Finally, if the FBXO11-BAHD1 axis is indeed a toggle to regulate erythroid output in the bone marrow, this may have implications for the clinical course and possibly also the treatment of patients with erythroid differentiation defects such as those associated with myelodysplastic syndromes and polycythemia vera. Recent studies demonstrating the feasibility of targeted protein degradation hold promise for future drug development based on tinkering with the ubiquitin-proteasome system.[2]

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Pleyer et al, page 185

Vaccinations in CLL: implications for COVID-19

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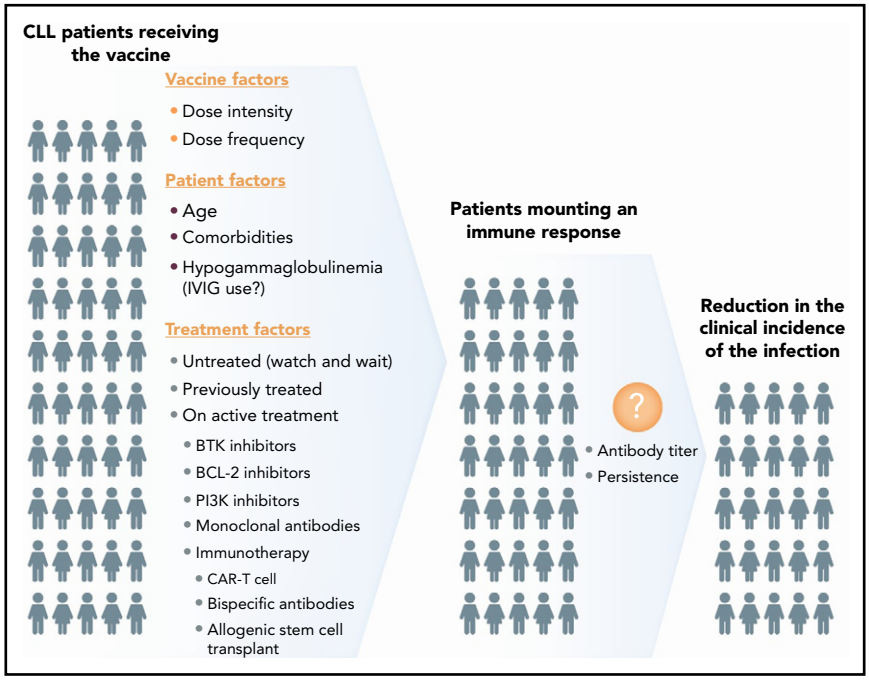
In this issue of *Blood*, Pleyer and colleagues report results from 2 studies assessing differences in the humoral response to 2 different vaccines in patients with chronic lymphocytic leukemia (CLL) on observation or receiving a Bruton tyrosine kinase inhibitor (BTKi).[1] Their findings have immediate clinical implications and call for research preparedness as we eagerly anticipate access to vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the near future.

Despite recent advancements in the treatment of CLL, our understanding of the potential impact of novel agents on the immune response to vaccinations is limited. Suboptimal humoral response to vaccination has been reported in CLL.[2] In recent years, novel agents, namely inhibitors of BTK, phosphoinositide 3-kinases, or the antiapoptotic protein, B-cell lymphoma-2, have changed the treatment landscape



Variables to consider when studying the efficacy of COVID-19 vaccine in patients with CLL. IVIG, intravenous immunoglobulin.

for CLL.[3] A growing proportion of patients now have indefinite or long-term exposure to these drugs that directly affect the immune system, potentially further dampening their ability to mount the appropriate response to vaccinations. Seroconversion after the seasonal influenza vaccine in patients receiving ibrutinib has been reported to be as low as 7% in 1 study evaluating the standard-dose vaccine and 26% in another in which a proportion of patients received a higher dose.[4,5] Given these alarming numbers, this remains an important area for investigation for the CLL community.

Pleyer and colleagues from the National Heart, Lung, and Blood Institute evaluated serologic responses with the adjuvanted recombinant hepatitis B (HepB-CpG) and zoster (RZV) vaccines in patients with treatment-naive (TN) CLL and those receiving BTKi's. Seroconversion was measured 6 months after vaccination. De novo immune response was assessed in the HepB-CpG study; investigators observed a significant difference in antibody response between the TN (28%) and BTKi (3.8%) cohorts. In contrast, when assessing for recall antibody response with the RZV vaccine, there was no difference in serologic response between the 2 cohorts (59% vs 41%). Given the lack of de novo humoral response to the HepB-CpG vaccine in the BTKi cohort, the authors appropriately suggested that vaccination against novel antigens may need to be considered well before initiating the BTKi therapy.[1]

The finding of comparable serologic responses to the RZV in patients receiving BTKi therapy is promising and confirms current recommendations. Notably, another recent study by Zent et al also showed a high rate of early (1 month) humoral and cellular responses in patients with CLL and lymphoplasmacytic lymphoma receiving BTKi's.[6] Together, these studies provide a strong basis for larger confirmatory trials to better inform practitioners regarding appropriate vaccination strategies for patients with CLL and other lymphoid malignancies. In the meantime, these data can be used to support the use of RZV vaccine for CLL patients on a BTKi. Given the various indications for first- (ibrutinib) and second- (acalabrutinib, zanubrutinib) generation BTKi's in lymphoid malignancies, this could have broader clinical implications.

