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Gene therapy — personalised medicine in action

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We all begin life as a single cell that divides and develops until we are a fully grown human being. Inside this cell, our DNA carries the complete set of instructions for this to happen. However, if your DNA sequence harbours an alteration — even a small, seemingly insignificant single-base sequence change — it can have detrimental consequences: inherited genetic disease.

Decades ago scientists hypothesised that gene therapy would be an effective treatment for inherited disease 'in the future'. Health and medical researchers have often said that 'one day' a cure will be found for serious genetic diseases. Doctors have said they will be able to treat these debilitating conditions 'at some stage'.

Well, the future is here and now — personalised medicine is front and centre on the international stage. Biotechnology, nanotechnology and affordable genome sequencing are already changing lives around the globe.

So, what exactly is gene therapy? Gene therapy is the introduction of new genetic material into cells to replace or repair missing or malfunctioning genes. The primary goal is to treat serious genetic diseases. Gene therapy is a potentially revolutionary innovation since it is possible that a one-time gene therapy could cure diseases that currently have no treatments that can delay onset or prolong survival let alone provide a cure.

Gene therapy success stories

Several hundred genetic therapies are currently in development as promising treatments, and many aspire to cure the ~5,000 diseases caused by errors in a single gene — referred to as 'monogenetic diseases'. These include conditions that affect the immune system, such as X-linked severe combined immunodeficiency (X-SCID) and Wiskott-Aldrich Syndrome, where affected individuals are more susceptible to infections; diseases that affect vision, like Leber congenital amourosis, which is a rare form of blindness; bleeding disorders like haemophilia; and neurological diseases such as Huntington disease, Rett syndrome and Friedreich ataxia. In these cases, the single-gene mutation causes certain cells in the body, such as immune cells or neurons, to develop abnormally and/or function poorly, resulting in disease.

Monogenetic diseases are appealing targets for gene therapy since there is only one gene that needs to be corrected for clinical benefit. Pioneering proof-of-principle clinical trials for cell and gene therapy have successfully improved symptoms in patients with:

- chronic granulomatous disease (CGD) — an immune disorder (Ott et al., 2006)
- Leber congenital amaurosis (Bainbridge et al., 2008)
- β-thalassaemia — a condition that results in anaemia due to lack of red blood cells (Cavazzana-Calvo et al., 2010)
- X-linked adrenoleukodystrophy — a degenerative condition that affects the brain and shortens lifespan and was the subject of the film Lorenzo's oil (X-ALD) (Cartier et al., 2009; Eichler et al., 2017)
- adenosine deaminase severe combined immunodeficiency (ADA-SCID) (Bordignon; 1995)
- X-SCID (Cavazzana-Calvo et al., 2000)

Table 1: The 12 human serotypes of AAV that can target different cells

AAV Serotypes	
Tissue	**Optimal Serotype**
Central nervous system (CNS)	AAV1, AAV2, AAV4, AAV5, AAV8, AAV9, AAV10
Heart	AAV1,AAV8, AAV9
Kidney	AAV2
Liver	AAV7, AAV8, AAV9
Lung	AAV4, AAV5, AAV6, AAV9
Pancreas	AAV8
Photoreceptor cells	AAV2, AAV5, AAV8
Retinal pigment epithelium (RPE)	AAV1, AAV2, AAV4, AAV5, AAV8
Skeletal Muscle	AAV1, AAV6, AAV7, AAV8, AAV9
12 human serotypes identified	
>100 non-human primate serotypes identified	
Hybrid and synthetic AAV also in development	

- haemophilia B (Nathwani, 2011)
- Wiskott-Aldrich syndrome (Aiuti, 2013)
- metachromatic leukodystrophy — another degenerative disorder of the brain (Biffi et al., 2013)

A number of these therapies have entered the commercialisation pipeline, with very recent entries to the market following regulatory approval, and many more are in pre-clinical development and ongoing clinical trials.

These success stories give hope to those with more serious and debilitating diseases. One example is Friedreich ataxia, a progressive neurodegenerative disease that usually begins in childhood. Symptoms worsen over time, and affected individuals need to use a wheelchair due to severe incoordination. Friedreich ataxia also results in heart complications, which markedly shorten lifespan. There is currently no proven treatment to slow or halt disease progression.

