cells, suggesting that stochastic stem-cell expansions during the early phase are sufficient to explain the clone size heterogeneity observed in the late phase. Radtke et al further challenged their predictions using CellTag, an expressed DNA barcoding system, which allowed them to query the clonal dynamics of $CD34^+$ cells using single-cell transcriptomics.[6] The single-cell nature of the assay allowed them to reliably quantify the clonal sizes while simultaneously verifying their exact molecular identity. These results confirmed that HSCs contained a collection of clonally expanded stem-cell pools, which are the cause for the observed distributions in mature progenitors. Thus, the researchers conclude that HSCs are sufficient to drive all phases of cell recovery during hematopoietic transplantation and that the fast cell divisions required to drive early regeneration result in the stochastic expansion of clonal stem-cell pools, which stabilize after the first year after transplantation.

Although such simple stochastic models may be sufficient to explain clonal dynamics, previous work in mouse HSCs has suggested that various regenerative behaviors, including lineage bias and stem-cell expansion, are partly deterministic, driven by intrinsic and heritable properties.[7-9] Importantly, in these cases, stochastic models were also sufficient to fit distributions of individual samples. Still, clones acted more similarly than expected when the same clone was measured across multiple independent parallel functional tests, suggesting that both stochastic and deterministic mechanisms contribute to HSC dynamics. Further experiments in nonhuman primates should be able to determine whether heritable, deterministic programs also influence regenerative behaviors in this model. Finally, although HSCs are clearly sufficient to drive blood formation across all phases, the relative contribution of other progenitors in a real transplantation scenario is still uncertain. Solving this will require distinctly labeling and tracing multiple stem and progenitor populations simultaneously.[10] These and future findings will be of extreme importance to a growing community of clinical and translational HSC researchers who need to consider the hematopoietic populations that they need to target to achieve the most efficacious results for patients.

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LYMPHOID NEOPLASIA

Comment on Jiang et al, page 44

Targeting the methylome to improve CLL outcome

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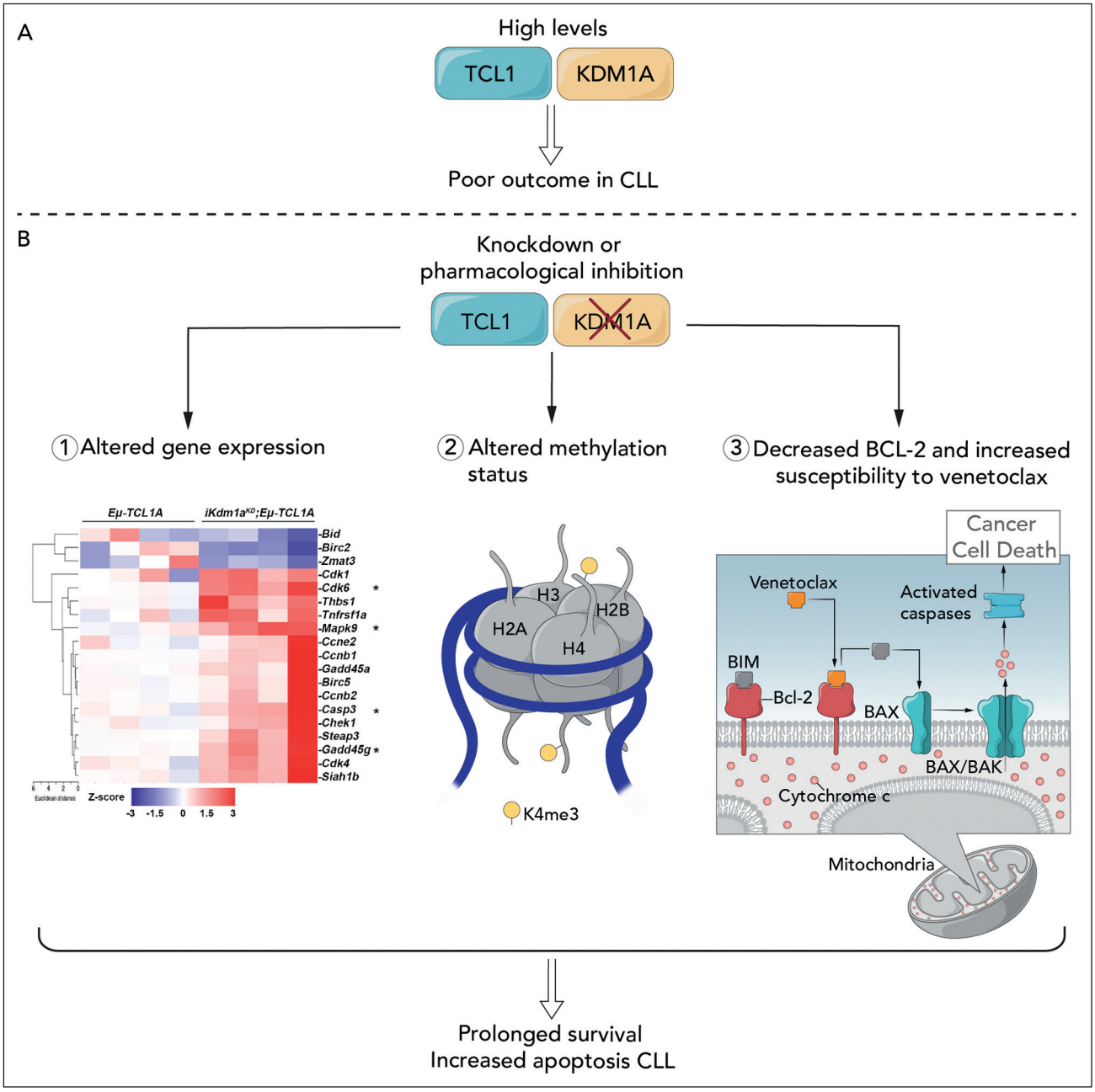
In this issue of *Blood*,[1] Jiang et al demonstrate that KDM1A is upregulated in malignant B cells and that this is associated with aggressive disease as well as adverse outcome in samples from the CLL8 clinical trial[2] (see figure panel A). They further show that knockdown of Kdm1a in a murine model altered the epigenome.