Lack of serologic response to the HepB-CPG vaccine in BTKi-treated patients is concerning not only for HepB prevention but also in regard to any vaccine designed against other novel antigens as well. The most relevant and prime examples of such vaccines are those for SARS-CoV-2. Although the COVID-19 global pandemic continues to be the leading public health issue, preliminary data indicating the efficacy of messenger RNA–based vaccines in immunocompetent patients have been promising.[7,8] However, an important and unanswered question is the efficacy of those vaccines in patients with an impaired immune state because of their underlying condition or/and CLL-specific therapies. In fact, the development of an adequate serologic response after SARS-CoV-2 infection is compromised in CLL, with only one-third of patients developing detectable immunoglobulin G antibodies after a median of ~2 months after infection, based on 1 study.[9]

Therefore, while we await the US Food and Drug Administration's approval of a SARS-CoV-2 vaccine(s), it is imperative to design studies to assess their efficacy in patients with lymphoid malignancies, including CLL. Such studies should be planned early to assure inclusiveness, as many patients are expected to receive the vaccine as soon as it becomes available. The CLL research community has already developed a COVID-19/CLL consortium and presented inferior outcomes in this population.[10] Ideally, we will extend these efforts to a comprehensive vaccine database that will allow for uniform data collection promptly. In order to be clinically informative, such a database should include (1) patient characteristics, (2) specifics of the vaccine(s) (type, intensity, frequency), and (3) anti-CLL therapy (see figure). The main emphasis should focus on the impact of CLL-specific treatments. Will "watch-and-wait" patients have a different response to the vaccine? Will there be a meaningful difference in seroconversion in patients receiving BTKi vs venetoclax? Should patients be strategically vaccinated prior to initiation of therapy, and if so, how much earlier? In patients with stable disease, is it reasonable to hold the CLL treatment temporarily to allow for an antibody response to the vaccine? If so, what is a reasonable duration for holding? Is there a significant advantage (or disadvantage) of a time-limited therapy before vaccination? What is the impact of previous treatment with monoclonal antibodies or cellular therapy approaches (allogeneic hematopoietic transplant or chimeric antigen receptor T-cell therapy)? More importantly, although timing and quality of a serologic response as a surrogate endpoint are critical, the main question is to understand the clinical impact of vaccination, including level of risk reduction for SARS-CoV-2 infection

and identification of possible predictors of such immune response in patients with CLL.

Although these questions should ideally be addressed in the setting of clinical trials, in the absence of such studies in the foreseeable future, the real-world evidence (RWE) platform seems to be a reasonable approach to answer some of these important practical questions. Given the successful experience of the CLL research community in collaborative efforts and utilizing the RWE in clinical practice, similar collaborations to answer these timely questions are expected in the near future.[10]

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HEMATOPOIESIS AND STEM CELLS

Comment on Ding et al, page 190

Remodel your way to fetal hematopoiesis

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In this issue of *Blood*, the study by Ding et al[1] describes a novel role for the chromatin remodeling factor *smarca5* during the development of hematopoietic stem and progenitor cells (HSPCs) as they emerge from dorsal aorta hemogenic endothelium[2-4] and then transition to fetal-like stage.[5,6]

Throughout vertebrate hematopoietic ontogeny there is always a fetal-like stage between HSPC emergence and the adult marrow.[7] Changes in the genetic program of HSPCs are apparent from the moment they leave the dorsal aorta[8] and as they transit through the fetal niche. However, how an HSPC undergoes these intrinsic changes is not well understood.

To explore these intriguing events, the authors follow chromatin and transcriptome changes in zebrafish HSPCs. The zebrafish is ideal to study these processes because of its highly conserved hematopoietic system and many available genetic tools. First, the authors used cell sorting to purify labeled HSPCs from transgenic zebrafish lines (*cd41:gfp*$^{+}$*/gata1:dsRed*$^{-}$), collecting cells from either nascent or fetal-like stages. In zebrafish, fetal-like hematopoiesis progresses in a vascular region of the embryonic tail called the caudal hematopoietic tissue (CHT),[5,6] which is equivalent to the mammalian fetal liver. Using nascent or fetal-like HSPCs, they performed assay for transposase accessible chromatin with high-throughput sequencing (ATAC-seq) and RNA sequencing (RNA-seq) to confirm a difference in chromatin accessibility and gene expression, respectively, between the 2 stages. The analysis revealed there is an overall increase in chromatin accessibility of hematopoietic genes during the nascent to fetal-like transition, suggesting progression in the developmental program.

To find the chromatin remodeling factors that may be responsible for this increase in chromatin accessibility, the authors examined the expression profile of 65 factors between nascent and fetal-like stages and then focused on the factors that were upregulated during the transition. They further narrowed these candidates by using morpholino knockdown, a rapid screening tool in zebrafish for testing gene function. There were 7 chromatin remodeling factors that upon knockdown had fewer HSPCs in the fetal-like CHT but had no effect on HSPC numbers at earlier emergence stages. After considering the spatial and temporal expression pattern of each factor, they decided to further pursue *smarca5* because of its increased and specific expression in fetal-like HSPCs.

A *smarca5* mutant generated by CRISPR/Cas9 recapitulated the morpholino phenotype and showed similar reduction in definitive HSPCs from fetal-like stages and