A critical protein called frataxin is present at much lower levels in affected individuals compared to those without

disease. This 'loss of frataxin' is the primary cause of Friedreich ataxia, so treatment strategies aim to increase frataxin, and that is where cell and gene therapies come in.

Different modified viruses that deliver frataxin to cells are in development as potential treatments for Friedreich ataxia. Pre-clinical studies have shown that gene and cell therapy can improve the inherent nervous system and heart damage using animal models. Whether these different gene therapies translate to clinical benefit in affected individuals remains to be seen.

How do you do gene therapy?

There are several ways to perform gene therapy, but most commonly, inactivated viruses are used to deliver new genetic material into cells. A therapeutic gene (called a transgene) is inserted into the viral genome and packaged into the 'outer shell' (called the capsid) of the inactive virus. This is now called a viral vector, which has corrective potential and can enter specific cell types to deliver the therapeutic gene. This 'Trojan horse' approach is proving effective in a number of inherited diseases, particularly those affecting the immune system, the eye and even the heart.

Viral-based gene therapy

A range of inactivated viruses have proven to be successful agents of delivery for gene therapy, including adenoviruses (which cause the common cold), adeno-associated viruses (no known diseases caused in humans) and retroviruses, including the lentivirus and human immunodeficiency virus (HIV).

Adeno-associated viral vectors (AAV)

AAV vectors have a relatively small packaging capacity and therefore are not ideal to use in every disease setting. Some genes are quite large and will not fit into AAV.

There are 12 human forms of AAV called serotypes (Table 1) and more than 100 non-human primate serotypes that allow AAV to target different cell types within the body. AAV can enter a wide variety of cell types, but they can be lost from actively dividing cells such as blood cells because they do not readily integrate into the cellular DNA (the host genome). AAV is effective in cells that have stopped dividing or slowly dividing cells. To increase the application of AAV vectors, expand their target cell types and increase their efficiency, a range of new 'synthetic' AAV serotypes designed by researchers is also in development.

Challenges in developing AAV gene therapy

A significant challenge for AAV gene therapy is the limited packaging capacity of AAV. This restricts the size of genes that can be incorporated into this vector. New forms of AAV have now been developed to increase the packaging capacity; however, they are not suited to every disease setting.

The primary safety concern with AAV is the potential immune response produced inside the human body. AAV gene therapy is administered directly to the patient undergoing gene therapy. Most people are naturally exposed to AAV in their lives, meaning they often have pre-existing, neutralising antibodies to different AAV serotypes. Around two-thirds of the population have antibodies to AAV. Prior to gene therapy, individuals must be tested for pre-existing antibodies to the AAV serotype used. Not only does an immune response present a potential risk to the individual, but an immune response targeting AAV-transduced cells reduces the amount of protein produced by the corrective gene and can therefore reduce the potential benefits of gene therapy.

Generally, only a single administration of each AAV vector is possible since once exposed to a certain type of AAV, treated

individuals develop an immune response to the AAV. Administration of the same AAV a second time will be less effective because the body's immune system will inactivate the AAV before it can deliver the therapeutic gene to cells. This can limit options for further treatments in the future. Several methods can reduce the immune response, including steroid treatments and immune cell depletion. Of note, there is a clinical trial of AAV gene therapy (AAV9) for Pompe disease, a condition that results in muscle weakness, to evaluate repeat applications of AAV gene therapy, and the results will be of great interest to the field.

Retroviral vectors

Using retroviral vectors for gene correction has been integral to the development of effective gene therapies. Derived from retroviruses, these vectors have several advantages over other vectors, including their ability to integrate into the host cell's DNA (the genome). This means retroviral vectors can permanently and stably modify and correct the host genome. Two types of retroviral vectors have been developed for gene therapy — oncoretroviral and lentiviral vectors.

