specific T cells for each patient. TCR gene transfer using retroviral vectors is an attractive strategy for redirecting the antigen specificity of polyclonal primary T cells to create tumor antigen-specific lymphocytes. This approach can potentially overcome the limitations of current adoptive T-cell therapies, which rely largely on the isolation and expansion of tumor-specific lymphocytes ex vivo from individual patients, and result in the generation of sufficient numbers of potent antitumor immune effectors for adoptive immunotherapy.

Pioneering work from Rosenberg and colleagues at the National Cancer Institute proved the feasibility of this approach in clinical trials of adoptive therapy with autologous TCR gene-transduced T cells directed against cancer-associated antigens for the treatment of metastatic melanoma and synovial cell sarcoma.[4,5] In the setting of hematologic malignancies, TCR gene therapy targeting a number of leukemia-associated antigens such as Wilms tumor gene product 1 (WT1),[6] and minor histocompatibility antigens such as HA-1 and HA-2[7] are currently being investigated in preclinical studies or in early-phase clinical trials.

Aurora kinase A is a candidate tumor-associated antigen that is widely expressed in various types of cancer, including hematologic malignancies; its expression in normal tissues is largely limited to the testis, making it an ideal target for immunotherapy.[8] Previously, Yasukawa and colleagues identified an immunogenic HLA-A2–restricted antigenic epitope of Aurora kinase A, $AURKA_{207-215}$, capable of inducing $CD8^+$ T cells with in vitro cytotoxicity against AURKA expressing leukemic cells.[9]

Here, Nagai and colleagues sought to examine the feasibility of generating engineered T cells bearing Aurora kinase-A specific TCR genes as a strategy for the treatment of leukemia. In elegant experiments, they clearly demonstrate that polycloncal $CD8^+$ T cells retrovirally transduced to express the HLA-A2–restricted $AURKA_{207-215}$ TCR α/β chains generated from the AURKA $CD8^+$ T-cell clone have specific recognition of AURKA-overexpressing human leukemic cells, both in vitro and in a xenogeneic mouse model of human leukemia. Importantly, $AURKA_{207-215}$–specific TCR-transduced $CD8^+$ T cells were selective in their recognition of leukemic cells and did not lyze HLA-A2 positive normal peripheral blood mononuclear cells or cord blood $CD34^+$ cells, suggesting that $CD8^+$ T cells targeting AURKA will not result in immune-mediated destruction of normal stem cells.

Intriguingly, Nagai et al show that $CD4^+$ T cells could be redirected, using the same TCR that recognized the HLA-A2–restricted $AURKA_{207-215}$ epitope, to recognize and secrete T helper 1 cytokines including IL-2, in response to $AURKA_{207-215}$–expressing targets. The cytotoxic antitumor effect of $CD8^+$ T cells is partly dependent on $CD4^+$ T cells, which provide $CD8^+$ T cells with growth factors such as IL-2 and can mediate the destruction of tumor cells either directly or indirectly.[10] It is therefore likely that the adoptive transfer of redirected $CD4^+$ T cells concurrently with $CD8^+$ T cells expressing the same tumor-specific TCR gene could enhance the in vivo expansion, persistence, and antitumor reactivity of adoptively transferred antigen-specific $CD8^+$ T cells in vivo. It remains to be determined whether such redirected helper $CD4^+$ T cells will also exert direct cytotoxicity against leukemic cells in vivo.

Taken together, these results suggest that adoptive therapy using redirected $CD4^+$ and $CD8^+$ T cells that recognize AURKA-derived epitopes, may be a promising strategy for the treatment of AURKA-expressing cancers. These encouraging preclinical data support the development of clinical trials to investigate the safety and utility of such an approach in patients with relapsed or refractory leukemia.

