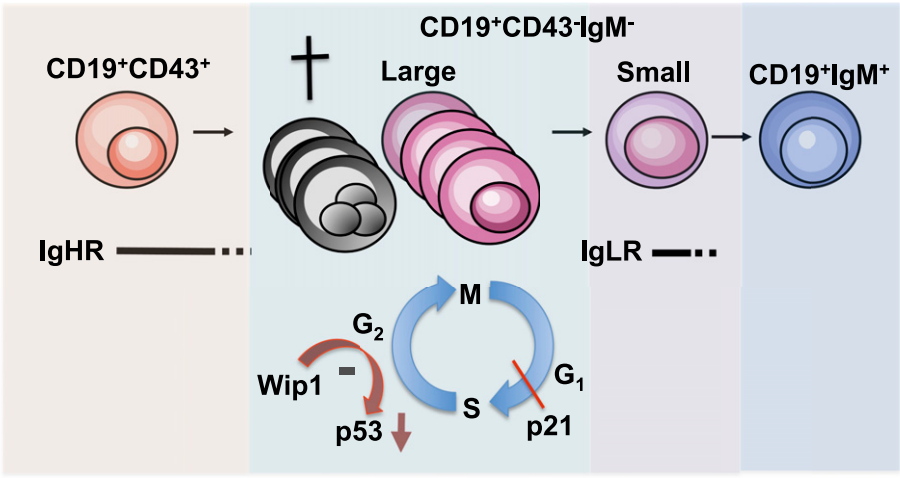

Stage-specific expansion in the early B-cell compartment. The figure displays a schematic drawing of early B-cell development indicating stage-specific recombination events of the immunoglobulin heavy-chain (IgHR) and light-chain (IgLR) genes and the expansion or deletion (apoptosis indicated by a cross) of early pre–B cells. The lower part of the figure indicates the specific stages of the cell cycle (G_1 [gap I], S [synthesis], G_2 [gap II], and M [mitosis]), as well as the cell-cycle block in G_1 induced by p21 and the ability of Wip1 to modulate the activity of p53 in G_2 phase.

long-lived hematopoietic progenitors in the BM.[9]

Although there exists an apparent need to harness p53 activity to achieve normal B-cell production, there is likely a need to balance rather than abolish the activity of this protein, because loss of p53 function is a common event in human malignancies. Of special interest in the context of this report is the finding that a mutated or deleted *TP53* gene is one of the strongest independent predictors of inferior treatment outcome in childhood B-lineage acute lymphoblastic leukemia.[10] In a situation where the functional dose of a transcription factor like p53 is of critical importance, the use of an autoregulatory loop such as that created by the induction of *Wip1* transcription by p53 presents an elegant solution to preserve a high output of normal cells while still preventing uncontrolled malignant proliferation of progenitor cells. Therefore, the extended insight to Wip1 function provided by this report is an important contribution to our understanding of regulatory events in early B-lymphocyte development.

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

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● ● ● LYMPHOID NEOPLASIA

Comment on Qin et al, page 629

TSLPR: a new CAR in the showroom for B-ALL

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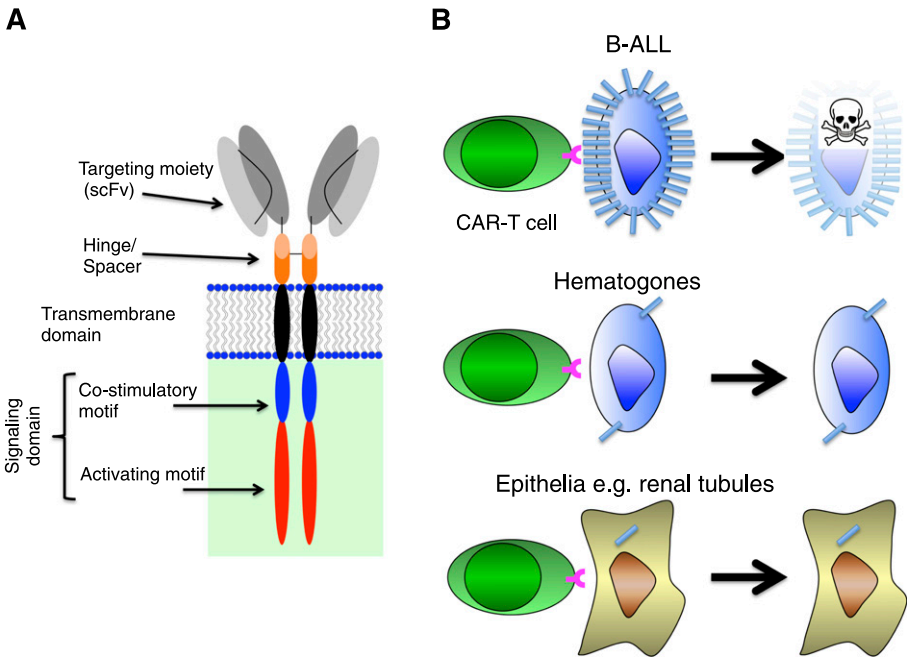
In this issue of *Blood*, Qin et al demonstrate the ability of chimeric antigen receptor (CAR)-engineered T cells targeted against thymic stromal lymphopoietin receptor (TSLPR) to eradicate disease in several models of B-cell acute lymphoblastic leukemia (B-ALL) that overexpress this protein.[1]