Oncoretroviral vectors

For oncoretroviral vectors, particularly those derived from the murine leukaemia virus (MLV), one of the greatest challenges is the risk of a side effect called insertional mutagenesis. This is where the vector inserts next to genes in the genome that when activated result in major problems such as cancer for the treated individual. Clinical trials using oncoretroviral vectors have provided clear evidence that gene integration into the treated person's genome can cause life-threatening complications. To reduce the risk, vector design has improved, and preclinical vectors are rigorously tested in animal models of disease such as mice. The aim of this is to ensure the gene will

insert into a safe region of the genome, minimising the risk of such severe side effects.

Lentiviral vectors

Lentiviral vectors derived from the human immuno-deficiency virus (HIV) can accommodate larger genes due to their increased packaging capacity. The 'gutted' HIV genome is used to make lentiviral vector particles. Human disease-causing segments of the HIV virus are removed, while the non-disease causing parts that allow DNA delivery to cells and insertion into the host genome remain intact. Therefore, there is no risk of HIV-related disease in those treated with gene therapy using lentiviral vectors.

There are several advantages to using lentiviral vectors for gene therapy, including sustainable 'life-long' correction, since they permanently correct diseased cells. Lentiviral vectors also become part of the genome and are therefore recognised as 'self'. This means there is a markedly lower risk of any immune response compared to AAV. Lentiviral vectors can also enter non-dividing cells such as nerve cells much more efficiently than AAV.

Safety concerns and solutions

The safety concerns for lentiviral vectors are similar to oncoretroviral vectors since both vectors insert into the genome. Various techniques have increased the safety profile of lentiviral vectors and almost negated the risk of insertional mutagenesis. Importantly, three strategies have significantly increased vector safety:

1. using third generation self-inactivating (SIN) vectors.
2. incorporating insulator elements into vectors.
3. using cellular promoters rather than viral promoters to drive gene expression.

SIN vectors are inactive and 'gutted' of many of the viral elements, making them safer to use in gene therapy. Insulators are regions of DNA where multiple proteins bind to isolate regions of DNA and limit any cross-talk between different DNA regions. Insulated and non-insulated lentiviral vectors are in development, and several different types of insulators are under investigation. Cellular promoters are regular promoters present inside all human cells. These switch on genes in cells. Using cellular promoters rather than viral promoters increases safety. In recently completed clinical trials, third-generation SIN lentiviral vectors using insulators and cellular promoters are proving safe.

Other strategies in development include 'non-integrating' versions of these vectors that do not insert into the host genome. Non-integrating versions of retroviral vectors can produce the therapeutic gene and can efficiently correct non-dividing cells, such as neurons or muscle, but they do not permanently correct all cell types.

A brief history of gene therapy

Compared to many other treatments and therapies, gene therapy is still relatively young. It is incredibly exciting to see how far the field has come in a relatively short time. It has gone from fundamental laboratory-based basic scientific experiments through to proof-of-concept in animal models, and onto highly successful clinical application in affected individuals all in less than 50 years. The development of gene therapy is no different to other therapeutics and medicines in that it has faced challenges and experienced setbacks alongside advances and success.

An early human gene therapy trial resulted in the death of 18-year-old Jesse Gelsinger in the United States. Jesse was injected with an adenoviral vector for ornithine transcarbamylase (OTC) deficiency. Adenovirus causes the common cold and, unbeknown to all, Jess carried pre-existing antibodies to the

type of vector used. He died within four days due to a severe immune response. The field moved forward, and in 2001, laboratory results were strong and another gene therapy trial was underway. A team in France treated 11 children with X-linked SCID using a standard oncoretroviral vector; however, two of the children developed leukaemia post gene therapy due to insertional mutagenesis. One of the two children responded to standard chemotherapy; the other did not respond to treatment and later died. The US Food and Drug Administration (FDA) promptly suspended 27 trials over safety concerns.

The field has overcome these major setbacks, with scientists persisting in their research to solve multiple issues, reduce side effects and increase the safety and efficacy of gene therapy for everyone. Over 30 individuals with SCID have now undergone gene therapy, and most of those children (>90%) have been cured of their disorder — a significant improvement on the 50% chance of recovery offered by the standard treatment of bone marrow transplant. Today, these children live normal disease-free lives thanks to gene therapy.

The gene therapy timeline

1960s: First evidence of successful gene transfer in human cells.

