mutants, antisense RNA, and interference oligonucleotides.[6] In a seminal paper in 2005, the authors showed that systemic administration of STAT3 inhibitors not only inhibited tumor growth but also reduced the production of immunosuppressive cytokines while increasing production of inflammatory cytokines and chemokines, leading to augmentation of DC function and cytotoxic T-cell induction.[7] They later showed that in the same way, ablating STAT3 in hematopoietic cells results in rapid activation of innate immunity by CpG (a TLR9 ligand), with enhanced production of interferon-γ, tumor necrosis factor α, and interleukin-12 and activation of macrophages, neutrophils, and natural killer (NK) cells associated with eradication of B16 melanoma tumors.[8]

STAT3 has also been shown to play a significant role in promoting AML cell proliferation and survival,[9] but whether it also plays a role in AML-induced immune evasion remains to be determined. In the present study, the authors extend from their previous work to investigate whether silencing STAT3 using small interfering RNA (siRNA) increases CpG-induced DC maturation, T-cell activation, and AML-induced immunity in a genetic mouse model of $Cbfb/MYH11/Mpl^+$-induced leukemia, which closely resembles human AML with inv(16)(p13:q22) gene fusion. The authors report that CpG-Stat3 siRNA-induced leukemia regression is primarily dependent on correction of the immunosuppressive microenvironment of AML rather than on direct tumor cell killing. In a series of elegant experiments, they showed that CpG-Stat3 siRNA facilitates differentiation of AML blasts to antigen-presenting cells (APCs) with DC phenotype, with upregulation of major histocompatibility class II and costimulatory molecules. Moreover, systemic STAT3 blocking/TLR9 triggering was shown to reverse immune tolerance by downregulating expression of the coinhibitory PD-L1 molecule and reducing numbers of Tregs while increasing recruitment of activated CD8 T cells into major leukemia reservoirs, such as spleen and bone marrow.

These findings show that STAT3 is a key molecule favoring persistence of AML through 3 mechanisms: promoting proliferation and survival, preventing AML differentiation to functional DCs, and blocking T-cell function through other pathways (see

figure). However, while the murine AML model may be an accurate representation of human core binding factor leukemias, the mouse immune milieu may be a less accurate representation of its human counterpart. Nevertheless, these findings encourage us to explore the role that STAT3 plays in our ability to achieve and maintain remissions in AML and raise the exciting prospect of therapeutically targeting STAT3 in conjunction with remission induction treatment. A diversity of abnormalities in NK cells, T cells, and APC function have been described in AML. Could they all be derived from STAT3 overexpression in the leukemia cell? More studies with human AML are now indicated to explore the downstream events following leukemia-cell STAT3 signaling. An important question is whether the least-differentiated leukemia-initiating cells, as well as the more mature AML blasts, can suppress immunity. Such findings might predict whether the immune suppression persists into remission. Critically, we need to explore the relationship between STAT3 activity and outcome in patients with AML. Could STAT3 expression serve as a predictive factor for the maintenance of remission? Do leukemia subtypes with more favorable outcomes have less STAT3 expression? Supporting data from human AML would form the basis for trials of STAT3 inhibition to improve treatment outcomes.

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

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● ● ● **IMMUNOBIOLOGY**

Comment on Didigu et al, page 61

Dual zinc-finger nucleases block HIV infection

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In this issue of *Blood*, Didigu et al explore a potential strategy for HIV cure that uses gene therapy to simultaneously inactivate both cellular coreceptors used for viral entry: CC chemokine receptor 5 (CCR5) and CXC chemokine receptor 4 (CXCR4).[1]

The first case of HIV cure was, in essence, a form of gene therapy. In 2007, a courageous patient was cured both of his acute myeloid leukemia and his HIV infection with a bone marrow transplant from a donor who was homozygous for a deletion in CCR5, the major cellular coreceptor used by HIV to infect CD4 T cells.[2] With this transplant, the patient received an immune system that was impenetrable to the most common variants of HIV that use CCR5 to enter cells, R5-tropic virus.

