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Editorial

Adenovirus-Based Vectors: Maximizing Opportunities and Optimizing a Rich Diversity of Vectors for Gene-Based Therapy

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THIS SPECIAL FOCUS ISSUE of *Human Gene Therapy* addresses many key aspects of adenovirus-based vectors and their utility for gene therapy and vaccine development, including detailed reviews and articles on adenovirus interactions with the host immune system, the complications of these effects, and how adenovirus can be developed and engineered to manipulate host immune responses. One review addresses the many challenges of manufacturing adenoviruses for clinical use, using adenovirus-based vaccines as the target disease area. Collectively, the reviews offer the latest insights into adenovirus biology, vectorology, and the intricate interactions of this virus with the host immune system. This issue also includes several original research articles on adenovirus, including articles on adenovirus-based vaccine development as well as basic adenovirus:host interactions and vector-based retargeting.

Adenoviridae is a large family of double-stranded DNA viruses with hosts including human and non-human primates, rodents, amphibians, reptiles, fish, and birds (for complete information on taxonomy and available sequences see the very useful website edited by Dr. Balazs Harrach, Institute for Veterinary Medical Research, Hungary, at www.vmri.hu/~harrach/AdVtaxlong.htm). In humans, the mastadenoviruses are pathogens that, in general, lead to self-limiting infections in immunocompetent hosts; however, they can be fatal in certain opportunistic situations, such as in children undergoing bone marrow transplantation and other immune-suppressed scenarios.

Adenovirus is a major virus-based vector system applied to human gene therapy and used in many past and present gene therapy trials worldwide (www.abedia.com/wiley/). Indeed, Dr. Ron Crystal has recently been presented with *Human Gene Therapy's* pioneer award for his efforts on early translation of adenovirus vectors into clinical gene therapeutics (Crystal, 2014). The vast majority of early studies used recombinant human serotype 5–based vectors (HAdV-5) because of the existing knowledge of its tropism *in vivo* as a viral vector and the creation of efficient vector systems for production of recombinant vectors, such as the vectors generated by Frank Graham in the 1980s. Many of these studies progressed to clinical trials, for example, in cardiovascular disease and cancer. HAdV5-based vectors offer high utility because of their versatile tropism, their large transgene carriage capacity, and its ability to achieve efficient transgene expression independent of cell cycle and without risk of insertional mutagenesis. The physical properties of adenovirus particles also highlight their suitability as potentially commercial products, being relatively hardy, non-enveloped ~110 nm monodisperse particles suitable for cGMP manufacturing.

Adenovirus vectors, mainly based on HAdV-5, have already been administered to several thousand patients with different diseases worldwide and, despite one well-publicized fatality involving a patient with compromised liver function who was administered a very large dose of HAdV-5 in 1999 (Raper *et al.*, 2003), the general safety record has been excellent. However, compared to other vector systems, such as retroviruses, lentiviruses, and adeno-associated viruses (AAV), the clinical efficacy data has, in general terms, been disappointing. This may be due to several reasons, including longevity of transgene expression using conventional ''first-generation/E1-deleted'' vectors and the prevalence of pre-existing immunity to HAdV-5 in the population. The disconnection between the efficacy in small and large animal models and the poor performance in clinical trials may also be impacted by fundamental differences between virus:host interactions among species that have often stirred the attention of many translationally minded adenovirus researchers. Efficient ''delivery'' of the virus is a major factor restricting progress of many important new genetic medicines, particularly those that lack direct access to the target tissue, either surgically or through *ex vivo* manipulation. Moreover, limited knowledge relating to host interactions that govern infectivity, biodistribution, and toxicity of the virus has perhaps restricted the refinement of adenovirus as a vector for medical applications. Notably, the

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use of these adenoviruses in early clinical trial applications has been in the absence of important knowledge relating to properties of the virus itself that have only recently come to light.

In recent years, many studies on adenovirus-mediated gene therapy have improved our understanding of the fundamental aspects of some adenoviruses, for example, improved understanding of virus structure, interactions with host factors via tropism-determining regions on the virus, and the virus's interaction with the immune system. In 2010, Glen Nemerow's group reported the crystal structure of HAdV-5 at 3.5Å resolution, providing new insights into both assembly and structure/function relationships that clearly impact vector design and utility. At the cell:host interaction level, the importance of coagulation factors in adenovirus-mediated gene transfer has unveiled a previously unknown function of the hexon protein in *in vivo* vector tropism (Kalyuzhniy *et al.*, 2008; Waddington *et al.*, 2008). Subsequent to this, the group of Andrew Byrnes has elegantly shown how this interaction is essential for protection of the virus from immune attack (at least in mice) upon intravenous injection (Xu *et al.*, 2013). Collectively, these pathways impact how adenoviruses can be used clinically as vectors for therapeutics. It is essential that we continue to improve our understanding of the function and consequence of these interactions if we are to optimize the clinical use of adenovirus vectors.

The improved understanding not only provides valuable information on the virus itself, but also how it can be tweaked for future therapeutic applications. It is very likely that further studies will bring other important interactions to light; hence, it is critical for additional fundamental research into adenovirus biology and function. This is true not only for HAdV5, where there is already a great deal known, but also other adenoviruses that are being used (or could potentially be used) for translation to human gene therapy. While HAdV-5-based vectors were developed for the aforementioned reasons, there are rich resources of alternative adenovirus-based vectors that have not been ''vectorized'' to date.

Use of vectors derived from alternate human or non-human adenoviruses is attractive for several reasons. These include alternate infectivity/tropism profiles, differential interaction with the immune system, and vastly different pre-existing immunity profiles (see the insightful review by Arnberg, 2012). Classically, switching to human adenoviruses that bind CD46 and other cellular receptors has led to improved targeting, particularly of cell types such as cancer, offering improved gene delivery (Gaggar *et al.*, 2003). There are examples also from non-human adenoviruses. Recent studies have highlighted the potential for canine adenovirus type 2 (CAdV-2) vectors for gene transfer in the brain, principally defined by the exquisite neuronal tropism of this vector (Salinas *et al.*, 2010). This is potentially the tip of the iceberg, and it will be interesting to see how the field progresses, with improved information available relating to the genome sequences, properties, and potential for alternate adenovirus vectors.

One of the most important areas in which a range of human and non-human adenoviruses are being evaluated preclinically and clinically is in vaccination. There has been substantial activity using HAdV-26 (Barouch *et al.*, 2012) and chimpanzee adenovirus-based vectors (Ewer *et al.*, 2013). Adenoviruses remain attractive for vaccine development since some of them prime innate and adaptive responses, and there have been clear advantages of maximizing and optimizing the breadth of properties within the Adenoviridae to define the most effective vectors in vaccine regimens.

Further focus on adenovirus biology and adenovirus-based vectors for gene therapy will take place at the 11th International Adenovirus Meeting in San Diego in July 2014 (http://iam.scripps.edu/). Sun, sea, sand, and adenoviruses—the perfect combination!

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