Correcting Immune Deficiency with Direct Injection Gene Therapy

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One method to correct genetic defects in blood-related diseases is through gene therapy, delivering corrective genes to stem cells through viral vectors. In *ex vivo* gene therapy, hematopoietic stem or progenitor cells are isolated from patients and transduced with viral vectors in an artificial environment, which requires highly specialized culture conditions and facilities. These manipulated cells are then returned to their host to repopulate the blood system with genetically corrected cells, though with a loss of stem cell repopulating potential. *In vivo* gene therapy mitigates these drawbacks through direct injection of viral vectors to transduce hematopoietic stem/progenitor cells in their natural environment. A new study published in *Blood* reports success of *in vivo* gene therapy, using a foamy virus vector to correct severe combined immunodeficiency (SCID) in a canine model. The study was led by postdoctoral fellow Dr. Christopher Burtner in the laboratory of Dr. Hans-Peter Kiem in the Clinical Research Division.

SCID is a combination of several genetic diseases, which together block the production of T cells, with some forms also affecting the development of natural killer cells and B cells. The lack of an intact immune system results in recurring opportunistic infections in children. Half of all SCID cases are due to an X-linked inherited mutation in the common gamma chain (*IL2RG* gene), which serves as a receptor for a variety of cytokines that control immune cell development and function. Hematopoietic stem cell transplant is the most common treatment for SCID-X1, but with significant side effects. Pioneering *ex vivo* gene therapy trials were conducted in the 1990s to reintroduce *IL2RG* expression, but were halted after one-fourth of patients developed leukemia due to viral integration near proto-oncogenes.

For the current study, Burtner and colleagues utilized a foamy virus to correct *IL2RG* expression for in vivo gene therapy. Foamy viruses largely resist serum inactivation so would have improved stability for *in vivo* gene therapy. Foamy viruses are also less likely to integrate near proto-oncogenes compared to other viral vectors, decreasing the risk for leukemia observed in clinical trials using a gammaretrovirus.

The researchers injected a single dose of a foamy vector encoding the human *IL2RG* gene intravenously in five newborn dogs with SCID-X1. T cells positive for *IL2RG* were detected in all dogs as early as 2 weeks. The authors characterized the reconstituted T cells by flow cytometry for cell surface markers and for T-cell receptor gene rearrangement, indicating normal development with diverse T-cell receptor repertoires. Furthermore, the researchers confirmed expression of functional common gamma chain in T cells by exposing peripheral blood mononuclear cells to a cytokine that binds the receptor, interleukin-2 (IL-2), and testing receptor activation of cell signaling proteins.

Next, the researchers examined the retroviral integration sites in the gene-modified cells. Multiple integration sites were found indicating the reconstituted immune cells derived from several

individually infected cells. Greater than 20% of the T cells had the same integration sites, suggesting clonal dominance in at least one time point examined for each dog.

Importantly, the researchers detected viral integration in some myeloid cells in addition to T cells, suggesting that immature hematopoietic stem cells could be transduced with *in vivo* gene therapy. Despite the broad tropism of the foamy virus vector, only one off-target viral integration occurred in the gut cells of one dog. Although all dogs showed immune reconstitution to some extent, they all succumbed to infections that limited the follow up time to 10.5 months.

According to Dr. Burtner, "*in vivo* gene therapy is portable and scalable, meaning multiple doses can be administered to patients based on the disease and individual response to therapy." Higher infectious titers, or serial injections over days, may increase the number of genetically modified cells and lower the clonal dominance. Furthermore, adjusting the dose and the strength of the promoter driving corrected gene expression could optimize clinical outcomes.

Burtner, C.R., Beard, B.C., Kennedy, D.R., Wohlfahrt, M.E., Adair, J.E., Trobridge, G.D., Scharenberg, A.M., Torgerson, T.R., Rawlings, D.J., Felsburg, P.J., Kiem, H.-P. 2014. Intravenous injection of a foamy virus vector to correct canine SCID-X1. *Blood*. Epub ahead of print, doi: 10.1182/blood-2013-11-538926

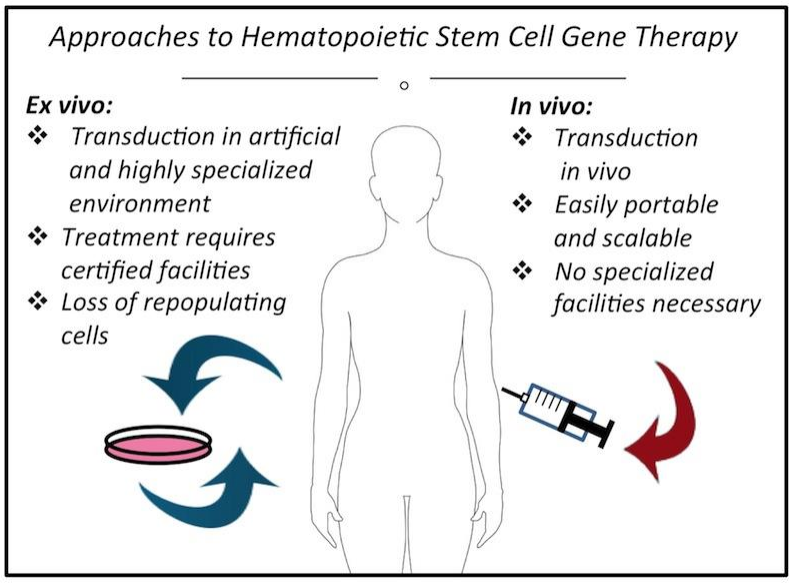

Image courtesy of Dr. Christopher Burtner and Grace Choi

Ex vivo gene therapy requires culturing and transducing hematopoietic stem/progenitor cells in an artificial environment, which requires highly specialized culture conditions and facilities and is associated with loss of stem cell repopulating potential. In vivo gene therapy mitigates these drawbacks by transducing hematopoietic stem/progenitor cells in their natural environment.