This case generated enormous interest in gene therapy approaches to cure HIV by

knocking out CCR5 expression. One of the most promising methods to achieve this is the use of zinc-finger nucleases (ZFNs). ZFNs are engineered nucleases that target and cut specific cellular DNA sequences. The cell attempts to repair the break using nonhomologous end joining, an error-prone mechanism that often results in a nonfunctional gene product. ZFNs can be delivered to CD4$^+$ T cells and/or hematopoietic stem cells using an adenoviral vector delivery system.[3]

Some strains of HIV have evolved to use a different cellular coreceptor, CXCR4. These X4-tropic viruses are rarely transmitted but develop within an infected individual over time. They are associated with a worse prognosis and rapid disease progression.[4] There is concern that the use of ZFNs that inactivate CCR5 (R5 ZFNs) will lead to the selection of X4-tropic HIV strains. As such, R5 ZFNs could not be used in individuals who harbor dual or mixed-tropic viruses, about half of individuals with AIDS.[5] ZFNs specific for CXCR4 (X4 ZFNs) have been developed[6] but it was previously unclear whether they could be successfully used in conjunction with R5 ZFNs. To fully protect a cell from both R5- and X4-tropic viruses would require simultaneous editing of 4 alleles.

In this study, Didigu et al provide convincing evidence that treatment with dual ZFNs achieves this goal, disrupting both CCR5 and CXCR4 within the same cell.[1] The authors show that treatment of primary CD4$^+$ T cells with the dual ZFNs leads to modification of ~20% of CCR5 and CXCR4 genes. After infection with a mix of R5 and X4 HIV strains, CD4$^+$ T cells that had been treated with the dual ZFNs continued to proliferate in culture, whereas untreated cells and cells treated only with an R5 ZFN died.

Functional effects of the dual ZFN gene therapy treatment were further explored in a humanized mouse model of HIV infection. Immunodeficient mice transplanted with CD4$^+$ T cells that had been treated with dual ZFNs had comparable engraftment to mice transplanted with unmodified CD4$^+$ T cells. More importantly, the mice

transplanted with dual ZFN-treated cells maintained CD4$^+$ T cell counts 200-fold higher than mice receiving untreated cells 2 months after challenge with a mix of R5 and X4 HIV viruses. In this model, the authors found that about 70% of CCR5 and CXCR4 genes showed evidence of gene editing 1 month after infection. These analyses demonstrate that simultaneous treatment with ZFNs for both HIV coreceptors can result in a pool of CD4$^+$ T cells that are resistant to both X4- and R5-tropic HIV strains.

These results are novel and exciting, and it is important to consider the challenges to moving this approach from the bench to the bedside. A gene therapy approach that included the use of a R5 ZFN was found to be safe and tolerable in a phase 2 clinical trial, but it was not effective in reducing levels of HIV plasma virus.[7] Low and transient engraftment of the genetically modified cells has been problematic. Overcoming this limitation seems likely as the technology continues to advance.

A more problematic issue is that uncontrolled HIV replication is required to select for the genetically modified cells, as illustrated in the mouse model in this study. Interrupting antiretroviral treatment in HIV-infected individuals may be difficult to justify given the growing evidence that delays and interruptions in therapy lead to clinical complications.[8]

Finally, new findings that suggest gene therapy may not be necessary to HIV cure in the context of allogeneic stem cell transplantation. Simply maintaining antiretroviral therapy during transplant may be sufficient to protect donor cells from acquiring HIV. In parallel, donor hematopoietic cells should replace all host hematopoietic cells over time due to the allogeneic or graft-versus-host effect, which will nonspecifically eradicate viral reservoirs. Proof of concept for this hypothesis was provided by Henrich et al who identified 2 HIV-infected individuals who had received allogeneic CCR5 wild-type stem cell transplants while maintaining antiretroviral therapy. Several years after transplant, Henrich et al did not detect HIV in the

plasma or peripheral blood cells.[9] Recently, antiretroviral therapy was carefully interrupted in these individuals, and early rebound of HIV viremia has not been observed.[10] Longer-term follow-up will be needed to determine whether these individuals are cured.

What is clear is that the field of HIV cure research is rapidly evolving. Multiple innovative strategies will likely be required. With this study, Didigu et al have demonstrated that dual ZFN treatment is another potential weapon in our arsenal.

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

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