2. Smyth MJ, Thia KY, Street SE, MacGregor D, Godfrey DI, Trapani JA. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. J Exp Med. 2000;192:755-760.

3. Stepp SE, Mathew PA, Bennett M, de Saint Basile G, Kumar V. Perforin: more than just an effector molecule. Immunol Today. 2000;21:254-256.

4. Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. Blood. 2004;104:735-743.

5. Voskoboinik I, Thia MC, Trapani JA. A functional analysis of the putative polymorphisms A91V and N252S, and 22 missense perforin mutations associated with familial hemophagocytic lymphohistiocytosis. Blood. Prepublished on March 8, 2005 as DOI 10.1182/blood-2004-12-4935.

6. Trambas C, Gallo F, Pende D, et al. A single amino acid change, A91V, lead to conformational changes which can impair processing to the active form of perforin. Blood. Prepublished on March 1, 2005 as DOI 10.1182/blood-2004-09-3713.

7. Davidson WF, Giese T, Fredrickson TN. Spontaneous development of plasmacytoid tumors in mice with defective Fas-Fas ligand interactions. J Exp Med. 1998;187:1825-1838.

PLENARY PAPER

Comment on Guzman et al, page 4163

The long and winding road may be getting shorter

Giorgio Zauli and Paola Secchiero UNIVERSITY OF TRIESTE, UNIVERSITY OF FERRARA

Guzman and collegues demonstrate that it is possible to specifically target leukemic stem/progenitor cells with the naturally occurring small molecule parthenolide. These findings open new avenues for the treatment of hematological malignancies.

The field of stem cell biology has recently provided new insights on the pathogenesis, self-renewal, and spreading of both solid cancers and hematologic malignancies. It is of great practical interest to better understand the role of these putative cancer stem cells, for obvious therapeutic and follow-up management purposes.[1-3] In this issue of *Blood*, Guzman and colleagues show, through standard colony-forming unit (CFU) in vitro assays, that $CD34^+CD38^-$ human leukemic stem/progenitor cells (HLSCs), obtained from different patients affected by acute and blast crisis chronic myeloid leukemia (AML and bcCML), are more prone to parthenolide-induced apoptosis than the normal counterparts (human hemopoietic stem cells [HSCs]) with remarkable specificity. Moreover, the authors demonstrated, with reconstitution studies in mice, that HLSCs are exquisitely sensitive to the action of the small molecule parthenolide in vivo, underscoring the pivotal role of HLSCs in the establishment of acute leukemia in vivo. In fact, parthenolide efficiently inhibited the engraftment of the human leukemic cell population in the bone marrow of sublethally irradiated nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice reconstituted by primary human cells obtained from leukemic progenitors. The ability of parthenolide to kill HLSCs while sparing normal HSCs is of particular relevance also in consideration of the fact that cytosine arabinoside (Ara-C), one of the most widely employed drugs in the treatment of AML, is much less specific than parthenolide for AML cells and in particular for the HLSC fraction. Consistently, Guzman and colleagues demonstrated that Ara-C is significantly more toxic to normal HSCs. In an attempt to gain insights on the mechanism of action of parthenolide, Guzman and collegues showed that nuclear factor κB (NF-κB) inhibition, p53 activation/phosphorylation, and induction of reactive oxygen intermediates concur in variable fashion to the effects of the drug on HLSCs. Although the mechanism of action of parthenolide requires further investigation, manipulation of these intracellular pathways might lead to potentiation of the antineoplastic activity of parthenolide. It is also suggested that parthenolide might synergize with tumor necrosis factor (TNF)–related apoptosis inducing ligand (TRAIL)[4] in inducing AML cytotoxicity. Although this possibility is particularly attractive considering the relatively low toxicity of TRAIL on normal HSCs and its therapeutic potential for the combined treatment of hematologic malignancies,[5] it remains to be demonstrated whether HLSCs express the death receptors TRAIL-R1 and/or TRAIL-R2. Although the low solubility in water and poor pharmacologic properties of parthenolide represent a major concern for its therapeutic use, Guzman and colleagues cite unpublished data demonstrating substantial improvement by medicinal chemistry of the molecule, with maintenance of the antitumor properties in several parthenolide analogues. Through this newborn field of cancer stem cell chemotherapy, the long road to a less toxic and more efficient and specific therapy toward AML can now be attained. Further studies will help in translating these very promising findings into clinical practice. ■

REFERENCES

1. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414:105-111.

2. Mihich E, Jaenisch R. Sixteenth annual Pezcoller symposium: stem cells and epigenesis in cancer. Cancer Res. 2004;64:8474-8477.