Epigenetic changes are known to drive clonal evolution and diversity in chronic lymphocytic leukemia (CLL), making CLL a good model to study epigenetic evolution over time.[3,4] However, the molecular mechanisms that drive these changes are poorly understood. To understand the mechanisms better, the authors generated a doxorubicin (Dox)-inducible *Kdm1a* knockdown in Eμ-TCL1 mice (*Kdm1a*-KD). *Kdm1a*-KD mice had reduced tumor burden, prolonged survival, upregulation of p53 and proapoptotic pathways, and changes in the tumor microenvironment (TME), indicating an important role for KDMIA. So, how does this occur and what, if anything, does this have to do with the CLL epigenome? In elegant and comprehensive experiments, the authors analyzed differences in global transcriptome by RNA sequencing as well as H3K4me3 marks by chromatin immunoprecipitation sequencing between Eμ-TCL1 and *Kdm1a*-KD mice and then confirmed their findings in human CLL samples. Their findings demonstrate that in CLL cells, KDM1A alters histone methylation patterns in pathways regulating cell death and motility, thereby acting as an oncogenic transcriptional repressor in this disease. More important, they were able



(A) KDM1A interacts with TCL1 and is increased in CLL. Higher levels are associated with more aggressive disease and shorter response to chemoimmunotherapy. (B) Knockdown of KDM1A in a mouse model alters gene expression, notably of genes regulating apoptosis and motility, alters methylation status, and works synergistically with agents, such as venetoclax, to increase CLL cell killing. BAK, BCL2 antagonist/killer 1; BAX, BCL2 associated X; BCL2, B-cell lymphoma 2; BIM, Bcl-2 interacting mediator of cell death. Professional illustration by Patrick Lane, ScEYEnce Studios.

to demonstrate this not only in their murine model, but also in primary human CLL cells. Last, they demonstrate marked synergisms of pharmacologic KDM1A inhibition with other currently available agents, which they suggest provides a strong rationale to investigate targeting of KDM1A in CLL (see figure panel B).