1990: First gene therapy attempt in a patient with adenosine deaminase severe combined immunodeficiency (ADA-SCID).

1998: Development of self-inactivating (SIN) lentiviral vectors.

1999: Death of Jesse Gelsinger in a US trial of an adenoviral vector for OTC — due to a severe immune response.

2000: Eleven children with X-linked SCID treated using a standard oncoretroviral vector delivered by bone marrow transplant. Two children developed leukaemia post-gene therapy due to insertional mutagenesis. The FDA promptly suspended 27 trials over safety concerns.

2002: Correction of ADA-SCID using a standard oncoretroviral vector delivered by bone marrow transplant.
2008: AAV gene therapy improves visual function in Leber's congenital amaurosis.
2009: Lentiviral gene therapy halted progressive brain damage in two 7-year-old boys with X-linked adrenoleukodystrophy (X-ALD).
2011: AAV gene therapy increased clotting factor in individuals with haemophilia B.
2012: uniQure's Glybera is the first AAV gene therapy approved by European regulators. The cost per treatment was $1 million!
2013: CRISPR-Cas9 gene-editing is published and the field has exploded.
2017: Novartis' tisagenlecleucel (Kymriah™) is the first CAR T-cell immunotherapy approved by the FDA in the United States.
2017: Successful treatment of 15 boys with X-ALD by transplanting bone marrow corrected with lentiviral vectors.

Cancer gene therapy — immunotherapy

The basis of this treatment is that cells undergo gene therapy to introduce cancer-specific particles that are reinfused into the body. The body then mounts an immune response to these particles. This immune response can then act against the cancer cells, resulting in death of these cells but not healthy cells. Kymriah is the first FDA-approved immunotherapy to treat acute lymphoblastic leukemia (ALL) — the most common childhood cancer. Multiple companies are now developing immunotherapies to treat a range of cancers, and immunotherapy is considered one of the most promising strategies to

effectively treat cancers that do not respond to traditional therapies.

Gene therapy into the future

Gene therapists dream about seamless, targeted correction that causes no other changes to the genome. Recent advances have seen this dream take a giant leap closer to reality with a new technology called gene-editing. The development of gene-editing for disease has exploded, with genetic diseases a primary target for gene-editing and correction studies.

Over the last decade, a number of tools for targeted gene-editing have been successfully developed. These include meganucleases, zinc finger nucleases, TALENs and the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas9 system.

A major advancement in the gene-editing field has been the highly successful application of the bacterial protein — the CRISPR-associated protein 9 (Cas9) nuclease — from a bacterium called *Streptococcus pyogenes*. CRISPR-Cas9 is guided to a specific DNA sequence inside the cell — the site of correction. Once at the desired target, CRISPR-Cas9 specifically changes the DNA and corrects the DNA mutation. A rapidly growing number of laboratory-based, pre-clinical studies are ongoing, and human studies are already underway in China and the United States.

Yet, as with every new technology in this dynamic scientific field, there are challenges to overcome. Off-target effects must be carefully evaluated in the context of gene-editing since they pose a risk for these therapies. Off-target effects occur when the technology damages other regions of the genome beyond the target DNA site — regions that are not involved in the disease being treated. Similar to insertional mutagenesis, this could result in undesirable side effects such as cancer. CRISPR-Cas9

has fewer off-target effects than previous gene-editing tools (TALENs and ZFNs), but is still not perfect, and CRISPR researchers aim to further improve its safety profile. It is important to screen for off-target effects, however, since they can cause changes in the DNA, including large deletions in the genome. Improved versions of CRISPR with greater specificity are under investigation. There are serious ethical discussions underway about gene-editing too. Potential fears around 'designer babies' of the future have sparked significant debate. As with any major technological advancement, we must take stock and consider the unintended consequences, and place policies and laws in place to protect against these outcomes from happening.

It is important to remember, however, that gene therapy offers hope to individuals living every single day with severe, debilitating diseases. Diseases that can seriously limit their lives and dramatically affect their families and friends. The value of hope through research to this community cannot be underestimated.

The potential for gene therapy to improve lives is real, but it must be approached with the wisdom of lessons learnt and the absolute rigour of the scientific process, every step of the way.

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