with the inherent limitations of such an analysis, including lack of uniform follow-up, response assessments, and toxicity management. In addition, the unusually short duration of first KI therapy and the fact that the toxicities leading to discontinuation were often serious (atrial fibrillation) and unusually frequent (17% pneumonitis among patients with discontinued idelalisib), testify to the selective nature of the cohort. The authors describe real world experience with regard to the outcomes, but it is notable that all centers involved are university hospitals with expertise in the management of CLL and KI toxicities.

Additionally, they report that “Moreover, outcomes did not appear to differ whether ibrutinib or idelalisib was selected as the first or second KI, suggesting that either sequence is appropriate.” However, we would urge caution in this interpretation, as no data are provided on response rates and PFS for the first KI. Data were only collected on the patients who discontinued the first KI; hence, the true response rate to the first KI and median PFS was not captured.

Resistance to KI remains a major clinical concern. Many patients who fail ibrutinib have an acquired mutation of BTK C481S, or rarely, PLCγ2 mutation. Resistance mechanisms to idelalisib and venetoclax remain unclear and are the subject of ongoing investigations. The current study provides valuable data on the outcomes after KI discontinuation and emphasizes the need to develop more effective therapies for KI failure.

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**GENE THERAPY**

Comment on Richter et al, page 2206

*Gene therapy simplified*

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In this issue of *Blood*, Richter et al report their work on an in vivo gene transduction system using the adenoavirus-based vector and hyperactive SB transposase (SB1000×) system. Their results show that this system is effective and safe and performs without needing ex vivo expansion and transduction of hematopoietic stem cells (HSCs). This system may overcome some of the difficulties associated with cell collection and manufacturing and provide technical advances for the field of gene therapy.

Gene therapies using HSCs for the treatment of immunodeficiencies and inherited diseases have demonstrated substantial clinical benefits. Currently, most clinical gene therapies start with HSC mobilization using granulocyte colony-stimulating factor (G-CSF) alone or along with AMD3100 (Plerixafor) followed by leukapheresis collection and the enrichment of HSCs by immunoselection. The purified cells are stimulated and transduced in a cell manufacturing center located in the same institution as the collection facility or shipped to another facility for manufacture. For the safety of patients and quality of the collected and manufactured cells, all these procedures must be conducted in accredited facilities following strict standards (ie, American Association of Blood Banks Standards for Cellular Therapy Services and standards of Foundation for Accreditation Cellular Therapy in the United States). In addition, the collection and production of HSCs for gene therapies must also follow the guidance of regulatory agencies like the European Union or US Food and Drug Administration. To meet these requirements, considerable investment is needed, particularly in establishing and maintaining the cell processing center. In addition, manufacturing the gene corrected autologous HSCs can be costly. In this paper, the autologous HSCs were mobilized into peripheral blood, and the HSCs were directly transduced with a novel transduction system in vivo, resulting in functional transduced HSCs in animal models. Therefore, HSC collection and cell manufacturing were not required. These results imply that the gene therapy may be conducted in 1 facility, follow a simplified regulation pathway, and should be less costly.

This new system circumvents the technical/medical limitations of leukapheresis collection. Leukapheresis is very time consuming, and unfortunately, collection yields and efficiencies are highly variable. The efficiency of the CD34 HSC collection may be influenced by many factors, including the volume of processed whole blood, flow rates of the blood separators, and the decision to perform single or multiple collections. Venous access is also a limiting factor of leukapheresis, especially for pediatric patients, for whom a central line may be necessary for the collection.

