Gene therapy for global brain diseases – One small step for mice, one giant leap for humans

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The gene therapy field has recently reached another significant milestone in its march towards revolutionising the treatment of genetic diseases. Zolgensma, an adeno-associated viral (AAV) vector -based gene therapy, has been approved by the FDA for the treatment of spinal muscular atrophy (SMA). This joins Luxturna, another FDA approved AAV-based therapy for the treatment of retinal dystrophy, in making gene therapy an effective and potentially commercially viable treatment option. It is also noteworthy that both these initial successes have been realised in rare conditions, an arena that has become ground zero for gene therapy clinical trials.

Zolgensma's effectiveness in making life-saving differences to SMA patients through mainly rescuing motor neurons located down the spinal cord has been impressive (Mendell *et al*., 2017). Importantly, it has provided evidence that AAV serotype 9 (AAV9) can cross the blood-brain barrier (BBB) in humans and deliver the therapeutic *SMN1* gene to motor neurons following a single non-invasive intravenous administration. This has provided hope to those studying other neurological diseases, especially the large number of rare intractable conditions that affect multiple regions of the brain. However, these conditions will represent a far more complex challenge than SMA. There is nothing simple about delivering therapeutic genes to multiple regions, various neural populations and at the required efficiency in what is arguably the most complex organ of the human body. Numerous pre-clinical studies in mouse models of monogenic brain disease suggest that an intravenous administration of an AAV9 vector is sufficient to cure the condition. However, the debate over whether this will work sufficiently in the larger and more complex human brain rages on. After all, the gyrencephalic human brain is >600 times larger in volume than that of the lissencephalic rat brain. The total surface area of the cerebral cortex in humans is 377 times larger than in a rat (Hofman, 1985; Hofman, 1988) . Ferrer *et al*. demonstrated that most of the cellular types were present in both smooth and convoluted brains (Ferrer *et al*., 1986; Ferrer *et al.*, 1986) (Figure 1). However, they observed the differences in the orientation of neurones as a result of folding, formation of the local circuit subsystem and a very characteristic of gyrencephalic brain development of ipsilateral cortico-cortical fibrillary system.

In this issue of Brain, Young Yoon and co-workers provide evidence in support of intravenous AAV-mediated gene therapy for brain diseases (Young Yoon *et al*., 2020). Initially, they identify the AAVhu.32 vector as mediating better levels of brain transduction following intravenous administration to adult mice compared to closely related AAV9. Using the larger gyrencepahlic feline brain as a 'stepping stone' towards modelling what will happen in the human brain, intravenous administration of AAVhu.32 mediated widespread reporter gene delivery in the brain. Interestingly, despite using a minimal human GUSB promoter that should express in all cell types, only neuronal expression was observed. The researchers then conducted a pre-clinical study in a cat model of the alpha-mannosidosis; a neurodegenerative lysosomal storage disease caused by mutations in the alpha-mannosidase gene (*MANB*) that results in global brain pathology. AAVhu.32 carrying a therapeutic copy of the feline *MANB* gene was administered to the cat model via either intravenous or intraarterial injection. The therapeutic response was dose dependent and intraarterial administration of vector provided better therapy. Survival, severity of ataxia and neuropathology were improved.

The findings of Young Yoon *et al.* are a step forward in developing a gene therapy strategy for alpha-mannosidosis. This may also help to enhance gene therapy for other similar conditions. Especially those involving soluble enzymes that can leak out of a transduced cell and then cross-correct the

defect in untransduced neighbouring cells, hence amplifying the therapeutic efficacy. The study does also provide us with some important reminders and interesting observations - which pose new questions. The first is the absolute importance of the delivery vector in dictating whether a gene therapy approach is going to work and the importance of continually enhancing and developing them. Another reminder is that you cannot make assumptions that a new vector will act in the way you may predict, it will require thorough evaluation in its own right. AAV9 and AAVhu32 are both phylogenetic clade F partners and Young Yoon *et al.* describe how the sequences of capsid proteins differ by 12 amino acids. However, the higher levels of transduction, strong neuronal tropism and shift of transduction patterns further forward in a larger gyrencephalic brain mediated by AAVhu32 relative to AAV9 is not necessarily predictable. Especially if one takes into account the fact that AAVhu32 does transduce other neural cells when injected directly into the brain parenchyma of mice (Cearley *et al.*, 2008).