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

REFERENCES

1. Schmitt TM, Ragnarsson GB, Greenberg PD. T cell receptor gene therapy for cancer. *Hum Gene Ther*. 2009; 20(11):1240-1248.
2. Nagai K, Ochi T, Fujiwara H, et al. Aurora kinase A-specific T-cell receptor gene transfer redirects T lymphocytes to display effective antileukemia reactivity. *Blood*. 2012;119(2):368-376.
3. Rezvani K, Yong AS, Tawab A et al. Ex vivo characterization of polyclonal memory CD8+ T-cell responses to PRAME-specific peptides in patients with acute lymphoblastic leukemia and acute and chronic myeloid leukemia. *Blood*. 2009;113(10):2245-2255.
4. Morgan RA, Dudley ME, Wunderlich JR et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*. 2006;314(5796):126-129.
5. Robbins PF, Morgan RA, Feldman SA et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol*. 2011;29(7):917-924.
6. Tsuji T, Yasukawa M, Matsuzaki J et al. Generation of tumor-specific, HLA class I-restricted human Th1 and Tc1 cells by cell engineering with tumor peptide-specific T-cell receptor genes. *Blood*. 2005;106(2):470-476.
7. Heemskerk MH, Hoogeboom M, de Paus RA et al. Redirection of antileukemic reactivity of peripheral T lymphocytes using gene transfer of minor histocompatibility antigen HA-2-specific T-cell receptor complexes expressing a conserved alpha joining region. *Blood*. 2003;102(10): 3530-3540.
8. Ochi T, Fujiwara H, Yasukawa M. Aurora-A kinase: a novel target both for cellular immunotherapy and molecular target therapy against human leukemia. *Expert Opin Ther Targets*. 2009;13(12):1399-1410.
9. Ochi T, Fujiwara H, Suemori K et al. Aurora-A kinase: a novel target of cellular immunotherapy for leukemia. *Blood*. 2009;113(1):66-74.
10. Walter EA, Greenberg PD, Gilbert MJ et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med*. 1995;333(16): 1038-1044.

● ● ● MYELOID NEOPLASIA

Comment on Frisch et al, page 540

Bad to the bone

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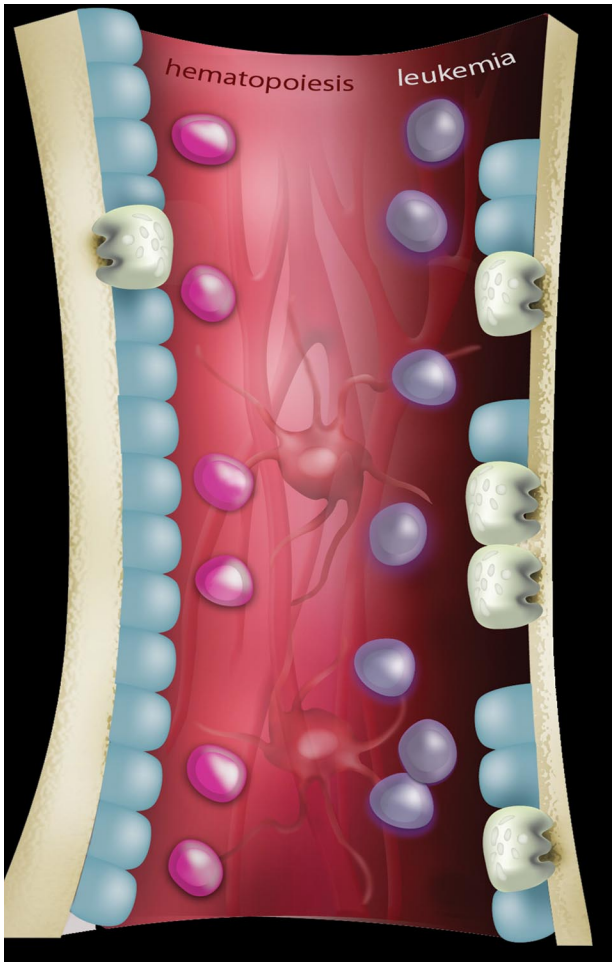
In this issue of *Blood*, Frisch and colleagues identify an unexpected effect of leukemia cells: alterations in bone homeostasis within the bone marrow hematopoietic microenvironment.[1]