Chimeric antigen receptors are genetically delivered fusion proteins that redirect the specificity of polyclonal T cells or natural killer cells against a chosen cell surface molecule. Constructs consist of a targeting moiety such as a peptide, ligand derivative, or antibody-derived single-chain variable fragment (scFv) that is coupled in series to a hinge/spacer, membrane-spanning element, and signaling endodomain (see figure, part A). Target antigen is engaged in its native conformation, rather than as a processed peptide displayed within the groove of a human leukocyte antigen (HLA) molecule. Consequently, CARs recognize target cells irrespective of a patient's HLA haplotype. Furthermore, their function is unhindered by a common immune evasion strategy that is deployed in acute lymphoblastic leukemia (ALL) and other malignancies, namely, the selective downregulation of some HLA allo-specificities. In so-called "first-generation" CARs, the endodomain generally contains CD3ζ alone, thereby providing signals that mimic those naturally provided by the T-cell receptor/CD3 complex. Second- and third-generation receptors are distinct in that they respectively contain either 1 or 2 additional costimulatory motifs, commonly



TSLPR-specific CAR. (A) A schematic diagram of a CAR expressed at the T-cell surface, illustrating the typical component parts found in a second-generation variant. (B) Cartoon depicting the elimination of B-ALL target cells with a *CRLF2* rearrangement by TSLPR-retargeted CAR T cells. By contrast, healthy B-lymphocyte precursors (hematogones) express TSLPR at low levels, which may protect them from immune attack. Some epithelial cell types also express low levels of TSLPR, although immunohistochemical analysis suggests that the target is not present at the cell surface.

derived from CD28 and/or tumor necrosis factor receptor families. Upon target ligation, integrated signaling by the CAR endodomain results in T-cell activation and target-cell killing (see figure, part B). The provision of costimulation in later-generation CARs ensures improved T-cell persistence in vivo,[2] a property that correlates strongly with enhanced efficacy.

Target selection is of fundamental importance in maximizing the therapeutic index of CAR-based immunotherapy. Few tumor-associated antigens are truly disease specific, mandating careful risk assessment and vigilance for the emergence of "on-target, off-organ" toxicity. In B-ALL, unprecedented complete remission rates approaching 90% have been repeatedly attained in phase 1 clinical trials involving CAR T cells that engage the ubiquitous B-cell antigen, CD19.[3] Because CD19 is expressed throughout B-cell differentiation, this success occurs at the expense of predictable toxicity in the form of B-cell aplasia and hypogammaglobulinemia. Although this is clearly undesirable, it can be managed effectively with IV or subcutaneously administered immunoglobulin replacement therapy. Of greater concern, however, is the risk of therapeutic failure or relapse with CD19 null disease.[4] The latter may reflect the lack of direct contribution of CD19 expression to disease pathogenesis in B-ALL and highlights the need to identify additional candidate target molecules.

Expression of the cytokine receptor–like factor 2 (*CRLF2*) gene may be perturbed by a number of genomic alterations in B-ALL. As a result, enhanced cell-surface expression of the encoded TSLPR protein is seen in as much as 15% of cases that lack typical chromosomal rearrangements.[5,6] This observation raises the possibility that TSLPR could represent an attractive new candidate for CAR T-cell therapy of some patients with B-ALL. In support of this, Qin et al demonstrate that T cells expressing a CAR targeted with a 3G11-derived scFv can be efficiently redirected against TSLPR$^+$ target cells. Potent antitumor effects were observed both in vitro and in vivo, even in the setting of established disease. Complete tumor eradication was also demonstrated in several patient-derived xenograft models, confirming efficacy in a more clinically relevant setting. Importantly, T cells expressing the TSLPR-specific CAR demonstrated in vivo efficacy comparable with CD19-specific CAR T cells, thereby further confirming the potential of TSLPR as a target in B-ALL associated with *CRLF2* rearrangements. In agreement with clinical findings, efficacy was associated with enhanced T-cell survival post–adoptive transfer. This was demonstrated by the fact that T cells expressing a CAR variant with a longer spacer exhibited satisfactory function in vitro, but failed to persist in vivo or to mediate antileukemic activity.

