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AAV9: Over the Fence and Into the Woods . . .

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One of the long-term aims of gene therapy is to deliver vectors to diseased organs directly from the circulation. Efforts toward this end with recombinant adeno-associated virus (AAV)-based vectors have been hampered by the fact that most of the vector ends up in the liver after systemic delivery. In addition, tissue-specific barriers such as the blood-brain barrier (BBB) can limit the entry of vectors from the blood to certain organs. Two papers in this issue of *Molecular Therapy* describe progress in addressing these obstacles with respect to a relative newcomer to the family of AAV vector serotypes, AAV9. These new reports complement recent articles published in *MT* and elsewhere suggesting that AAV9 may soon take its place alongside AAV2 in the field of AAV-based gene therapy.

In one of the two papers in this issue, Pulicherla and colleagues[1] show that variants of AAV9 can be engineered to reduce dramatically the transduction of liver relative to cardiac and skeletal muscle without reducing the overall high transduction efficiency of vectors based on this serotype. These results and those of others[2,3] suggest that detailed rational engineering of AAV capsids may lead to AAV variants optimized for specific target tissues. In the other study, Gray *et al.*[4] compare the transduction efficiency of AAV9 in rodents and nonhuman primates (NHPs) and report that, upon systemic delivery, the pattern of cellular transduction in the NHP brain differs significantly from that observed in mice, although they caution against the temptation to extrapolate transduction data in rodents directly to humans. In NHPs, astrocytes are the principal target cell, whereas neuronal transduction predominates in mice. Thus, although AAV9 might be engineered to detarget the liver at the expense of specific organs of interest, the species-specific pattern of target cell transduction should be fully characterized in detail before further clinical development.

The clinical development of AAV for the treatment of hemophilia[5] and Parkinson's disease[6] has until now made use of vectors based on AAV2, which displays a natural tropism toward skeletal muscles, neurons, vascular smooth muscle cells, and hepatocytes. AAV2 became the preeminent vector in translational programs for a multitude of reasons, not the least of which was that it was the first to be commercially produced at scale in support of clinical gene therapy programs. This early developmental lead created a powerful precedent that rendered AAV2 the frontrunner for clinical development, despite its comparatively low transduction efficiency. Of course, transduction efficiency is only one of several variables that must be considered in choosing an appropriate vector for a particular application.

Isolated originally by James Wilson and colleagues, AAV9 is one of a very large family of AAV clades containing more than 100 new serotypes that remain poorly characterized.[7,8] Of these, AAV8 and AAV9 stand out; vectors carrying capsids from these serotypes transduce rodent muscle, liver, and lung about 100-fold more efficiently than AAV2. In terms of the treatment of neurological diseases, a central reason for the emerging popularity of AAV9 is its remarkable ability to cross the BBB.[9] Children with diffuse brain degeneration due to lysosomal storage disorders could be treated effectively with gene therapy today if not for the BBB, which forces workers to deliver the vector directly to the brain via intracranial injection. The prospect of delivering therapeutic genes directly to the brain through the vasculature promises a more straightforward approach to treating a number of neurological diseases, particularly genetic diseases in infants that affect the structure and function of large areas of the brain.[10] Because AAV9 also efficiently transduces both spinal motor neurons and dorsal root ganglia following systemic delivery, this vector may merit accelerated clinical development for diseases also affecting the structure and function of the spinal cord, such as amyotrophic lateral sclerosis, neuropathic pain, spinal injury, and certain ataxias.[11]

The relatively poor transduction of neurons by vascular AAV9 represents another limitation. Many neurological diseases are neuronal in origin and require anatomically localized expression of therapeutic agents in neurons. For example, AAV9-mediated vascular delivery of glia-derived neurotrophic factor for Parkinson's disease might be therapeutic but would also carry risks such as weight loss as a result of transgene expression in other parts of the brain.[12,13] However, the use of astrocytes to deliver secreted transgene products into the brain has, in our view, considerable potential if the problem of humoral immunity to AAV9 can be avoided—clearly a strong possibility in neonates. Finally, the issue of density of expression is concerning. Although Gray *et al.*[4] show widespread transduction, the number of green fluorescent protein-expressing cells in the NHP brain per unit area is modest, and it is not clear whether this level of transduction will be sufficient for therapeutic purposes; it will ultimately depend very much on the specific transgene being delivered.

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Gray *et al.* also report that very low neutralizing antibody titers in the blood of NHPs blocked the otherwise impressive transduction of glial cells throughout the central nervous system.[4] Although only 47% of adults are seropositive for antibodies to AAV9, compared with about 70% for AAV2 (refs. 14 and 15), it is striking how even very low titers are able to block transduction.[16] Hence, pediatric populations may represent the most fruitful target population for gene therapy by systemic delivery of AAV9 because infants are more likely to be seronegative.[17]

A final challenge highlighted by both papers concerns the actual amounts of AAV9 required to achieve effective transduction via systemic vascular delivery in mice: approximately 1×10^{13} vg/kg. For the average 70- to 80-kg human, this would equate to a dose of approximately 1×10^{15} vg, a very large amount of vector, equivalent to a full good manufacturing practices production run per single patient using current technology. Clearly, clinical development of AAV9 will require a much more robust scalable technology for such studies to become feasible.[18] Thus, although some barriers have been breached and certain hurdles can be avoided by developing AAV9 gene transfer directly from the vasculature, important challenges remain to be overcome on the way to safe and efficient gene therapy from a systemic vascular route with AAV9.

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