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GENE THERAPY – THE FUTURE HAS ARRIVED!

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When I started at Medical School in 1974, the structure of DNA had only been identified some 21 years earlier in 1953 by Watson and Crick. During my undergraduate training, we only had one lecture on DNA and I remember quite clearly the lecturer concluding at the end of the lecture that 'DNA might be useful in some way to treat patients'. In fact, in Watson and Crick's seminal paper on the structure of DNA that was published in Nature in April of 1953 they stated that 'This structure has novel features which are of considerable biological interest'. Little did they know that some 40 years later, the first clinical study using DNA as a basis of a therapeutic agent would be carried out and then some 20 years after that, the first medical product for the treatment of a genetic disease would receive regulatory approval to available to be prescribed to patients.

In this lecture, I will give the background as to how DNA was researched and developed to form the basis of a treatment that has become to be known as 'Gene Therapy'. I will talk about the principles of gene therapy and how DNA can be administered to humans using viral vectors and other delivery systems. I will also give details about how gene therapies have been developed to treat various diseases and clinical indications and finally I will give examples of specific gene based therapies and how they are being now utilised for the benefit of patients.
The basis of gene therapy started back in 1868, when a Swiss physician, Friedrich Miescher isolated a substance from the nuclei of cells which he called 'Nuclein'. Today this is what is now known as 'nucleic acid' as in deoxyribo-nucleic-acid or DNA. Around this time Gregor Mendel, the father of genetics, completed a series of experiments with pea plants and showed that certain traits in peas, their shapes and colours, were inherited in what he called 'different packages'. These 'packages' are what we now call 'genes'. However, the connection between nucleic acid and genes was not known for a long time until 1944, when Dr Oswald Avery, working at the Rockefeller University Hospital in New York, managed to transfer the ability to cause disease from one strain of bacteria to another and that the previously harmless bacteria could pass on the trait to the next generation. What was transferred was 'nucleic acid' and in doing this work confirmed that 'genes' were made up of nucleic acid.

In the late 1940's scientists were aware that DNA was most likely the 'molecule of life' but no one knew what it looked like. Many groups were working on the problem including people like Linus Pauling, who published a paper in early 1953 where he proposed that DNA had a triple-helical structure. In 1953, James Watson and Francis Crick, working in Cambridge together with Rosalind Franklin and Maurice Wilkins, at Kings College in London solved the problem of DNA structure using X-ray diffraction techniques and identified the 'Double Helix', which they realised translated biological information.

DNA is a very large molecule that contains the 'instructions' that an organism needs in order to develop, live and reproduce. It also determines what the organism looks like and what diseases they may develop. These instructions are found inside nearly every cell of the organism, being passed down from parents to children. The structure of DNA itself is that it is made up of much smaller individual molecules called 'nucleotides', with each nucleotide containing a phosphate group, a sugar group and a nitrogen base. There are four types of nitrogen 'bases' called Adenosine (A), Thymine (T), Guanine (G) and Cytosine (C). The order of these bases determines the DNAs instructions, known as the Genetic Code and a specific sequence forms a 'gene'. Any changes in

these bases in a specific sequence can change the structure and nature of the 'gene' and is what is known as a 'gene mutation'.

Genes are then used by a cell to make protein by using another type of nucleic acid known as ribonucleic acid or RNA, which facilitates the translation of the genetic information from DNA into proteins. The Human Genome contains about 3 billion bases and about 20,000 individual genes.

Gene Therapy is defined as the use of DNA/RNA as a pharmaceutical agent to treat disease. Gene therapy can be used in two ways. It can either be used to replace a mutated gene in diseases such genetically inherited blindness like retinitis pigmentosa, or it can be used to generate a specific therapeutic protein drug (rather than a natural human gene) to provide a specific treatment for a patient.

The first mention of gene therapy in the medical literature is believed to have been in a publication in Science in 1972 by Friedmann and Robin entitled 'Gene Therapy for human genetic disease?' and they proposed 'that exogenous 'good' DNA' could be used to replace defective DNA in those who suffer from genetic based diseases. The first gene therapy clinical study was in the United States and took place on September 14, 1990, at the National Institute of Health, Washington DC. It was performed on a four year old girl named Ashanti DeSilva, as a treatment for a genetic defect that caused her to have an immune system deficiency (SCID). The treatment effects were only temporary, but successful. Since then, over 2,400 clinical trials have been conducted using a number of techniques for gene therapy and the numbers of clinical trials have increased annually since, with a total of 170 clinical trials being initiated in 2015 as an example. In terms of where most clinical trials have been conducted, the United States have conducted about two-thirds of them with Europe conducting about one-quarter. It is of interest that the UK leads the way in Europe in terms of both the research and development of gene therapies.