Although upregulation of TSLPR is restricted to a subset of patients, the demonstration that it can be targeted effectively using CAR-engineered T cells is important for 2 additional reasons. First, patients with B-ALL bearing *CRLF2* rearrangements and TSLPR overexpression have particularly poor relapse-free and overall survival rates.[7] Second, there is significant evidence that TSLPR contributes directly to disease pathogenesis in B-ALL, a factor that may protect against antigen loss. Overexpression of TSLPR in lymphoid progenitors is sufficient to promote their growth.[5] Moreover, patient samples that express high levels of this receptor proliferate in response to TSLP, unlike samples lacking *CRLF2* rearrangements.[8] However, it should be noted that leukemias in which TSLPR is upregulated also exhibit a number of cooperative genetic changes, most notably activating mutations in Janus kinase 2 (*JAK2*).[9] Although expression of mutated *JAK2* was insufficient to enable immortalized Ba/F3 cells to grow in the absence of exogenous cytokine,[6] it has been suggested that the signaling through mutated *JAK2* may be responsible for the continued, albeit slower, growth of a B-ALL cell line in which TSLPR expression had been silenced.[5] Consequently, it remains to be determined whether signaling provided by mutated *JAK2* or related kinases will be sufficient to allow TSLPR loss in response to the selective pressure mediated by CAR T cells in vivo.

An additional caveat to the potential use of TSLPR as an immunotherapeutic target is the lower-level expression of this receptor in a number of other cell types, notably CD4$^+$ T cells and dendritic cells.[1] Although the level of TSLPR required for activation of the CAR$^+$ T cells is unknown, the potential for on-target, off-organ toxicity is of concern and warrants close attention during phase 1 clinical evaluation. The CAR tested in this study did not crossreact with the mouse ortholog of TSLPR. This contrasts with some other CARs directed against cytokine receptors and that have triggered cytokine release syndrome when evaluated in mouse xenograft models, owing to target engagement in healthy tissues.[10]

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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● ● ● RED CELLS, IRON, AND ERYTHROPOIESIS

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 Gγ- and Aγ-globin, a point that explains why SCD and β-thalassemia patients first experience major symptoms in late infancy when the fetal γ-globin genes become developmentally extinguished.[2] Furthermore, rare mutations that lead to persistence of fetal γ-globin expression in adults significantly ameliorate SCD and β-thalassemia symptoms, highlighting the clinical benefits of elevated levels of HbF.[2] Therefore, a major research objective is the development of methods to reactivate fetal γ-globin in adult erythroid cells.

The β-like globin genes reside in a single cluster where they are arranged in the order of their expression during development. High-level expression of these genes is mediated by the locus control region (LCR), a distal array of multiple enhancers that act in an additive manner to increase the rate of transcriptional elongation.[3] During development, when the embryonic, fetal, and adult β-globin genes undergo sequential phases of expression followed by gene silencing, the LCR alters its spatial positioning within the nucleus to remain in close proximity to the promoter of the developmentally appropriate, active β-like globin gene through a 3-dimensional looping of chromatin.[4] Although the mechanism through which looping is established is not entirely clear, the authors have previously identified the Lim-domain binding 1 (LDB1) protein as a key factor that mediates loop formation.[5] Furthermore, it has been shown that in adult erythroid cells, tethering the dimerization domain of LDB1 to the fetal γ-globin gene promoters via an artificial zinc-finger protein brings the LCR in close proximity to the fetal genes and stimulates their expression.[6] Although this shows that forced looping through an artificial transcription factor allows reactivation of HbF in adult erythroid cells (see figure), such an approach requires genetic manipulation of erythroblasts, which may complicate its application in a clinical setting.

Here, Krivega et al describe a novel pharmacologic approach to modulate β-globin gene expression where they use a small molecule inhibitor of the histone H3 lysine 9 (H3K9) methyltransferase enzymes G9a and G9a-like protein (GLP) to reactivate HbF production in adult erythroid cells.[1] Interestingly, the authors show that this reactivation is associated with spatial reconfiguration of the locus whereby the LCR alters its nuclear positioning to gain proximity to the fetal γ-globin genes (see figure). This finding is important because it provides proof-of-principle that structural reconfiguration of the β-globin locus can be achieved through pharmacologic modification of its chromatin state. In addition, the study provides new insights into the mechanism of long-distance enhancer-gene communication by showing that the chromatin-modifying enzyme G9a, previously shown to spread across the β-globin locus,[7] 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 di-methylate H3K9. Furthermore, G9a and GLP possess ankyrin repeat domains, which allow them to bind to their own substrate, albeit with different specificities (ie, 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, and spreads across the β-globin locus.[7] Furthermore, knocking down G9a through RNA interference in murine erythroid cells,[7] or inhibiting its enzymatic activity in human hematopoietic progenitors,[8] leads to reactivation of the embryonic/fetal β-like globin genes,