Nonetheless, the data presented suggest that TSLPR represents a promising immunotherapeutic target in a high-risk and poor-prognosis subset of B-ALL, and therefore merits further clinical development.

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

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**Comment on Krivega et al, page 665**

Pharmacologic control of chromatin looping

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In this issue of Blood, Krivega et al establish a new method to reactivate fetal hemoglobin (HbF) production in adult human erythroid cells through pharmacologic manipulation of chromatin looping at the β-globin locus.¹

Sickle cell disease (SCD) and β-thalassemia are widespread genetic disorders that result from inherited mutations in the adult β-globin gene. An important aspect of SCD and β-thalassemia is that disease-causing mutations affect the adult β-globin gene but leave intact its fetal counterparts. For example, Krivega et al previously showed that the chromatin-modifying enzyme G9a, previously shown to spread across the β-globin locus,² contributes to the regulation of chromatin loop formation. This finding offers the first clue that chromatin spreading and looping may be functionally linked.

G9a and its paralog GLP are methyltransferases that can mono- and dimethylate H3 lysine 9 (H3K9) and H3K9me2 for G9a). It has been previously shown that G9a, previously shown to spread across the β-globin locus,² contributes to the regulation of chromatin loop formation. This finding offers the first clue that chromatin spreading and looping may be functionally linked.

G9a and GLP are methyltransferases that can mono- and dimethylate H3K9. Furthermore, G9a and GLP possess ankyrin repeat domains, which allow them to bind to their own substrate, albeit with different specificities (i.e., H3K9me1 for GLP and H3K9me2 for G9a). It has been previously shown that G9a is recruited to the β-globin LCR by the transcription factor NF-E2,³ and spreads across the β-globin locus.⁴ Furthermore, knocking down G9a through RNA interference in murine erythroid cells,⁴ or inhibiting its enzymatic activity in human hematopoietic progenitors,⁵ leads to reactivation of the embryonic/fetal β-like globin genes,
suggested that pharmacologic inhibition of G9a could be used to counteract fetal γ-globin silencing.

To determine the phase of erythropoiesis at which inhibition of G9a is most efficient to increase levels of HbF, the authors used a 3-stage ex vivo differentiation system with human CD34⁺ hematopoietic progenitors from adult donors. They demonstrate that inhibition of G9a/GLP methyltransferase activity with the small molecule inhibitor UNC06389 leads to a pronounced increase in HbF (up to 30% of total hemoglobin) when applied at the time of erythropoietin-mediated induction of erythroid differentiation. This effect is mediated through upregulation of fetal γ-globin and downregulation of adult β-globin expression. At the molecular level, the authors show that the drug leads to a locus-wide decrease in H3K9me2, which is accompanied by complex changes in G9a binding (ie, increased binding at the fetal promoter, decreased binding at the adult promoter, and no change at the LCR). Similarly, they observed a shift in binding of the looping factor LDB1 from the adult to the fetal gene promoters. Finally, they show that the fetal γ-globin gene relocates to achieve closer proximity to the LCR.¹

Taken together, these results establish G9a as a major player in the maintenance of γ-globin silencing in adult erythroid cells. Furthermore, it suggests a mechanism whereby the G9a-mediated H3K9me2 mark on the γ-globin promoter prevents spatial proximity with the LCR through inhibiting binding of the “looping factor” LDB1. Testing this model will require additional experiments to determine the sequential order of events following H3K9 methyltransferase inhibition.

Finally, a surprising finding is that G9a remains bound to the β-globin locus upon drug treatment, despite a widespread loss of the H3K9me2 mark. This result is at odds with the current model of G9a spreading on chromatin through its ankyrin domain-mediated recognition of H3K9me2. However, it is consistent with the absence of a visible phenotype in knock-in mice carrying a mutant form of G9a that is unable to bind to H3K9me2.¹⁰ Although these results converge to suggest that the interaction of G9a with its substrate does not play a dominant role in the maintenance of G9a binding to chromatin in vivo, we cannot exclude the possibility that the interaction with H3K9me2 is important for the initial establishment of G9a binding (ie, spreading). A candidate factor for retaining G9a binding to the β-globin locus in the absence of H3K9me2 is G9a-heterodimerization partner GLP, whose H3K9me1 binding activity appears to play a dominant role in vivo.¹⁰ In that regard, it will be interesting to analyze GLP binding and H3K9me1 enrichment on the β-globin locus upon inhibition of H3K9 methyltransferase activity.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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THROMBOSIS AND HEMOSTASIS

Comment on Mammadova-Bach et al, page 683

Polymerized fibrin activates glycoprotein VI

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In this issue of Blood, Mammadova-Bach et al show that polymerized fibrin binds to the platelet receptor glycoprotein VI (GPVI), amplifying thrombin generation and enhancing thrombus growth.¹
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