

Swapping the serotype: A novel helper-dependent adenoviral vector platform for *in vivo* HSC gene therapy

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Gene therapy of hematopoietic stem cells (HSCs) has been successfully applied to ameliorate monogenic blood disorders, such as severe combined immunodeficiencies and β -hemoglobinopathies. However, the treatment is resource intensive and limited to patients with access to specialized facilities. The development of *in vivo* HSC gene therapy could help enable wider distribution, including to the Global South, where diseases such as sickle cell disease are especially prevalent. Previously, Andre Lieber and colleagues developed *in vivo* HSC gene therapy protocols using helper-dependent adenoviral vectors and demonstrated proof-of-concept in mice and non-human primates.^{1–3} Their approach relies on the mobilization of HSCs from the bone marrow niche followed by the intravenous administration of the adenoviral vector, which includes a transposase to stably integrate the therapeutic transgene into the host genome, and *in vivo* selection of transduced cells using low-dose chemotherapy.⁴ By *in vivo* delivery, these protocols circumvent the need for HSC harvest, *ex vivo* HSC cultivation, and myeloablative conditioning, which may greatly reduce the toxicity and complexity of the procedure.

Previously, this research group had focused on the HDAd5/35++ modified helper-dependent adenoviral vector system. This vector platform is based on species C Ad5 serotype and has been modified with mutated Ad35 fiber knobs to increase affinity to CD46 entry receptor, a target antigen that is highly expressed on HSCs. In the present study (see [Figure 1](#)), Wang et al. switched to the species C Ad6 serotype, which has a similar genome structure compared with Ad5 but is associated with

reduced prevalence of neutralizing antibodies in humans.⁵ The authors demonstrate that Ad6- and Ad5-based vectors transduce human and rhesus CD34+ hematopoietic stem and progenitor cells (HSPCs) at similar rates *in vitro* (on average 25%). The novel HDAd6/35++ vector was tested in short-term and long-term *in vivo* gene therapy experiments using a hCD46-transgenic mouse model. In both settings, HDAd6/35++ performed comparably or slightly better than the original HDAd5/35++ vector. Wang et al. used humanized NBSGW mice as an additional model, demonstrating that the HDAd6/35++ novel vector platform is capable of transducing *in vivo* human HSCs and not only hCD46-transgenic murine HSCs. Again both vector platforms performed comparably (8% transduced CD34+ cells in the bone marrow in a short-term experiment). Overall, the transduction rates were higher in CD34+ HSPCs compared with lineage-committed populations, which the authors attributed to the higher density of hCD46 on HSPCs. Finally, the vectors were tested in Ad5 pre-immunized mice, which mimic pre-existing immunity against HDAd5/35++. In this context, the novel HDAd6/35++ outperformed the HDAd5/35++ vector, confirming that neutralizing antibodies against serotype Ad5 are not cross-reactive. This lack of cross-reactivity could encourage future experiments to test if sequential dosing with different serotypes might improve HSC gene transfer.

Besides better evading pre-existing immunity, the novel HDAd6/35++ vector also showed less liver off-target tissue transduction compared with HDAd5/35++ (liver vec-

tor copy number of 0.5 compared with 4, respectively).⁵ The reduction in off-target tissue transduction could be important to improve the safety and efficacy of the gene therapy. Moreover, by avoiding sequestration of viral vectors by off-target tissues, viral vector doses could potentially be decreased to achieve requisite transduction levels in HSCs, which could help reduce the overall cost of the therapy.

The most pressing challenge in the *in vivo* HSC gene therapy field is to achieve higher HSC-specific transduction to create an efficient and cost-effective gene therapy product. Unfortunately, there is not a single, unique antigen for HSCs. CD46 as well as other antigens such as CD117 and CD90 are highly expressed on HSCs but also on other hematopoietic and non-hematopoietic cell types, which ultimately results in potential off-target cell transduction and sequestration of viral particles. In this vein, recent work from the Lieber lab has explored desmoglein 2 as an alternative receptor for *in vivo* gene therapy of NHP CD34+ cells, showcasing the flexibility of the adenoviral vector system for different HSC target receptors.⁶ Other efforts have investigated the co-expression of multiple targeting moieties such as through scFv domains on enveloped particles or protein switches to achieve cell type-specific targeting.^{7,8}

An area of concern regarding adenoviral vectors is their immunogenicity, which may necessitate immunosuppressive treatment. Relatively high MOIs and vector doses may also be required to achieve cell transduction (2,000 viral particles/cell *in vitro* or doses of 10^{12} viral particles/kg body weight). Similar to retroviral vectors, there is a small chance of insertional mutagenesis associated with transposase-mediated semirandom integration, which might only be avoided by targeted integration or genome editing. Also the

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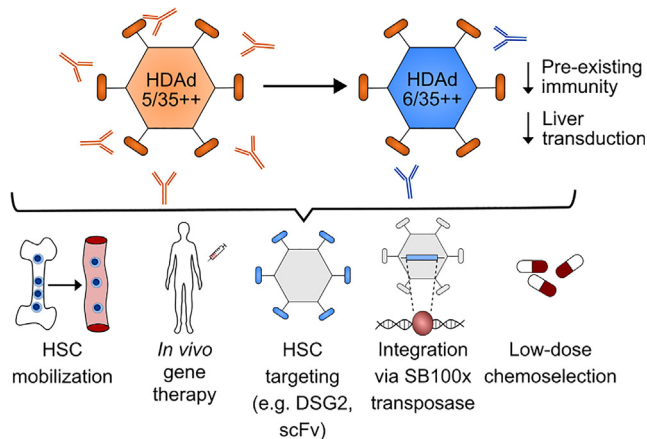


Figure 1. Swapping the serotype of HDAd5/35++ adenoviral vectors to HDAd6/35++ overcomes the limitation of pre-existing immunity and reduces off-target transduction of hepatocytes

Upon mobilization of HSCs from the bone marrow to the peripheral blood, this novel vector platform is suitable to transduce HSCs for *in vivo* gene therapy. HSC specificity can be modulated by introduction of specific targeting moieties on the viral particle. Using the Sleeping Beauty SB100x transposase, the adenoviral vector genome may be stably integrated into the host genome of transduced cells, which can be subsequently enriched to a therapeutic level using low-dose chemotherapy selection.

requirement for *in vivo* selection with MGMT-P140K overexpression and low-dose chemotherapy (O6BG/BCNU) to achieve high gene marking rates raises questions about possible long-term adverse events due to chemotherapy. Notwithstanding these remaining challenges, the current study⁵ represents a forward step toward the holy grail of *in vivo* gene therapy that could be distributed worldwide.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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