Gene Therapy (2005) 12, 1539

www.nature.com/gt

Adeno-associated vectors

Are we there yet?

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Gene Therapy (2005) **12,** 1539. doi:10.1038/sj.gt.3302605;
published online 18 August 2005

In a field where the promise of effective gene therapy has outweighed performance, a spate of recent publications offers the tantalizing possibility that there is a light at the end of the tunnel. Recent papers by Sarkar *et al*[1] and Wang *et al*[2] suggest that sustained therapeutic levels of coagation factors FVIII and FIX, respectively, were achieved in murine knockout and canine deficient animals. These results stem from previous observations[3,4] and the isolation of new adeno-associated virus (AAV) serotypes in primates.[5]

The recent ASGT meeting in St Louis saw notable abstracts listing the use of AAV serotypes, as well as the unveiling of new serotypes (AAV10). It would seem that we are now in a period not unlike the automotive industry, where a new and improved model/serotype is revealed every year.

As we are painfully aware, the results obtained in animal models are not predictive of success in clinical trials. Two clinical trials using the now antiquated AAV type 2 failed because the virus did not infect the target organ (skeletal muscle) and a loss of the transgene expression was observed in the liver, presumably by an ill-defined immune response. The doses of vector used in those clinical experiments were on the order of 10^{11-12}/kg. The genome copy (gc) doses of AAV8 vectors used in canines were of the order of 10^{12-13}/kg.[1,2] The initial promise of the new AAV serotypes was that less virus could generate more secretable coagulation factor, with less risk for potential immune side effects. It would appear that in the recent studies extremely high gc doses were still required.

An alternative approach using self-complementary AAV that can package half the wild-type transgene size ($\sim$2.3 kb) improves transgene expression dramatically in the case of AAV2 (100–1000-fold), but only modestly (3–10-fold) for most of the other isolated serotypes.[6] One explanation for these results lies in the transit of the recombinant virus from the cell membrane into the nucleus, where the uncoated virus undergoes second-strand synthesis. The data imply that this last step is markedly limited for AAV2 and to a much lesser degree with other serotypes. The implication is that transit from the cell membrane into the nucleus is enhanced with the new serotypes. Why relatively small changes in the capsid structure provide such an improvement remains to be clearly delineated.

Where does this leave the field? Several approaches have been taken. One is to define newly identified AAV serotypes derived from primates infected with wild-type adenovirus and hope that newer finds are 'better' than existing AAV. This restricts the ability of many investigators who do not have access to such models. A second approach is to build AAV specifically to target defined organs/tissues. Inserting small sequences into the AAV cap genes[7,8] can be tailored to suit one's purposes. Alternatively, hybrid viruses can be produced to define critical motifs in the capsid structure that facilitate improved gene transfer.[9]

In its infancy 15 years ago, the ability to produce 10^{4-5} gc per preparation was considered reasonable for rAAV. Now, the production of 10^{3-4} gc/cell is routine. Although it has always been perceived that more virus is better, the results of the clinical trials for inherited disorders with adenovirus and AAV to date suggest that this may not be the case. Inflammatory/immune responses that are not observed at low gc doses are elicited at higher doses and would suggest that 'new and improved' should relate to more transgene protein expressed/gc vector delivered. Although there will likely be a rush to test these new vectors in the clinic, the question still remains as to which model/serotype one wants to use. The recent publications by Sankar and Wang give biologic insight, but, until the mysteries of this vector are fully understood, empiricism reigns. Since basic science of vectoring will always be several steps of ahead of clinical experience, this argues that future clinical trials should be designed in a manner to test for all outcomes (positive and negative). ■

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Published online 18 August 2005

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