Ever since descriptions of the various functional components of the hematopoietic stem cell niche, leukemia researchers have wondered whether leukemia stem cells (LSCs) require similar cell-extrinsic support for long-term maintenance of leukemia.[2] LSCs differ from normal hematopoietic stem cells (HSCs) in that they have activation of key pathways regulating self-renewal, proliferation, and survival. Activation of these pathways is sufficient in many models for full leukemogenesis, suggesting that the LSC niche may be an attractive, but functionally redundant contributor to LSC survival. However, recent key



A schematic representation showing the differences in bone homeostasis between normal hematopoiesis and leukemia. In normal hematopoiesis (left side of figure), hematopoietic stem cells (HSCs; pink) are in balance with components of the hematopoietic microenvironment including osteoblastic cells (blue), osteoclasts (gray), mesenchymal cells, and vascular structures (background; red). In leukemia (right side of figure), invasion of leukemia cells (purple) results in osteopenia mediated by an expansion of osteoclasts causing increased bone resorption and a concomitant reduction of osteoblastic activity. The effect, if any, on other components of the HSC niche has yet to be determined. Artwork produced by Ms M. Kersting (QIMR).

findings point to cell-extrinsic factors in the development of leukemia,[3] LSC engraftment into the hematopoietic microenvironment,[4] survival after chemotherapy,[5] and even the determination of leukemia phenotype,[6] suggesting that components of the normal HSC niche, including osteoblastic cells, may have important roles in leukemia pathogenesis.

Frisch and colleagues now describe the somewhat unexpected finding that acute leukemia cells have direct effects on host bone formation and turnover (see figure).[1] Using a retroviral bone marrow transplantation model of blast-crisis chronic myeloid leukemia, the authors observed that mice with leukemia had a reduction in bone trabeculae and thinning of cortical bone compared with wild-type, non-leukemic controls. These bony changes were mediated through both reduced bone formation and increased osteoclastic bone resorption, and were partially reversible by treatment with bisphosphonate therapy (to inhibit osteoclast function). The authors identified that leukemic mice but not normal controls had increased expression of the soluble proinflammatory cytokine CCL3 (also known as macrophage inflammatory protein-1α), a factor known to inhibit in vitro bone formation and previously implicated in myeloma-induced pathologic bone resorption.[7] Finally, they were able to demonstrate increased expression of CCL3 in a significant proportion of human acute myeloid leukemia (AML) samples, suggesting that similar mechanisms may be active in patients with AML.

These findings are quite provocative and lead to many interesting and potentially clinically relevant questions. For example, is osteoporosis a common finding in acute leukemia and will screening for this provide any useful information in the acute management of the patient with newly diagnosed leukemia? In contrast to myeloma and other cancers, osteolytic lesions are not frequently encountered in patients with AML, suggesting that if these findings are present, then perhaps diffuse osteopenia, rather than discrete osteolytic lesions, is the more likely pathologic entity. Given the association with elevated CCL3 levels in AML, it is tempting to speculate that blocking this pathway may be useful in preventing bony changes. Ongoing functional work is needed to determine whether CCL3 is in fact the pathogenic molecule or whether this is one of many dysregulated chemokines in the leukemic milieu.

What, then, is the effect of these bony changes on normal host hematopoiesis? We know that osteoblastic function is an integral component of the normal HSC niche,[8] and it would logically follow that impaired osteoblastic function through leukemia-secreted factors such as CCL3 may contribute to delayed hematopoietic recovery after chemotherapy. However, others have shown that a reduction in osteoblast number may not necessarily impair hematopoiesis,[9] and it remains to be seen whether these leukemia-induced bony abnormalities have a negative effect on normal HSC function and recovery after chemotherapy. Along these lines, it would be important to know what, if any, effects leukemia cells have on other putative components of the HSC niche, including nestin-positive mesenchymal cells[10] and vascular structures. These experiments, although technically challenging, will be valuable to further our understanding of postchemotherapy myelosuppression and potentially provide therapeutic avenues to ameliorate it.

