Gene therapy revisited

In spite of problems and drawbacks, gene therapy moves forward

Since the death of Jesse Gelsinger 2 years ago during clinical trials at the University of Pennsylvania, gene therapy has maintained a low profile and receded from the public eye. But away from the headlines, researchers are, in fact, quietly making progress and are confident that, within the next decade, gene transfer will be elevated from its current experimental status to a therapeutic modality.

At the recent Emerging Technologies in Gene/Drug Therapy and Molecular Biology meeting, sponsored by the Regulon

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company (Mountain View) and the International Society of Gene Therapy and Molecular Biology, a group of about 100 scientists gathered in Corfu, Greece, to discuss advances in bringing gene therapy to the clinic. They still face major challenges: targeting the right gene to the right location in the right cells and expressing it at the right time, all while minimising any adverse reactions. But the scientists presented data on the development of viral and non-viral gene vectors, tissue- and disease-specific gene delivery and cell cycle control that indicate that the clinical use of gene transfer is becoming a tangible possibility.

Much research has concentrated on finding the best vector for a specific application. Eugene Redmond of Yale University (New Haven, CT) compared four viral vectors to determine which prolonged gene expression the most and which was the least destructive of brain tissue in treating neurological diseases: 'A comparative study of viral vectors could be useful for evaluating vectors for human clinical use for somatic gene therapy, where long-duration gene expression might be necessary, and inflammatory, demyelination and cytotoxicity is undesirable for diseases such as Parkinson's, Alzheimer's, or other diseases involving the brain'.

Comparing lentiviral, adenoviral, adeno-associated and retroviral vectors in

primates, Redmond found that the adeno-associated vector was the safest and most effective vector in producing long-lasting gene expression when delivered directly to the brain. In contrast, adenovirus, the vector that was used in the Gelsinger trial and caused the fatal toxic reactions, produced the most apoptosis and inflammation—both of which are undesirable in treating neurological conditions, although potentially beneficial in treating cancer. Redmond also concluded that, after comparing gene expression and inflammation at the site of administration, cell death, demyelination and cytotoxicity seen with adenoviral vectors resulted in minimal gene expression.

Indeed, much of the clinically orientated work in gene therapy has been concentrating on neurological diseases. David Fink of the University of Pittsburgh (Pittsburgh, PA) is exploiting the natural tropism to the central nervous system of the herpes simplex virus (HSV-1) in devising potential treatments for Parkinson's disease, neuropathy and chronic pain. Fink and his colleagues demonstrated that HSV containing the sequence for bcl-2, a human anti-apoptosis gene, preserved the expression of tyrosine hydroxylase, a critical enzyme for the synthesis of the neurotransmitter dopamine, when injected into the substantia nigra in a rat model of Parkinson's disease.

In a subsequent experiment, Fink showed that injecting an HSV vector expressing glial-derived neurotrophic factor (GDNF) and bcl-2 improved both cell survival and the presence of tyrosine hydroxylase in brain lesions: 'While further studies will be needed to define the mechanism through which GDNF and Bcl-2 act together to prevent neurotoxicity to dopaminergic neurons, our research shows that the two factors act together more effectively than either factor alone in blocking toxicity, and thus support the possible utility of a combination therapy to treat Parkinson's disease'.

Fink injected the genes into the spinal cords of rats 30 min after damaging the spinal nerve root, which had caused

apoptotic death of motor neurons. Neither gene alone preserved the neurotransmitters in damaged neurons; but, injected together, GDNF and bcl-2 acted synergistically to preserve choline acetyltransferase in the damaged motor neurons.

The team also tested the HSV vector in animal models of pain. When the gene for proenkephalin, an endogenous analgesic, was targeted to a rat's paw, the resulting expression and release from neurons in the dorsal root ganglia relieved inflammatory-induced and neuropathic pain. Fink now hopes to use the HSV vector to treat pain in cancer patients within the next year.

With the goal of treating Parkinson's disease, Patrick Aebischer of the Ecole Polytechnique Federale de Lausanne (Lausanne, Switzerland) and Jeffrey Kordower of Rush Presbyterian Hospital (Chicago, IL) used a lentiviral vector to express GDNF in the brains of monkeys. While GDNF is well known for its ability to restore dopamine-producing cells damaged through this disease, Aebischer says that the problem has been pinpointing an efficient method of delivery. Although an early clinical trial was abandoned due to adverse effects, Aebischer and Kordower now plan to administer GDNF directly to the site of damage in Parkinson's disease, and thereby bypass the problems seen with less exact delivery. They are testing both an equine-derived vector with GDNF in animals and an HIV-derived vector with encapsulation technology for long-term expression.

Aebischer hopes to reach the clinic with one of these Parkinson's treatments within 2 years, but he says that additional studies will be needed to define the mechanisms through which GDNF acts to prevent neurotoxicity. They must also develop a switch for regulating gene expression to ensure that treatment can be stopped if necessary. 'We will also have to establish sensitive methods to evaluate vector distribution throughout the body, and to detect whether viral particles can recombine to produce replication-competent virus', Aebischer said.