This system also avoids the potential concerns associated with the ex vivo expansion and transduction of CD34 HSCs. At present, HSCs are typically stimulated and expanded using a cocktail of cytokines, such as stem cell factor, thrombopoietin, Fms-like tyrosine kinase 3 ligand, and interleukin 3 and then transduced with viral vectors. All of these steps may affect the phenotype, long-term viability, and homing and repopulation
capacity of HSCs once they are transplanted in vivo. In this paper, the murine HSCs were transduced directly in vivo without ex vivo manipulation. The study found that 2.45% of bone marrow cells and 7.7% of progenitor LSK cells in hCD46tg mice expressed the transduced gene 4 weeks after transduction; ~0.5% of huCD45 + cells and 1.5% of CD34 + cells in the bone marrow of humanized mice expressed the transduced gene 3 days after transduction, and 2.5% of huCD45 + cells in bone marrow expressed the transduced gene 4 week after transduction. The transduction efficiency was not as high as those reported in clinical trials using lentiviral vectors and ex vivo cell processing. However, gene-corrected cells may have a selective growth advantage over parental HSCs with defects, and gene therapy can be successful even when only a small fraction of the transplanted HSC has been transduced by the vector. Therefore, this system may prove to be a valuable method of clinical gene therapy.

Several questions must be addressed before translating the findings of this paper to clinical trials. The mobilization regimen in this paper used 4 doses of G-CSF and 1 dose of AMD3100, which resulted in variable numbers of mobilized CD34 + cells in hCD46tg mice and humanized mice. Because Plerixafor (AMD3100) and G-CSF mobilize different CD34 + cell populations from bone marrow, it would be interesting to test the transduction efficiency of this system on HSCs that are only mobilized by G-CSF. The increase of CD34 + HSCs in blood is transient, and the CD34 + cell counts may fall quickly if a daily dose of G-CSF is not given, so monitoring the white blood cell count and CD34 + counts daily will be helpful in evaluating the mobilization efficiency and deciding on the best time to conduct gene transduction.

The safety of the newly developed transduction system needs to be comprehensively evaluated. Early-stage gene therapies with retroviral vectors have been associated with side effects, such as leukemia, although lentiviral vectors are more efficient and safer. In this paper, the authors identified 155 distinct SB100×-mediated integration sites and no integration within or near a proto-oncogene, but it appears that chromosome 13 had more integration sites than the others, so it would be beneficial to further investigate the genotoxicity of this system. In addition, the toxicities of the adenoviral vectors must be carefully assessed. Although the Ad5/35 + vector was not detected in the liver, the genomes for some adenoviral vectors can be found in the lung, liver, heart, kidney, and spleen. Off-target toxicities are especially worrisome for adenoviral vectors due to the death of a patient in a gene therapy protocol using adenoviral vectors in 1999.

In summary, the work of Richter provides promising results in the development of in vivo gene transduction system, which may simplify gene therapy by eliminating autologous cell collection and ex vivo cell manipulation. After further characterization and proof of the method’s safety, it may prove to be useful for clinical gene therapy.

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REFERENCES


Comment on Rauch et al, page 2253

Down for the count in acute myeloid leukemia

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In this issue of Blood, Rauch et al provide evidence for a novel mechanism to explain a fundamental yet enigmatic observation that has plagued hematologists for decades: the decline in nonleukemic hematopoiesis in the bone marrow of patients with acute myeloid leukemia (AML). The authors found that high expression of the thrombopoietin (TPO) receptor MPL on AML blasts predicts neutropenia and thrombocytopenia, and that AML blasts expressing high levels of MPL deplete TPO in cell culture and in mouse models. Rather than crowding out normal bone marrow hematopoietic stem cells (HSCs), might MPL-expressing AML blasts impair hematopoiesis by stealing the cytokine TPO?"}

Most patients with AML present with cytopenias in 1 or more cell lineages. However, the mechanisms by which AML impairs normal hematopoiesis have remained elusive. Is it simple competition for nutrients or space? Why do some patients with a bone marrow full of blasts have relatively preserved blood counts, whereas others experience life-threatening cytopenias with far less bone marrow involvement?

Previous studies reveal multiple mechanisms by which AML blasts might induce cytopenias, including inhibition of normal HSC proliferation and differentiation. Leukemic cells also alter the stromal microenvironment, creating abnormal malignant niches that sequester normal
Gene therapy simplified

Jiaqiang Ren and David F. Stroncek