The route of administration used to deliver the gene delivery vector is an important consideration. Young Yoon *et al*. demonstrate that an intraarterial administration of AAVhu32 has greater therapeutic efficacy than when administered via intravenous administration. The authors point out that this is despite a limited number of studies in non-human primates showing that there is little difference in transduction efficiency between the two routes of administration using the AAV9 vector. Systemic routes of administration have the added advantage of providing therapy to affected visceral organs that accompany the life-threatening degeneration in the brain. However, what we cannot gauge from this study is whether the optimal intraarterial route of administration is superior to administration of the vector via the cerebrospinal fluid (CSF). This could be through intracerebroventricular, intracisternamagna or intrathecal administration and using the CSF to maximise delivery throughout the CNS. There are ongoing clinical trials using intravenously administered AAV9 vectors for other neurodegenerative lysosomal storage disorders (e.g. in Mucopolysaccharidosis IIIA, ClinicalTrials.gov Identifier: NCT02716246). But there are more researchers that opted for intra-CSF administrations (e.g. in Mucopolysaccharidosis IIIA via intracerebroventricular administration, EudraCT number 2015-000359-26; in CLN6 Batten disease via intrathecal administration, ClinicalTrials.gov Identifier: NCT02725580). This also raises the question of whether bypassing the BBB and injecting directly into the CSF will alter the neuronal tropism of the AAVhu32 vector? Indeed, would broadening the tropism of the vector to include glial cells be advantageous and would this provide more cells to act as enzyme producing 'factories'? It is interesting to observe that there is a significant decline over time in levels of alpha-mannosidase in the plasma and in the CSF of treated cats. A decline in plasma levels could be attributed to turnover of cells in visceral organs and dilution of the non-integrating AAV genome. However, the decline in enzyme levels within the CNS is surprising given that expression is coming exclusively from a stable population of non-dividing post-mitotic neurons.

The challenge now lies in evaluating whether the AAVhu32 vector has the ability to be effective in the even larger and more complex human brain. A logical step forward would be testing it in non-human primates. Information is also required on the prevalence of pre-existing neutralising antibodies against AAVh32 in paediatric and adult human populations. The lack of any measurable adverse effects in the administered cats and normal serum chemistry is encouraging. Particularly as high doses of intravenously administered AAV vectors in SMA (Mendell *et al.*, 2017) and haemophilia B (Nathwani *et al*., 2011) clinical trials have resulted in elevated serum liver enzymes and have required transient corticosteroid treatment to normalise.

The promise of gene therapy lies in the potential to cure genetic diseases from a single administration. To realise this for global brain disorders, the hunt for more effective and efficient vectors must go on. Therefore, the study by Young Yoon *et al.* is welcome, particularly by scientists and clinicians working in the rare paediatric neurodegenerative arena where the prognosis for patients is bleak. No effective treatments are available but novel approaches such as gene therapy are desperately required and are a light at the end of a dark tunnel.

Figure 1. Neuronal architecture in lissencephalic and gyrencephalic mammals. A) Neuronal types in layer VI of the cerebral cortex of the mouse (*Mus musculus*). LP, large pyramidal neuron; SP, small pyramidal neuron; IP, inverted pyramidal neuron; FP, flattened pyramidal neuron; HP, horizontal pyramidal cell; H, horizontal neuron; S, multipolar neuron with locally arborising axon, M, Martinotti cell; ax, axons; asterisks, multiapical pyramidal cells. Bar, 100microM
B) and C). Camera lucida drawing of neurons of the sixth layer in the gyral (B) and fissural (C) regions of the cat cerebral cortex. P, pyramidal neuron; aP, atypical pyramidal cells; F, fusiform neurons; B, bipolar cells; LC, local circuit neuron. Asterisks show an inverted pyramidal cell in the gyrus and a horizontal pyramidal cell in the fissural region. Small arrows point out the course of the axons and collaterals. **D) and E).** Neurons of the sixth layer in the gyrus (D) and sulcus (E) of the cerebral cortex of the human infant. B, bipolar neuron; LC, local circuit neuron; sMp, spinous multipolar neuron with long descending axon. Asterisks show an inverted pyramidal cell in **D),** and a tangential (horizontal) pyramidal cell in **E)** (adapted from Ferrer *et al.,* 1986; Ferrer *et al.,* 1986)

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Competing Interests

The authors report no competing interests.