It is always important to understand if the murine model being used is a good model for the question being asked. Eμ-TCL1 transgenic mice are an established, well-characterized model of aggressive, unmutated immunoglobulin heavy chain variable CLL.[5] The changes in the TME closely resemble those seen in human disease.[6,7] This mouse model also recapitulates changes in the methylome in CLL.[8] The authors' starting point was to study the interactome of T-cell leukemia/lymphoma 1 (TCL1), so it is of course logical to start with this TCL1 transgenic mouse model. In their studies, they identified 1000 TCL1-interacting proteins, some of which were chromatin-modifying enzymes. For several reasons outlined in the article, they focused attention on KDM1A and verified the expected interaction with TCL1. On the basis of the observation that increased TCL1A expression led to increased histone methylation activity, they postulated that increased TCL1A expression in CLL cells affected the epigenetic signature by modulating

KDM1A-mediated demethylase activity. The findings are novel as there are no previous reports on the role of KDM1A in CLL, and they were able to confirm their findings by demonstrating higher KDM1A levels of expression in CLL and other leukemia cells compared with healthy B cells and that levels of expression increased with disease progression. Their studies in the Dox-induced KDM1A knockdown model demonstrated the potential importance of this pathway as the *Kdm1a*-KD mice had longer survival and slower disease progression. Changes in the T-cell and TME environment in Eμ-TCL1 mice have been shown to closely mimic that seen in human disease.[6] In both Eμ-TCL1 mice and in human cell lines in which KDM1A expression was knocked down, the observed results suggest a direct effect not only on CLL cells but also on the interaction of CLL cells with various components of the TME. Their studies further suggest that KDM1A expression regulates global transcriptional activity in CLL and that KDM1A knockdown led to differential H3K4me3 enrichment occupancy in areas regulating genes involved in apoptosis pathways and cell migration/adhesion. Their analyses from the CLL8 trial patient samples show that KDM1A is associated with more aggressive disease and poorer clinical outcome.

But why is any of this work of importance to us in thinking about treating CLL, a disease in which recent advances in treatment have revolutionized outcomes and in which there is no longer much relevance for the use of chemo-immunotherapy?[9] As shown in figure panel B, when the authors employed a variety of KDM1A inhibitors, they found that at least some of these, notably C12, had not only the expected activity in terms of changes in apoptotic cell death in CLL cells, H3K9me3 levels, and poly (ADP-ribose) polymerase cleavage, but also, and of more potential interest, had synergistic activity with agents, such as venetoclax, which is one of the mainstays of our current targeted treatment approaches in CLL. KDM1A inhibitors are already being explored in other malignancies, both alone and in combination.[10] Obviously, much work needs to be done to characterize potential KDM1A inhibitors, including their safety profile in early-phase clinical trials, and that the selected inhibitors recapitulate the synergistic activity with agents such as venetoclax.

In conclusion, I agree fully with the authors' conclusions that they have provided sufficient evidence to support that KTM1A appears to be a target worthy of further investigation in CLL.

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MYELOID NEOPLASIA

Comment on Bamezai et al, page 90

Breaking the loop in AML

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In this issue of *Blood*, Bamezai et al[1] reveal an unexpected, moonlighting (ie, working a second job, typically secretly in addition to one's regular employment) function of the germ cell–associated RNA binding protein (RBP) PIWIL4 in acute myeloid leukemia (AML). The authors show a tumor-specific requirement for PIWIL4 in R-loop homeostasis. Loss of PIWIL4 in AML cells led to uncontrolled R-loop formation, transcriptional stalling, DNA damage, and enhanced sensitivity to ATR inhibition, findings that may inform future therapeutic aprproaches.

R-loops form when a nascent RNA transcript hybridizes to its single-stranded DNA template behind the progressing RNA polymerase complex.[2,3] This creates an RNA-DNA hybrid structure, displacing the nontemplate strand as a single-stranded DNA loop. These transient structures are common, occupying as much as 5% to 10% of the genome, and may contribute to physiological processes such as transcriptional regulation and DNA repair. However, they also contribute to processes detrimental to the cell, including replication stress, DNA damage, and genomic instability. This may represent a particular vulnerability for cancer cells where high rates of both transcription and replication increase the risk of collision between R-loops and replication forks.[4] As such, cells employ multiple strategies to