So how does a Gene Therapy work? A 'carrier vector' is typically used to deliver the therapeutic gene to the patient's target cells. The majority of these vectors are viruses that have been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. So vectors are utilised to take advantage of this capability and the virus genome is manipulated to remove their disease-causing genes and insert therapeutic genes in their place. Target cells in the patient are the infected with the viral vector, where the vector then unloads its genetic material containing the therapeutic human gene into the target cell and generates of a functional protein product from the therapeutic gene that restores the target cell to its normal state.

There are four types of viral vectors that are mainly used as the basis of a gene based therapeutics. These are Adenoviruses, Adeno-associated viruses (AAV), Retroviruses and Lentiviruses and depending upon the clinical indication being treated the viral used is selected on the bases of its physiological and pathological features. As examples, Adenoviruses and Retroviruses are widely used in the treatment of caners, whilst nervous system, eye and muscle disease treatments utilise AAVs and the treatment of many inherited genetic diseases rely on lentiviruses and retroviruses.

The development of gene therapies follows the standard process that is used to develop chemically based therapies. The gene therapy product needs to be manufactured under strict regulatory compliance to ensure its required quality, purity and potency. Animal toxicology studies need to be performed for safety assessments prior to its administration to humans and finally clinical trials need to be undertaken to determine the dose of the product and its safety and efficacy in the specific disease being treated.

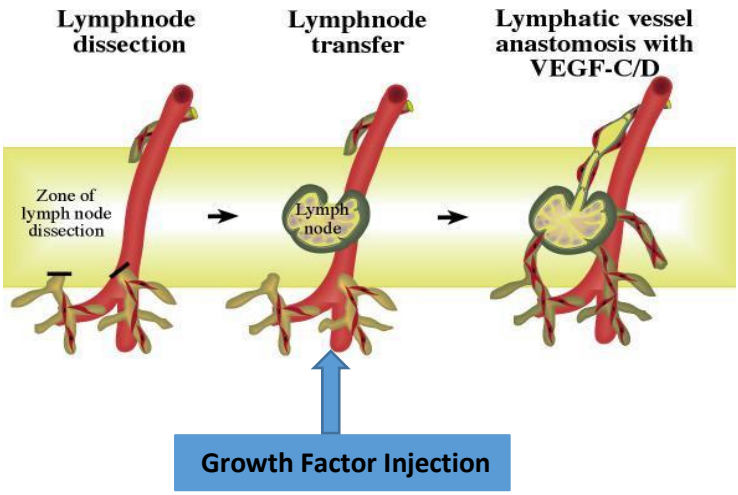
One of the main barriers to the development of gene therapy is ensuring that it reaches the target cells that need treating. All the gene therapy products that have reached an advanced stage of clinical development require special routes of delivery and administration. In order to get them to the site where they are needed and to bring about their therapeutic effects locally where they are needed. Individual gene therapy products that require simple intravenous administration are in the minority and the majority need to be delivered in a targeted way, often using specialised delivery techniques that may include medical devices.

Having given the back ground to the origins of gene therapy and how the area has grown over the past twenty years or so, I would now like to give details of some specific gene therapy development programmes and their

products which will further illustrate what has happened in the gene therapy space and what the future might hold.

The first development programme that I would like to discuss is that of a product under development called Lymfactin® (Herantis Pharma plc), which is being developed as a gene therapy for the treatment of secondary lymphedema. Lymphedema is a disabling, disfiguring and debilitating condition severely affecting quality of life. It is a chronic, progressive swelling of the affected tissues, typically in the arm caused by dysfunction of the lymphatic vasculature. Secondary lymphedema is a consequence of disease, trauma, surgery or radiotherapy and most commonly occurs in women who have been treated for breast cancer. There is no cure or satisfactory treatment for lymphedema and as an example, in the USA there are over 18,000 diagnosed new cases of breast cancer associated lymphedema each year, plus over 150,000 already diagnosed cases of this condition. Similar numbers of patients are diagnosed and exist in Europe.