3. Clarke MF. Chronic myelogenous leukaemia—identifying the hydra's heads. N Engl J Med. 2004;351:634-636.

4. Nakshatri H, Rice SE, Bhat-Nakshatri P. Antitumor agent parthenolide reverses resistance of breast cancer cells to tumor necrosis factor-related apoptosis-inducing ligand through sustained activation of c-Jun N-terminal kinase. Oncogene. 2004;23:7330-7344.

5. Secchiero P, Vaccarezza M, Gonelli A, Zauli G. TNF-related apoptosis-inducing ligand (TRAIL): a potential candidate for combined treatment of hematological malignancies. Curr Pharm Des. 2004;10:3673-3681.

GENE THERAPY

Comment on Thrasher et al, page 4255

Old before its time: age-related thymic dysfunction may preclude efficacy of gene therapy in older SCID-X1 patients

Ruth Seggewiss and Cynthia E. Dunbar NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Gene therapy can not be reserved as a "salvage therapy" in SCID-X1 patients failing bone marrow transplantation.

Thrasher and colleagues report in this issue of *Blood* on the failure of gene therapy to correct immune function when attempted in 2 patients aged 15 and 20 years with X-linked severe combined immunodeficiency (SCID-X1). Besides the implications for the timing of gene therapy, the study also further demonstrates the rapid and irreversible aging of the thymus, and highlights the difficulties of completely recreating an immune system beyond early childhood.

Children with severe forms of SCID, in particular SCID-X1, usually die at less than 1 year of age from opportunistic infections. Long-term survival is possible only with allogeneic stem cell transplantation (SCT), but delayed diagnosis can result in severe infections that preclude successful transplantation.[1] Even with early allogeneic transplantation, in particular with haploidentical parental donors, there is incomplete engraftment, with progressive defects in T-cell immunity and a complete lack of donor B cells, resulting in dependency on monthly intravenous infusions of immunoglobulin. These poor outcomes stimulated intense interest in gene therapy approaches to SCID.

In about 50% of all cases, SCID is inherited as an X-linked recessive disease characterized by a block in T and natural killer (NK) lymphocyte differentiation. The defective gene encodes the common cytokine receptor γ-chain, located on the X chromosome. The report of complete immune correction of young SCID-X1 boys by transduction of their $CD34^+$ bone marrow cells with a retroviral vector encoding the common γ-chain, and reinfusion of transduced cells without conditioning therapy, was very encouraging and represented the first unequivocal demonstration of gene therapy efficacy.[2,3] However, worryingly, 2 children in this trial developed T-cell leukemia due to insertion of the retroviral vector near the promoter of the proto-oncogene LMO2,[4] and a third child out of a total of 11 entered in the trial was recently also reported to have T-cell leukemia. Thus, at present the risks and potential benefits of gene therapy for this disease are being reconsidered.

Thrasher and colleagues now report the failure of gene therapy to produce therapeutic effects and T-cell reconstitution despite successful $CD34^+$ cell transduction in 2 patients with SCID-X1 aged 15 and 20 years, both enrolled in the trial at these advanced ages due to gradual failure of immune function following bone marrow transplantation (BMT). Whether the apparent impossibility for productive thymopoiesis in these patients was the result of previous infections, graft-versus-host disease (GVHD), or physiological aging of the thymus remains hypothetical. It mirrors the observed correlation between patient age at the time of BMT and poor immune outcome in SCID-X1 (eg, Patel et al[5]). This is a very important finding regarding the current discussion about the optimal treatment strategy in the absence of human leukocyte antigen (HLA)–matched donors for SCID-X1 patients. It appears that gene therapy can not be reserved as a "salvage therapy" in SCID-X1 patients failing or not maintaining immune function following BMT. There is a need to compare the oncogenic potential of different vectors in relevant animal models in order to develop safer gene therapy approaches based on their potential for insertional activation of oncogenes, but, as the work of Thrasher and colleagues demonstrates, additional efforts are also required to better understand the biology of diseases that are candidates for therapeutic genetic intervention to design optimal treatment strategies. ■

REFERENCES

1. Buckley RH, Schiff RI, Schiff SE, et al. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. J Pediatr. 1997;130:378-387.

2. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science. 2000;288:669-672.

3. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med. 2002;346:1185-1193.

4. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science. 2003;302:415-419.

5. Patel DD, Gooding ME, Parrott RE, Curtis KM, Haynes BF, Buckley RH. Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med. 2000;342:1325-1332.