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

REFERENCES

1. Frisch BJ, Ashton JM, Xing L, Becker MW, Jordan CT, Calvi LM. Functional inhibition of osteoblastic cells in an in vivo mouse model of myeloid leukemia. *Blood*. 2012; 119(2):540-550.

2. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. *Blood*. 2009;114(6):1150-1157.

3. Raaijmakers MH, Mukherjee S, Guo S, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature*. 2010;464(7290):852-857.

4. Lane SW, Wang YJ, Lo Celso C, et al. Differential niche and Wnt requirements during acute myeloid leukemia progression. *Blood*. 2011;118(10):2849-2856.

5. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft

within the bone-marrow endosteal region. *Nat Biotechnol.* 2007;25(11):1315-1321.

6. Wei J, Wunderlich M, Fox C, et al. Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. *Cancer Cell.* 2008;13(6):483-495.

7. Han JH, Choi SJ, Kurihara N, Koide M, Oba Y, Roodman GD. Macrophage inflammatory protein-1alpha is an osteoclastogenic factor in myeloma that is independent of receptor activator of nuclear factor kappaB ligand. *Blood.* 2001;97(11):3349-3353.

8. Adams GB, Scadden DT. The hematopoietic stem cell in its place. *Nat Immunol.* 2006;7(4):333-337.

9. Kiel MJ, Radice GL, Morrison SJ. Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. *Cell Stem Cell.* 2007;1(2):204-217.

10. Mendez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature.* 2010;466(7308):829-834.

Comment on Milanov et al, page 602

FIXing Factor VIII inhibitors

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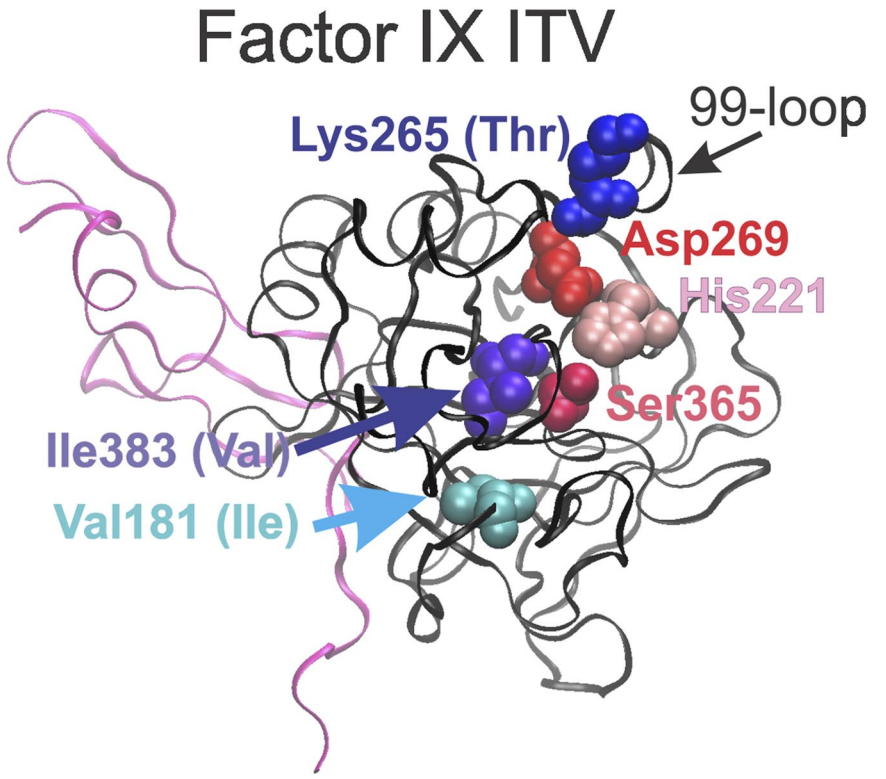
In this issue of *Blood*, Milanov and colleagues demonstrate that a Factor IX (FIX) variant that does not require activated Factor VIII (FVIIIa) for activity induces coagulation in hemophilia A mice with FVIII inhibitors.[1] This protein might be developed as a bypass agent.



