EDITORIAL

Safety of Adeno-associated virus-based vector-mediated gene therapy—impact of vector dose

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Gene therapy has become a realistic option for the treatment of various genetic diseases [1]. It involves the use of a vector (viral/non-viral) to deliver the required transgene for augmentation or correction of gene function [2]. Viral vectors have taken the center stage in gene therapy applications due to their evolutionarily fine-tuned ability to infect host tissue/cells [3]. Adeno-associated virus (AAV) [4] based vectors, being relatively safe [5, 6] have been evaluated in several clinical trials ($n = 263$, date of access 28th October 2021) (https://clinicaltrials.gov/). AAV based products such as Luxturna© and Zolgensma© have been approved by the US Food and Drug Administration (FDA) for the treatment of Leber congenital amaurosis type 2 (LCA2) and spinal muscular atrophy (SMA) [7] apart from several others that are available or in pipe-line [7]. Beside these successful attempts, some safety concerns have also emerged due to the mortality reported in a phase 2 clinical trial of a rare neuromuscular disease [8]. The etiology of such severe adverse events [9] in clinical settings and the basis of vector-related cytotoxicity [10], needs to be understood *in toto*.

To design an optimal gene transfer approach for a particular disease, a thorough understanding of the clinical data available with respect to safety and efficacy is essential. Herein, we have analyzed the retrospective and published data available from completed/ ongoing AAV-based clinical trials (https://clinicaltrials.gov/). We compared the clinical studies with respect to the disease targeted, route of vector delivery, dose administered, number of patients, adverse events, and major outcomes reported (Supplementary Table S1). While analyzing the data from clinical trials involving systemic vector administration, the target diseases included hemophilia A and hemophilia B, alpha-1 antitrypsin (AAT) deficiency, SMA, and lipoprotein lipase deficiency. Different AAV serotypes (AAV1, AAV2, AAV5, AAV8, and AAV9) have been used for gene therapy of these inherited disorders at vector doses ranging from 2×10^{11} to 2×10^{14} vector genomes per kilogram (vgs/kg) per patient. There is also an evidence for dose-dependent manifestation of the adverse events. In one of the first reports, at a lower dose (2×10^{11} vgs/kg) of AAV2-Factor IX vector, mild adverse events like increased neutralizing antibody titres was noted in hemophilia B patients, but a CD8+ T-cell response against the capsid and increased liver transaminases were noted at a higher dose of 2×10^{12} vgs/kg (Supplementary Table S1). In case of AAT deficiency, vector dose ranging from 6×10^{11} to 6.9×10^{13} vgs/kg generated a neutralizing antibody response and T-cell response while the phenotypic outcome was sub-therapeutic (Supplementary Table S1). For treatment of SMA, AAV9 vectors were used at a dose of 6.7×10^{13}–2×10^{14} vgs/kg, which led to a mild elevation in liver transaminase but led to improved survival and motor function, after gene therapy (Supplementary Table S1).

For localized gene therapy involving the delivery of the vector directly into the affected organ, diseases pertaining to eye/retina (retinoschisis, X-linked retinitis pigmentosa, LCA2, choroideremia), heart (cardiac failure), muscle (muscular dystrophy), and brain (Parkinson's) have been targeted with a vector dose ranging between 1×10^{9} and 1×10^{13} vgs/patient. Ocular diseases (retinoschisis, X-linked retinitis pigmentosa, LCA2, choroideremia) have been majorly treated *via* AAV2 or AAV8 serotype-based vectors (1×10^{9}–1×10^{12} vgs/eye). While at the lower doses of 1×10^{9}–1×10^{10} vgs/eye, none to only mild adverse events were noted, at higher doses (1×10^{11}–1×10^{12} vgs/eye) adverse events like inflammation and antibodies against AAV capsid with substantial improvement in the phenotypic outcomes were observed (Supplementary Table S1). In case of heart failure, AAV1 has been employed so far within a dose range of 1.4×10^{11}–1×10^{13} vgs/patient. The outcome data showed no significant adverse events when vectors were given to patients at lower doses (1.4×10^{11} vgs/patient), while a patient who had received AAV1-SERCA2a vectors at the higher dose of 1×10^{13} vgs/patient had a neutralizing antibody response (Supplementary Table S1). In patients with Becker and Duchenne muscular dystrophy, AAV1 and mutant AAV2 capsids have been used (6×10^{11}–3×10^{12} vgs/patient) causing no major adverse events, with an exception of neutralizing antibodies against AAV2 mutant capsid, along with an improvement in the phenotypic outcomes such as walking ability (Supplementary Table S1). In Parkinson's disease, rAAV2-hAADC, AAV-GAD, or rAAV2—Neurturin vectors when administered within a dose range of 5×10^{9}–4.7×10^{12} vgs/patient, had no significant adverse events (mild neutralizing antibodies only) with a dose-dependent improvement in the phenotypic outcomes, except in one study [11] (Supplementary Table S1).

Taken together, these data highlight that gene therapy is relatively safe in diseases that require either localized/systemic delivery and at a lower threshold of vector dose administered ($\leq 10^{12}$ vgs/kg/patient). However, further long-term follow-up data is required and as this information becomes available from the present clinical trials, a comprehensive understanding of the safety of AAV-based gene transfer will emerge. The efficacy of AAV vectors in these clinical trials was also largely dose-dependent [7] with the outcomes varying significantly based on the disease and serotype employed. Few preclinical studies on dog and mouse models have reported integration of recombinant AAV vector transgene into the host genome leading to clonal expansion [12] and other adverse events [13, 14] but this has not been observed in humans thus far. Nonetheless, establishing a more robust dose-response relationship during pre-clinical gene therapy and assessment of potential genotoxicity with the use of optimal viral vectors [15, 16] may further improve the safety and efficacy of AAV gene therapy in humans.

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AUTHOR CONTRIBUTIONS

SM, PS, and JGR analyzed data and wrote the manuscript. All the authors participated in a critical review of the manuscript and contributed to the final manuscript product.

CONFLICT OF INTEREST

The authors declare that IIT-Kanpur has filed non-provisional patents on improved AAV vectors for gene delivery.

ADDITIONAL INFORMATION

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