In Vivo Hematopoietic Stem Cell Transduction

Maximilian Richter, PhD[a,1], Daniel Stone, PhD[b,1], Carol Miao, PhD[c,d], Olivier Humbert, PhD[e], Hans-Peter Kiem, MD, PhD[e,f], Thalia Papayannopoulou, MD[g], André Lieber, MD, PhD[a,h,*]

KEYWORDS

• Intravenous • Intraosseal • Viral vectors • Mobilization

KEY POINTS

- Current protocols for hematopoietic stem gene therapy, involving the transplantation of ex vivo genetically modified hematopoietic stem cell (HSC), are complex and not without risk for the patient.
- HSCs in the bone marrow are intricately connected with the bone marrow stroma, which creates a physical barrier to transduction with intravenously injected gene transfer vectors.
- Intraosseal injection of viral vectors has been shown to result in in vivo transduction of HSCs in mice. It might be more feasible and efficient in large animals and humans.
- A new approach that involves the mobilization of HSCs from the bone marrow, their transduction in the periphery, and return to the bone marrow has shown first promising results.

INTRODUCTION

Hematopoietic Stem Cells in the Bone Marrow

Most hematopoietic stem cells (HSCs) reside in so-called stem cell niches regions of the bone marrow in which nonhematopoietic cells interact with HSCs and regulate their dormancy and self-renewal or their differentiation and expansion.[1] However, a

The authors declare no commercial or financial interest.

[a] Division of Medical Genetics, University of Washington, 1705 NE Pacific Street, Seattle, WA 98195, USA; [b] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, Seattle, WA 98109, USA; [c] Department of Pediatrics, University of Washington, 1705 NE Pacific Street, Seattle, WA 98195, USA; [d] Center for Immunity and Immunotherapy, Research Institute, Seattle Children's Hospital, 1900 9th Avenue, Seattle, WA 98101, USA; [e] Clinical Research Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Aveune N, Seattle, WA 98109, USA; [f] Department of Medicine, University of Washington School of Medicine, 1705 NE Pacific Street, Seattle, WA 98195, USA; [g] Division of Hematology, University of Washington, 1705 NE Pacific Street, Seattle, WA 98195, USA; [h] Department of Pathology, University of Washington, 1705 NE Pacific Street, Seattle, WA 98195, USA
[1] Equal contribution.
* Corresponding author. Division of Medical Genetics, University of Washington, Box 357720, Seattle, WA 98195.
E-mail address: lieber00@uw.edu

Hematol Oncol Clin N Am 31 (2017) 771–785
http://dx.doi.org/10.1016/j.hoc.2017.06.001 **hemonc.theclinics.com**

small fraction of HSCs circulate in the peripheral blood under steady-state conditions.[2] HSCs that leave the bone marrow under steady-state conditions provide a means of exchange between different stem cell niches as well as a way to react to local damage within hematopoietic tissues. Even under normal conditions, circulating HSCs appear to be able to rapidly re-engraft in bone marrow so that there is a cycle of constant egress and re-engraftment of HSCs.[3] HSC regulation and retention within the bone marrow stem cell niche are mediated through multiple interactions between HSC surface receptors and their respective ligands expressed or secreted by surrounding cells, including osteoblasts and sinusoidal endothelial and perivascular cells.

Hematopoietic Stem Cell Mobilization

The induced egress of HSCs is referred to as stem cell mobilization. Although egress of HSCs from the bone marrow can be observed in response to stress, for example, following injury of hematopoietic organs, stimulated mobilization is commonly achieved through drug administration. The most commonly used mobilizing agent is recombinant human granulocyte colony-stimulating factor (G-CSF), which is given in the form of subcutaneous injections for 5 days and leads to efficient mobilization of both HSCs and more differentiated cells.[2] Another class of mobilizing agents is CXCR4 antagonists, most prominently the US Food and Drug Administration–approved drug Plerixafor/AMD3100. CXCR4 antagonists lead to more rapid mobilization of HSCs than G-CSF[4] and are thought to cause mobilization solely through disruption of the SDF-1-CXCR4 axis. AMD3100 has been shown to synergize with G-CSF mobilization. Because of its higher mobilization power, the combination of G-CSF and AMD3100 is used as a regimen in poor HSC mobilizers such as chemotherapy patients.[5] In a similar manner, soluble stem cell factor (SCF) is able to interrupt the connection between membrane-bound SCF and c-kit, even though SCF is considered to be a slow mobilizing agent and has to be administered for several days.[6] Another class of mobilizing agents target VLA-4, and both VLA-4 binding antibodies[7] and small molecules[8] are able to rapidly mobilize HSCs. VLA-4 binding agents are also thought to have a synergistic or additive effect when used together with G-CSF and AMD3100.[9] One VLA-4 inhibitor, the small molecule BIO5192, was shown to efficiently and rapidly mobilize HSCs. Furthermore, BIO5192 can be combined with G-CSF alone or with AMD31000 to increase levels of mobilized HSCs. BOP (*N*-(benzenesulfonyl)-L-prolyl-L-O-(1-pyrrolidinylcarbonyl)tyrosine), a small molecule targeting $\alpha 9\beta 1/\alpha 4\beta 1$ integrins, also rapidly mobilizes long-term multilineage reconstituting HSC.[10] Synergistic engraftment augmentation is observed when BOP is coadministered with AMD3100.

EXAMPLES FOR IN VIVO HEMATOPOIETIC STEM CELL GENE THERAPY

Example 1: Intravenous Injection of Triplex-Forming Peptide Nucleic Acids

Peptide nucleic acids (PNAs) are designed to bind site-specifically to genomic DNA via strand invasion and formation of PNA/DNA/PNA triplexes with a displaced DNA strand. PNAs consist of a charge-neutral peptide-like backbone and nucleobases enabling high-affinity hybridization with DNA (**Fig. 1**). PNA/DNA/PNA triplexes can be used to modify DNA by recruiting endogenous DNA repair proteins to initiate site-specific modification of the genome when single-stranded "donor DNAs" are codelivered as templates containing the desired sequence modification.[11]

A recent study reported in vivo HSC gene editing in mice using intravenously (IV) injected triplex-forming PNAs in combination with SCF given intraperitoneally 3 hours before the PNAs.[12] Treatment of transgenic mice carrying a β-globin/GFP reporter transgene with PNAs and single-stranded donor DNA yielded gene editing in mouse