The stucture of FIX is based on that of Wang et al.[6] The ribbon structure of the heavy chain of activated FIX appears in black and part of the light chain in pink. The amino acids of the catalytic triad (His221, Asp269, and Ser365) are identified in reds and pinks. The amino acids of normal FIX that were mutated to generate FIX-ITV are identified in blues and purples, with the amino acids present in FIX-ITV shown in parenthesis. PDB code is 3LC3. Jay W. Ponder assisted in preparing the figure.

Inhibitors of FVIII are antibodies that block its coagulation function. For congenital hemophilia A, inhibitors develop in ~ 20% of all patients, for an overall incidence of 1:50 000 of the general population, or ~ 6000 people in the United States. Acquired FVIII inhibitors can develop spontaneously in patients with a normal *FVIII* gene, and occur in ~ 1 per 1 million people.[2] Bleeding episodes in such patients are often treated with so-called bypass agents, which allow coagulation to occur in the absence of FVIII activity.[3] One bypass agent is recombinant FVIIa, which activates FX of the common pathway of coagulation, and FIX of the extrinsic pathway. A second bypass agent is Factor 8 Inhibitor Bypassing Activity (FEIBA), a prothrombin complex concentrate that contains activated vitamin K–dependent coagulation factors. FVIIa and FEIBA appear to be roughly equivalent in their efficacy for minor bleeding episodes.[4] FVIIa is given up to every 2 hours at a cost of ~ $8000 per dose for an adult, while FEIBA is given up to every 8 hours at a cost of ~ $8500 per dose.[5] One of the major drawbacks for both of these treatments is the short half-life of the active protein(s), and the cost of treatment for a single major bleed or surgery can easily exceed $250 000. The development of a hemostatic agent that is active in the presence of an inhibitor but has a longer half-life could reduce the cost of treating bleeding episodes.

Here, Milanov et al report the development and in vivo testing of a FIX variant with activity in the absence of FVIIIa.[1] A FIX protein designated FIX-ITV that contained 3 mutations (V181I, K265T, and I383V; see figure based on the wild-type FIX structure[6]). FIX-ITV was demonstrated to promote coagulation in vitro in an aPTT assay when present at wild-type FIX levels in the absence of FVIII at ~ 16% of the activity found when normal levels of wild-type FIX were present with normal levels of wild-type FVIII. The secreted form of wild-type FIX has 415 amino acids, and is cleaved between Arg145 and Ala146, and between Arg180 and Val181 to generate the activated 2-chain FIXa protein for which the heavy chain (181-415) contains the proteolytic domain and the light chain (1-145) contains γ-carboxyglutamate (GLA) and epidermal growth factor (EGF) domains.[3] The amino acids for the catalytic triad are residues His221, Asp269, and Ser365 (see figure). It is believed that FVIIIa functions by altering the conformation of the so-called 99-loop, which are amino acids 94 to 99 using the chymotrypsin numbering system, which represent amino acids 259 to 266 of FIX.[7] The 99-loop is proposed to block the active site in the absence of FVIIIa. Although it was not definitely proven in this or prior studies with related proteins, the alterations in FIX-ITV were proposed to move the 99-loop and open up the FIX active site in the absence of FVIIIa, which would allow FIX to have access to its substrate, FX, and cleave FX more efficiently.[8,9]