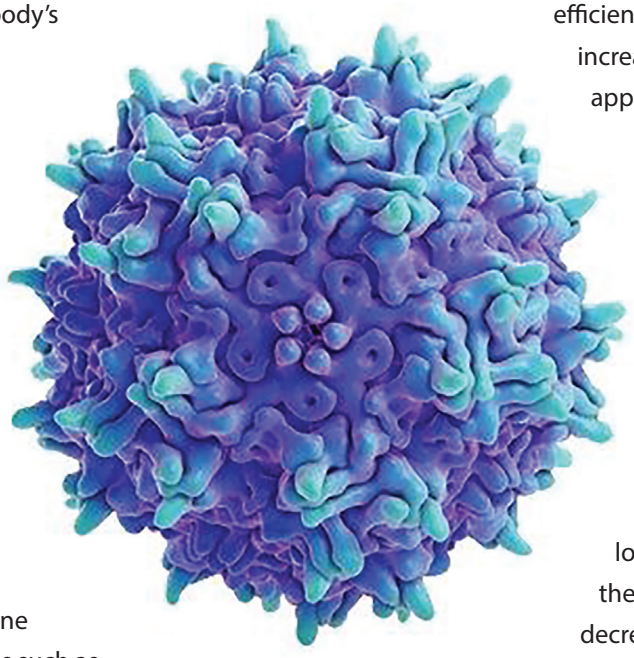
Transformative Technology That Overcomes the Challenge to **Effective Gene Therapy**

Recombinant adeno-associated viral vectors (AAV) are among the most popular gene delivery systems for gene therapy. While AAV vectors were initially thought to be minimally immunogenic, innate and adaptive immune responses have been observed and implicated in the loss of transgene expression in human gene therapy clinical trials.

NxGEN Vector Solutions has a technology that can reduce or eliminate the body's immune reaction toward your AAV vectors, leading to safe, effective, and durable therapeutic gene expression. Our technology builds gene therapy vectors that are stealthy, coming in under the immune radar, permitting AAV vector delivery without activating the body's immune defenses. The breakthrough came by identifying the precise reason that the AAV vectors were setting off the body's alarm bells: unmethylated CpG dinucleotide motifs in the AAV vector expression cassette. By removing the CpG dinucleotide motifs, AAV vectors could be designed to deliver the curative genes without activating the body's immune system.



Clinical Evidence

A recent publication in *Molecular Therapy* detailed the data from eight gene therapy clinical trials, including variables such as the AAV serotype employed, the CpG dinucleotide content in the open reading frame of the therapeutic gene, the vector production method, the vector dosage and estimated total capsid dose, the immunosuppressive drugs administered, the correlation of AAV gene transfer with a cytotoxic T lymphocyte response (CTL) response, the outcome peak of the therapeutic transgene, and the durability of the transgene. The power of this clinical trial information is that it is the single largest AAV vector clinical data set for a single disease—AAV gene therapy for hemophilia B—and allowed researchers to gain a clear insight into the key determinant for clinical success.

Among the variables, ***CpG content was revealed to be the only factor that correlates well with clinical outcome and long-term transgene expression, with unmethylated CpG content in AAV vectors being the key attribute that triggers transgene expression-limiting immune responses in humans***.

Codon modification to reduce the wild-type Factor IX (FIX) CpG content in the cDNA of the AAV vector expression cassette from 19 to 0 CpG motifs resulted in sustained FIX expression with the absence of or minimal CTL responses that were easily controlled by transient immune suppression for all four trials (33 combined subjects) reported. By contrast, in three clinical trials, a codon optimization approach to enhance transcriptional and translational efficiency of the expression cassette which increased the number of CpG motifs by approximately 5-fold over wild type cDNA resulted in a strong CTL response that was uncontrollable even with high dose immune suppression. In the two studies that published outcomes, a complete loss of FIX transgene expression was reported for all but one of 14 subjects. Clearly, CpG dinucleotide motifs in the AAV vector can trigger activation of a CTL response and therapeutic transgene loss in human clinical trials of AAV gene therapy, and vector design strategies to decrease the number of CpG motifs in the vector expression cassette are imperative to gene therapy clinical success. Partner with NxGEN Vector Solutions to apply NxGEN Technology to your AAV vectors and eliminate the immune response that limits transgene durability. Contact us at partnership@nxgenvectorsolutions.com. ■

For more information visit:
www.nxgenvectorsolutions.com

NxGEN VECTOR SOLUTIONS