Regarding the choice of the vector, retroviruses, such as HIV or herpes viruses, are clearly superior for expressing a therapeutic gene over the long term. Indeed, Ralph Dornburg of the Thomas Jefferson University (Philadelphia, PA) believes that they are the best choice to treat genetic disorders that require lengthy expression and the incorporation of large therapeutic genes. He is modifying vectors from C-type retroviruses, avian reticuloendotheliosis virus strain A (REV-A) and spleen necrosis virus (SNV) to target specific cells. As these viruses are non-pathogenic to humans, Dornburg thinks they are a good choice: 'REV-derived vectors efficiently transduce genes into human cells when they are pseudotyped with hetero-

polyethylenglycol (PEG) or by incorporating a transferrin ligand. His team is using the surface-shielded transferrin-PEG system to deliver tumour necrosis factor (TNF) gene directly to tumour cells. 'Shielding reduces plasma protein and erythrocyte binding, resulting in prolonged blood circulation and extravasation of DNA complexes in areas of vascular leakiness of the tumour tissue', Kircheis said. He says that the systemic application of surface-shielded complexes with the TNF- α gene in animal studies resulted in pronounced tumour necrosis and inhibition of tumour growth with no systemic toxicity. Boehringer plans to start human trials soon to treat patients with melanoma or sarcoma of the

shown to have lower toxicity, better efficacy and longer circulating times in mice, as well as avoiding the immune system and reaching distant tumour types. One of the drugs reduced tumour size in SCID mice with human breast, prostate and other cancer types after six injections over 2–3 months. Regulon is also testing second-generation therapies with tumour-specific regulatory DNA sequences against a variety of human malignancies.

Bouliskas has also modified semliki forest virus (SFV) by introducing two point mutations, which resulted in increased transgene expression and substantially prolonged survival of cultured cell lines. 'Because of the low cytotoxicity and absence of the shut-down of host cell protein synthesis typically seen for the conventional SFV vector, it is now possible to apply them for antisense, ribozyme and RNA interference technologies', said Kenneth Lundstrom from Hoffmann-LaRoche (Basel, Switzerland), who is collaborating with Regulon. They are also encapsulating SFV particles into liposomes to allow systemic delivery. Early studies show that the SFV virus could be targeted to human prostate tumours on SCID mice, with high doses of the viral particles showing no toxicity in normal tissue.

Much of the work in gene transfer currently concentrates on viral vectors, but even some of those who are using them in the clinic, like Estuardo Aguilera-Cordova of the Harvard University Gene Therapy Initiative and member of the US NIH's gene therapy oversight body, believe that the future of gene therapy lies

Even some of those researchers who are using viral vectors in the clinic believe that the future of gene therapy lies with safer non-viral vectors

with safer non-viral vectors. But Jesse Gelsinger's death has also brought greater caution and scrutiny to the field of gene therapy, so those working on viral vectors are paying more attention to safety and are only advancing slowly, one step at a time.

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logous envelope proteins such as the rabies, vesicular stomatitis or murine leukemia viruses, or when they are given cell-type-specific targeting envelopes'.

Recently, Dornburg's team developed SNV- and REV-A-derived vectors that can transfect quiescent human cells by engineering them to express single-chain antibodies (scAs) or other targeting ligands on the surface. Using phage display, they developed a system by which scAs can be screened and selected, and they subsequently produced the first vectors specifically targeted to human macrophages, T-cells and neurons by the addition of a nuclear localisation signal sequence to the matrix protein.

Dornburg is collaborating with TheraCyme (Irvine, CA), a company that manufactures implantation devices for the slow delivery of therapeutic cells for long-term treatment. Their devices have already been used for *in vivo* delivery of Factor IX, an antitumour antigen, human growth hormone and insulin in SCID mice, and Dornburg believes they have additional applications for gene therapy. The aim is to use such devices for slow vector delivery in order to prevent immunological reactions that would result from the delivery of a large quantity of vector.

Non-viral gene therapy is also making headway. Ralf Kircheis of Boehringer Ingelheim (Ingelheim, Germany) described their development of a surface-shielded gene delivery system to target metastases by either coating DNA with

extremities.

Babek Alizadeh from Somagenics (Santa Cruz, CA) presented a twist on antisense technology that can be utilised for therapeutic gene delivery and in diagnostics, drug target validation and research: 'RNA lassos' to inhibit gene expression at the RNA level. 'RNA lassos form knots around target nucleic acids and provide superior binding strength compared to ordinary antisense-based methods', said Alizadeh in describing their advantages. While conventional antisense molecules can be displaced during translation by the helicase action of the ribosome, those knots can only be removed by breaking the circle that forms the knot.

Lassos are also superior to traditional antisense RNA as they have minimal toxicity, can be regulated and have rapid and stable binding to the target, said Brian Johnson, CEO of Somagenics. The company's first therapeutic target is the inhibition of TNF- α for rheumatoid arthritis, with additional indications including septic shock, cachexia, type 1 diabetes, multiple sclerosis and lupus. It hopes to reach the clinic by 2006 and is currently working with both non-viral and viral delivery systems to deliver anti-TNF- α lassos.

Regulon is also working with both non-viral and viral vectors and is testing two drugs against five tumour types in European Phase I/II clinical trials. These are encapsulated forms of the cisplatin and interleukin-12 gene, which have been