Lymfactin® is a gene therapy product that consists of an adenoviral vector coding for Vascular Endothelial Growth Factor C (VEGF-C). VEGF-C is the growth factor that stimulates and maintains the lymphatic system in humans. Its application in this condition is being used to increase the levels of VEGF-C locally in the axilla or the arm-pit area to help facilitate the growth of a new lymphatic system in the patient. Therapy with Lymfactin® involves a surgical operation where a lymph node is removed from the patient's lower abdominal wall, injected with Lymfactin®, and then transplanted in to the axillary region of the patient.



To date, it has been demonstrtaed in animal models of the disease that the application of VEGF-C as a gene based therapy induces lymphatic vessel growth and improves lymphatic vessel function. In addition, it appears to have been well tolertaed in the animals and preserves the follicular structure in transferred lymph nodes. Recently a small clinical study in humans has been initiated to investigate the safety and the preliminary efficacy of the product. This study is aimed at treating patients that are independently undergoing surgery aiming to repair the damaged lymphatic tissue by transplanting their own abdominal tissue containing lymph nodes into the axilla with the injection of the gene therapy that produces VEGF-C. The initial results from this study are expected in early 2018 and this programme is a good example of how a gene therapy is being used to deliver a therapetic protein that should be of benefit to patients in treating their disease and medical condition.

The next programme that I would like to discuss is for a gene therapy that was deleoped for the treatment of malignant glioma, a severe form of brain cancer. The was called Cerepro® and was developed by a company called Ark Therapeutics Ltd, of which I was the Resarch and Development Director. Malignant glioma is a cancerous tumour that is confined to the brain. There are approxiamtely 38,000 cases per year in the USA and Europe. The standard care for this tumour is its surgical removal, followed by radiotherapy and chemotherapy. It is of interest that the most recently licensed pharmaceutical product that has been approved for this cancer only extended life by an average of 10 weeks. Most patients with this tumour die within one year of diagnosis. Cerepro was developed for the treatment of operable primary & recurrent high grade glioma and from clinical studies it had clear survival benefits, extending life on average by 7 months. It was also given in addittion to standard therapy. This was also the first gene therapy product that was submiitted to the European Medicines

Agency for consideration for registration and approval as a medicinal product that could be prescribed to patients. The way that Cerepro was administered and subsequently worked was as follows: brain surgery was first performed to remove as much of the tumour mass as possible. Cerepro, is an adenoviral vector which contained the gene that would produce a protein call thymidine kinase (this is an enzyme that does not occur in humans) was injected into wall of tumour cavity, which mainly consisted of healthy brain cells. After sugery was completed, another medicine called Ganciclovir was then given given intravenously from 5 days post surgery for 14 days. In the brain the gene protein product, the thymidine kinase enzyme acted upon the Ganciclovir and turned it into a toxic product. This activated ganciclovir will only kill any proliferating tumour cells and does not harm healthy brain cells or neurones within the brain. This is an example of a gene based therapy that is providing a specific protein, namely the thymidine kinase enzyme, that has no activity in humans on its own but when combined with another agent produces a therapeutic benefit.

As part of the development pathway, animal models of malignant gloma were developed that were used to demonstate proof-of-concept for the therapy. The product was manufactured to the required standards and followin toxicology work in animals, clinical trials in humans were commenced. The first small clinical trial that was done investigated the safety of the product and assessed if the administration proceedures could be undertaken as required. The survival of the these patients was also assessed and was shown to improve survival by about 7 months. Following this a much larger randomised clinical trial was performed in approximatelt 300 patients. The results showed that in the group that received Cerepro in addition to the standard care, had a significant improvement in their survival of around 7-8 months, compared with the group that received standard care alone. As mentioned previously, all this data and information was submitted to the European Medicines Agency for consideration for approval as a prescription product. However they decided not to grant approval and requested that additional data be genertaed for the gene therapy. The Cerepro programme is an excelent example of the use of a gene therapy that makes a protein that is used directly to treat a disease such as cancer by its action in killing tumour cells but not harming healthy cells.

