Transduction of antigen-presenting cells in the brain by AAV9 warrants caution in preclinical studies

To the editor:

Recently, Hinderer *et al.*[1] published the results of a study in cynomolgus macaques that we believe is deserving of comment. In this study, adeno-associated virus serotype 9 expressing green fluorescent protein (AAV9-GFP) was injected intrathecally into these animals at either the lumbar region of the spine or into cisterna magna. The macaques were then analyzed 2 weeks after injection for expression of GFP in the brain and spinal cord. The authors nicely document significantly stronger transduction of primate brain after cisternal as compared to lumbar infusion, in agreement with our unpublished findings. For this reason, we have reported only cisternal delivery of AAV7 and AAV9 (refs. 2, 3), as the difference in central nervous system expression is so strikingly in favor of cisternal delivery.

In our studies we have documented in both rodents and nonhuman primates a robust immune response against foreign (but not self) antigens like GFP, which was originally derived from the jellyfish *Aequoria victoria*. AAV9 transduces antigen-presenting cells in nonhuman primate brain[3] and liver.[4] Lest this be seen as a phenomenon specific to GFP, we also showed that human aromatic L-amino acid decarboxylase (AADC) in an AAV9 vector triggers exactly the same kind of cytotoxic responses in rats as GFP does.[5] To the rodent immune system, human AADC is just as foreign as GFP. In contrast, encoding either human AADC or GFP in the highly neuron-specific AAV2 yields no such cytotoxic response, because neurons are not professional antigen-presenting cells, although they clearly—like nearly all mammalian cells—present antigen via the major histocompatibility complex class I.

In experiments in which AAV9 (and other broader specificity serotypes like AAV5 and AAV7) triggers such immunotoxicity, we see brisk upregulation of the major histocompatibility complex class II on astrocytes and microglia within 2 weeks after vector administration. However, the full immunotoxic effect is not visible for at least a month. Thus, the apparent discrepancy between our published data and the present study can be explained entirely in terms of the acute nature of the experiment performed by Hinderer *et al.* We agree that 2 weeks would not reveal significant signs of a classic immune response against a foreign antigen, and we caution other investigators that safety studies designed to reveal possible immunotoxicity should extend well beyond 6 weeks. We have settled on a 90-day experimental period for such studies. It should also be noted that this potential problem with central nervous system transduction exists only for the expression of non-self proteins with AAV serotypes that are not neuron-specific. Bacterial proteins such as tetracycline transactivator,[6] CRISPR,[7] or channel rhodopsins,[8] and similar foreign genes must be presumed immunologically guilty before proven innocent.

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REFERENCES

1. Hinderer, C, Bell, P, Vite, CH, Louboutin, J-P, Grant, R, Bote, E *et al.* (2014). Widespread gene transfer in the central nervous system of cynomolgus macaques following delivery of AAV9 into the cisterna magna. *Mol Ther Methods Clin Dev* **1**: 14051.
2. Samaranch, L, Salegio, EA, San Sebastian, W, Kells, AP, Bringas, JR, Forsayeth, J *et al.* (2013). Strong cortical and spinal cord transduction after AAV7 and AAV9 delivery into the cerebrospinal fluid of nonhuman primates. *Hum Gene Ther* **24**: 526–532.
3. Samaranch, L, San Sebastian, W, Kells, AP, Salegio, EA, Heller, G, Bringas, JR *et al.* (2014). AAV9-mediated expression of a non-self protein in nonhuman primate central nervous system triggers widespread neuroinflammation driven by antigen-presenting cell transduction. *Mol Ther* **22**: 329–337.
4. Gao, G, Wang, Q, Calcedo, R, Mays, L, Bell, P, Wang, L *et al.* (2009). Adeno-associated virus-mediated gene transfer to nonhuman primate liver can elicit destructive transgene-specific T cell responses. *Hum Gene Ther* **20**: 930–942.
5. Ciesielska, A, Hadaczek, P, Mittermeyer, G, Zhou, S, Wright, JF, Bankiewicz, KS *et al.* (2013). Cerebral infusion of AAV9 vector-encoding non-self proteins can elicit cell-mediated immune responses. *Mol Ther* **21**: 158–166.
6. Gossen, M and Bujard, H (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci USA* 89: 5547–5551.
7. Swiech, L, Heidenreich, M, Banerjee, A, Habib, N, Li, Y, Trombetta, J *et al.* (2015). *In vivo* interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat Biotechnol* **33**: 102–106.
8. Yizhar, O, Fenno, L, Zhang, F, Hegemann, P and Deisseroth, K (2011). Microbial opsins: a family of single-component tools for optical control of neural activity. *Cold Spring Harb Protoc* 2011; doi:10.1101/pdb.top102.

In vivo secretion of anti-CD3 × anti-tumor bispecific antibodies by gene-modified cells: over a decade of T-cell engagement

To the editor:

The recently published paper by Iwahori *et al.*[1] describes the generation of T cells secreting a bispecific anti-CD3 × anti-EphA2 tandem single-chain variable fragment antibody, or BiTE (bispecific T-cell engager). The authors claim that gene-modified T cells secreting bispecific antibodies present a "new class of antigen-specific T cells" with the unique ability to redirect bystander T cells to tumor cells in an antigen-dependent manner.

In 2003 our group reported for the first time a cancer immunotherapy strategy based on the secretion of a bispecific anti-CD3 × anti-carcinoembryonic antigen (CEA) diabody by gene-modified human cells that activate and redirect T cells to the tumor *in vivo*.[2] As a result of this proof-of-concept article, our group has been considerably productive in this field. Since then, we have published 12 scientific (original and review) articles[3–14] in which we have validated this bispecific antibody–based gene therapy strategy using different formats of bispecific antibodies (diabody and BiTE), various types of cell carriers (human T-cell lines and primary T cells, human mesenchymal and hematopoietic stem cells, and human endothelial cells), several gene transfer systems (plasmids and lentiviral vectors), and several mouse cancer models.[3–14]

In 2007 we reported in *Cancer Gene Therapy* the usefulness of lentiviral vectors for the sustained expression of a bispecific anti-CD3 × anti-CEA diabody in human primary peripheral blood T lymphocytes.[7] In this paper we specifically stated that the recruitment of both gene-modified and nonmodified T cells, present at the tumor site, would amplify the effector response. Moreover, gene-modified human stem cells embedded in synthetic extracellular matrix scaffold and implanted in a location distant from the primary tumor, were able to secrete the anti-CD3 × anti-CEA diabody, which significantly reduced the tumor growth and resulted in effective and