A Novel Computationally-Designed AAV Rep Gene for the Efficient Generation of rAAV Vectors

Genetically recoded AAV Rep enables the generation of Ad/AAV hybrid viruses that can tolerate Rep expression and provides a unique opportunity to place all genetic elements into one virus for the purpose of safely integrating a transgene into a region of the human genome and for efficient production of rAAV vectors.

Background:
Sustained phenotypic correction of genetic defects requires a safe means of gene replacement. To date, many gene therapy strategies use integrating lentiviruses or retroviruses for long-term gene replacement, although their clinical applications remain limited because of potential for viral-associated onogenesis. Gene therapy strategies have attempted to use hybrid Adenovirus/Adeno-associated viruses (Ad/AAV) to combine the capacity, tropism and ease of production of adenovirus (Ad) with adeno-associated virus’s (AAV’s) ability for site-specific integration (SSI) into chromosome 19 AAVS1. Although the AAV Rep78 protein is required for SSI, the AAV Rep78 protein has the disadvantage of an inhibitory effect on Ad replication, particularly when co-expressed within the Ad backbone. This has lead to difficulty in generating an integrating transgene within the back-bone of a single hybrid virus, such as Ad/AAV. While an Adenovirus carrying the AAV cis acting elements can be constructed, construction of an Adenovirus carrying the Rep expression cassette has met with only limited success.

Technology Description:
Dr. Wadie Bahou and colleagues identified a 3’ Cis acting genetic element within the Rep gene sequence as responsible for the dominant acting sequence inhibiting Adenoviral replication. They genetically recoded the AAV Rep gene using synonymous codon pair re-engineering to overcome Rep’s inhibitory effects on adenoviral replication. The re-engineered Rep protein maintains wild-type amino acid sequence and endonuclease activity while dramatically increasing Adenovirus replication and viral titer yields to levels observed in Adenovirus lacking co-expressed Rep. This technology will enable the assembly of a single-backbone delivery system retaining the requisite genetic elements necessary for site-specific integration, and the generation of one-step packaging systems for recombinant AAV (rAAV) viral production.

Patents and Publications:
- Patent pending covering composition of matter and methods of use.
- Computationally-designed AAV Rep 78 is efficiently maintained within an adenovirus vector.
- Sitaraman et al. Submitted

Advantages
- Ability to generate generate Ad/AAV hybrid viruses that can tolerate expression of Rep
- Generation of one-step packaging systems for rAAV viral production

Applications
- New Gene Therapy Vectors: The technology enables the generation of novel Ad/AAV vectors that can stably integrate into the genome.
- Improved rAAV production methods: The technology enables the generation of a single backbone virus for easy production of rAAV.