I would now like to provide details of another gene therapy that this time is targeted at treating a genetic disease which, due to a particular gene mutation, results in a disease that is fatal in young children. The disease in question is known as Adenosine Deaminase Deficiency which leads to a Severe Combined Immunodeficiency in very young children. The disease name is usualy abbreviated to ADA-SCID. This disease is a rare disorder occurring in approximately 15 patients per year in Europe. It is caused by a gene mutation that results in the absence of an essential protein called adenosine deaminase (ADA), which is required for the production of lymphocytes or white cells. Due to this condition children born with ADA-SCID do not develop a healthy immune system, so cannot fight off everyday infections, which results in severe and life-threatening illness. Without prompt treatment, the disorder often proves fatal within the child's first year of life. The main symptoms of ADA deficiency are pneumonia, chronic diarrhoea, and widespread skin rashes. Affected children also grow much more slowly than healthy children and some have developmental delays. Most individuals with ADA deficiency are diagnosed with SCID in the first 6 months of life and historically, because they need to be kept in isolation to prevent them becoming infected, have become to be known as 'Bubble Children'.

The standard treatment of ADA-SCID is for the child to have a Matched Related Bone Marrow Transplant (BMT) Or a Matched Unrelated BMT. An Enzyme Replacement Therapy is also available which consists of weekly injections with ADA Enzyme which has been manufacted synthetically. The main problems with these treatment approaches is that the bone marrow transplants do not always work and the enzyme replacement therapy is only suitable for some patients and does also involve weekly trips to the hospital for the infusions. However, in April 2016 the Committee for Medicinal Products for Human Use of the European Medicines Agency endorsed and recommended for approval a stem cell - gene therapy combination product called Strimvelis® (GSK plc), for children with ADA-SCID for whom no matched related bone marrow donor is available.

The way that Strimvelis is administered also involves the use of the patients own stem cells from their bone marrow. The first step in the procedure is that the patient has a sample of bone marrow taken from them. From this specific stem cells (CD43 cells) are isolated and multiplied up in the laboratory under sterile conditions. As these cells carry the mutated gene they are then transfected with the gene that expresses the ADA enzyme and is contained within a lentiviral vector. By the nature of the lentiviral vector it actually inserts the correct gene that will produce the ADA into the chromosomes of the cell. The patient then is given

chemotherapy which destroys all their bone morrow and removes all the cells that carry the mutant gene. Following this treatment, the CD34 cells that now contain the correct form of the ADA gene are then injected into the patient via an intravenous infusion and these cells will re-establish the bone marrow in the patient, which in turn will produce the white cells that the patient would normally have.

From the clinical studies that have been undertaken with Strimvelis, from the 18 patients that have been treated they have all survived much longer than expected, with the average survival time being around 7 years so far and increasing. Also the rate of severe infections that these patients have suffered has reduced dramatically. This has been accompanied by an increase in their white cell counts to normal levels and the children have subsequently grown and developed as would be expected for children of their age.

The Strimvelis gene therapy programme is a good example of using the patients own stem cells to deliver the gene therapy to where it is needed. This approch is called 'Ex-Vivo Gene Therapy' as the cells of interest where you want the gene therapy to be active are removed from the patient, grown up in number, then transfected with the gene of interest and then the cells are returned to the patient to bring about the benefit as detailed above.

The final gene therapy I would now like to cover is for an inherited genetic disease which involves the eye and if untreated results in blindness. The eye disease involves a mutated gene for a protein that is involved in the visual cycle and the chemicals at the back of the eye, which sense light, and transmit signals to the nerves that connect to the brain that allows people to see. The mutation affects a gene called 'RPE65' and this mutation leads to an eye disease known as 'Retinal Dystrophy'. The eye is a good target for gene therapy as it is a small, enclosed compartment, which accommodates small amounts of a gene therapy to act and also keep side effects to a minimum. The eye's structure allows for non-invasive assessment via direct observation of the retina with an ophthalmoscope and imaging techniques. In addition, because the eye is a self-contained, immune-privileged organ, the potential for the gene therapy to disseminate outside of the eye is minimised and the risk of an immune response to the gene therapy is reduced.

The RPE65 gene encodes the RPE65 enzyme and this enzyme plays an important role in the 'visual cycle'. Mutations in the RPE65 gene lead to partial or total loss of RPE65 enzyme function and eventually causes the accumulation of toxic by-products in the retina.This leads to a range of visual impairments such as: night blindness, reduced visual fields and reduced visual acuity, which all get progresively worse with age. Currently there are no approved treatments are available for this condition and children suffering from this condition are typically registered as blind by the time they are teenagers.

