# Genes go incognito into brain

**Vida Foubister**, freelance writer

A group of scientists led by William M. Pardridge, Professor of Medicine at the University of California, Los Angeles (UCLA; http://www.medsch.ucla.edu), have found that pegylated immunoliposomes (PIL) can be used to transfer genes from the blood into the brain of primates. Their results, which also demonstrate the exogenous gene is expressed at high levels, build on earlier studies in rodents and suggest that this approach might be applicable in humans.

## The delivery problem

Gene therapy holds tremendous promise for the treatment of many human diseases. However, the field has undergone significant setbacks because of the potentially lethal side effects of the viral vectors used to deliver genes. An adenovirus vector used in a clinical trial at the University of Pennsylvania is believed to have caused the death of 18-year-old Jesse Gelsinger in 1999 (http://www.med.upenn.edu). More recently, the US Food and Drug Administration (http://www.fda.gov) placed all active trials using retroviral vectors to insert genes into blood stem cells on 'clinical hold' after news that a second child treated for X-linked severe combined immunodeficiency disease in France had developed a leukemia-like condition.

Pardridge's team avoids this problem by using PILs as a delivery system. This so-called 'artificial virus' encapsulates the therapeutic gene inside a liposome, which is then coated with polyetheylene glycol (PEG). The liposome prevents degradation of the gene by endonucleases, and the pegylation process reduces the absorption of serum proteins to the liposome surface, which minimizes their uptake by cells lining the liver and spleen (the reticulo-endothelial system).

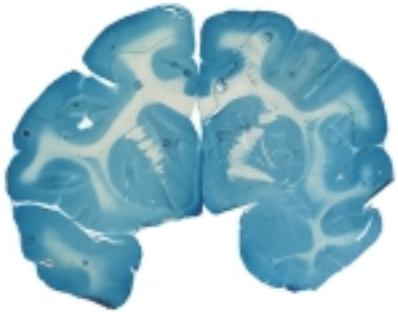

**Figure 1.** A section of adult rhesus monkey brain showing global expression of an exogenous bacterial β-galactosidase gene. The bacterial gene was encapsulated in pegylated immunoliposomes (PIL) that target the human insulin receptor and delivered by a single intravenous injection. Reproduced, with permission, from Ref. [1].

The final step is to target the PIL, in these experiments to neurons, by attaching a monoclonal antibody, to 1–2% of the PEG strands, that recognizes receptors expressed on both the blood–brain barrier (BBB) and the brain cell plasma membrane (BCM). Pardridge refers to these targeting monoclonal antibodies as a 'molecular Trojan horse'. He explains, 'It enables the PIL to first cross the blood–brain barrier and subsequently to cross the plasma membrane of the neuron. Once inside the neuron, it moves across the nuclear membrane, where the gene is expressed.' How this last step occurs is not understood.

## Blood–brain barrier

The approach used by the UCLA researchers is a novel way to get drugs through the BBB. 'This is a monumental problem for drug development because 98% of all small-molecule drugs and 100% of all large molecule drugs, including recombinant proteins, antisense RNA and gene medicines, do not cross the blood–brain barrier,' said Pardridge.

In their most recent study, a single intravenous injection of PIL, targeted to the brain with a mouse monoclonal antibody to the human insulin receptor, led to global expression of an exogenous gene coding for bacterial β-galactosidase in the adult rhesus monkey brain within 48 hours (Fig. 1). Furthermore, they showed that expression of an exogenous gene encoding luciferase was 50-fold higher in the rhesus monkey than seen in the rat, which used PIL targeted to the brain with a mouse monoclonal antibody to the rat transferrin receptor [2]. 'This particular work is the most innovative and potentially the most ground breaking that anybody in blood–brain research has done,' said Eain Cornford, Professor of Neurology at UCLA. 'It could well revolutionize the field.'

Furthermore, most inherited diseases that affect the brain result from defects in metabolic pathway genes. 'The ability to get the gene in cells through the brain is important to these processes,' added John Wolfe, Professor of Pathology and Medical Genetics at the University of Pennsylvania. 'This [research] is an important step towards getting widespread delivery.'

Although expression of the two exogenous genes also occurred in other organs, such as the liver and spleen, Pardridge has shown that this can be eliminated in mice through the

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use of a brain-specific promoter [6]. In the primate experiments, the exogenous genes were on plasmids under the influence of a widely expressed SV40 promoter.

However, the fact that the exogenous genes do not integrate into the genome, and thus their expression is short lived, could limit the therapeutic applications of this synthetic vector. 'If you're trying to treat an inherited disease, you're looking for permanent expression,' said Wolfe. Transient expression might be preferable for some diseases, and it also might be possible to deliver the genes using a vector that results in permanent expression, he added.

### Promise in rats

Pardridge's team has already demonstrated they can use this delivery system to get a pharmacological effect in rodents. A plasmid that expresses antisense mRNA to the human epidermal growth factor receptor injected intravenously in a PIL led to 100% increase of survival time in mice with brain cancer [3]. In addition, the effect of a neurotoxin that causes Parkinson's like symptoms in rats was reversed by using PIL to deliver the affected enzyme, tyrosine hydroxylase [4]. 'We showed that gene therapy not only normalizes the biochemistry but also eliminates the motor abnormality of the disease.'

The team is currently working to develop a brain-specific promoter for Parkinson's disease and also to use an RNA interference-based approach in the mouse brain cancer model: the combination of gene therapy and RNAi removes aberrant expression of the gene that is specific to the cancer, however, according to Pardridge, the problems preventing this from happening are 'delivery, delivery and delivery!'

### References


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# Gene therapy success for Alzheimer's?

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Using gene therapy, the levels of a protein implicated in Alzheimer's disease have been dramatically reduced in mice. Researchers from the Salk Institute (http://www.salk.edu) and the University of California, San Diego (UCSD; http://www.ucsd.edu) have introduced the gene for neprilysin into the brains of transgenic mice, reducing levels of amyloid protein by up to 50% [1]. Their findings offer hope to the millions of Alzheimer's sufferers, and their families, around the world.

### More common than you might think

Alzheimer's disease (AD) is the most common form of neurodegenerative disease in people over 65 years. It is estimated that >15 million people are affected by the disease worldwide and almost half of those over 85 years show signs of the disease [2]. The cognitive areas of the brain are the first to be affected, leading, amongst other things, to memory loss and behavioural abnormalities. The disease then spreads to the parts of the brain that control movement, and the patient requires constant care. Eventually, the loss of brain function becomes so severe that it can be the primary cause of death. As the average lifespan of people in the Western world increases, so too will the numbers of people affected by neurodegenerative illnesses. Needless to say, a tremendous amount of time, effort and money has been poured into finding a means to cure or prevent the disease.

### A complicated aetiology

Despite great strides in AD research, the precise mechanisms that lead to the disease are not fully understood and many genetic, cellular and molecular irregularities are implicated. One of the most established factors, however, is a build-up of amyloid proteins around brain cells to form harmful plaques. Mutations in the amyloid-precursor protein (APP) gene can result in increased production of $A\beta_{1-42}$, the form of amyloid protein that clusters into the harmful plaques. However, in rarer forms of AD, other factors might be contributory. For example, amyloid build up might be caused by a decreased $A\beta_{1-42}$ clearance, or from a shift in the balance of production and degradation.


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