The solution to this problem is a novel gene therapy that targets the specific gene mutation and is called 'Voretigene Neparvovec' gene therapy, that has been developed by Spark Therapeutics Inc in Philadelphia, USA. The product uses an adeno-associated virus as its viral vector. The advantages of using the AAV viral vector is that it is non-pathogenic in humans and it has the ability to penetrate photoreceptor cells at the back of the eye where the enzyme is needed. It also establishes long-term gene transfer for the gene and the vector, which means that only a single injection into the eye is needed to produce its benefits over a sustained period. Also it has a low immunogenicity potential and therefor shoud reduce the number of side effects that might occur. To date over 170 plus human clinical trials had been conducted with AAV on a worldwide basis, so there is much information known about AAVs and there use as vectors.

One issue that ths development programme has faced is that of the assessment of the gene therapies effectiveness in this disease. Because the eye disease affects functional vision including the ability to navigate, a novel test of functional vision was needed to quantify any beneficial change during treatment in the gene therapy clinical trials. Therefore Spark Therapeutics developed 'The Multi-Luminance Mobility Test' (MLMT) to assess ambulatory vision at light levels encountered during activities of daily living. A mobility course was designed to be navigable by children as young as age 3 & subjects were evaluated for accuracy and speed on the MLMT at 7 standardized light levels, ranging from 1 to 400 lux.

Using the MLMT assessment in clinical trials it has been possible to assess the effectiveness of the use of the gene therapy product, Voretigene Neparvovec, in patients. The main clinical study enrolled 31 subjects aged ≥4 years with confirmed RPE65 gene mutations and sufficient viable retinal cells to allow them to be treated. 2

subjects withdrew prior to treatment so in total 29 paients were treated in the study. Subjects randomized 2:1 to the treatment or control groups. Subjects in the treatment group received the gene therapy in each eye, with the second eye being injected within 6 to 18 days after the first. The control group subjects had the option to cross over to receive the gene therapy following 1 year from baseline evaluation. In relation to the age distribution in the study 43% of the treatment group were under 10 years of age vs 40% of the control group. The main endpoint of the clinical study was the mean change in the Mobility Test from baseline to 1 year compared between the treatment and control groups.

The results from this study showed that the patients treated with Voretigene Neparvovec showed a significant improvement in their visual function as assessed by the Mobility Test and also in their light-sensitivity threshold. For the patients who were in the control group who subsequentky went on to also receive treatment with Voretigene Neparvovec, they too showed similar improvements in their visual function.

The results of the clinical studies together with the manufacturing process details and all of the pre-clinical animal assessments that have been undertaken by Spark Therapeutics have recently been submitted to the FDA in the USA for consideration for Marketing Approval. A submission to the European Medicines Agency will also take place this year too. This gene therapy development programme is a good example of using a gene therapy to target and treat a specific gene mutation for a localised disease. Without such treatment the consequences for the patient are severe and would have an enormous impact on their and their families quality of life.

In terms of looking forward to the future, we now have three gene therapies that have been approved by the Regulatory Agencies either in Europe or in the USA. Although Cerepro was the first gene therapy to be submitted for approval, it was not finally approved. However it paved the way for other gene based therapies to be reviewd and receive regulatory approval. The three gene therapies that have been approved to date are: Glybera® developed by uniQure plc in the Netherlands for the treatment of lipoprotein lipase deficiency (Approved by the EMA); T-Vec™ from Amgen Inc for the treatment of malignant melanoma (Approved by the EMA and the FDA); and Strimvelis® from GSK for the treatment of ADA-SCID (Approved by the EMA). There are many other gene therapies under development and several reaching the stage whereby they will be submitted for regulatory approval, with the treatment for the RPE65 mutation that leads to blindness by Spark Therapeutics being a good example.

Little did Watson and Crick and all the other individuals who have worked over the many years, starting with Friedrich Miescher in the 19th century, perhaps appreciate how their work would lead to the kind of treatments that I have described during this presentation. Some of them will be life-changing for the individuals concerned and as far as gene therapy based products, although they have been a long time in coming they have certainly arrived and the future looks bright.

Thank you for your attention and I would like to acknowledge the help that Herantis Pharma plc, GSK plc and Spark Therapeutics Inc have given to me in preparing for this lecture.