

## REVIEW ARTICLE

# Gene therapy: An updated overview on the promising success stories

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### Abstract

Gene therapy is a method of treatment of disease aimed at its molecular level. The progress of gene therapy, however, was as promising as it was tardy mainly due to the limitations in the resources and financial part of its development as well as owing to the rarity of most diseases it can offer its benefits to. The methods of gene therapy can vary depending on factors such as the physiology of tissue of interest, affinity of vectors to a certain type of cells, depth and accessibility of the tissue of interest, and size of the gene to be replaced or edited. The concept behind gene therapy has inspired scientists and clinicians alike leading to a rapid expansion of its clinical utility that has become so widespread to not only include diseases of monogenic origin, but also polygenic diseases, albeit not so commonly. This article delves into notable success stories of gene therapy which has been regarded as the beacon of medical novelty expected to blossom in the near future to provide a holistic, targeted, precise, and individualistic personalised-medicine as well as laying out the future hopes of gene therapy in the treatment of debilitating diseases such as solid tumours, AIDS, Tuberculosis, Diabetes Mellitus, psychiatric illnesses, which are still at a standstill, from a gene therapy point of view.

**Keywords:** gene therapy, overview, transgene, update, vector

### INTRODUCTION

Gene therapy involves the use of genes as medicine to treat or prevent diseases. In other words, the introduction of nucleic acids into cells using a vector with the intention of altering gene expression, to prevent, halt, or reverse a pathologic condition, is gene therapy. Gene therapy has been largely an enigma for many years ever since its first conception in the 1980s. The concept, however, is not new, and it progressed rapidly up until the early 1990s where a monumental history was made when two young girls with adenosine deaminase severe combined immunodeficiency (ADA-SCID) had a successful gene transfer. Ever since that, the novelty in treatment modalities and possibilities have expanded to not only common diseases such as cancer and inherited lipid disorders, but also to rare ones such as limb ischemia. As of recent, almost 2600 gene therapy, clinical trials have been either completed or still in progress worldwide<sup>1</sup> covering a wide range of diseases such as immunodeficiency disorders

like severe combined immunodeficiency (SCID), haematological diseases like haemophilia and sickle cell anaemia, cancer like acute lymphoblastic leukaemia (ALL), muscular dystrophies like spinal muscular atrophy (SMA), metabolic diseases like mucopolysaccharidoses (MPS IIIA) as well as monogenic diseases like cystic fibrosis and alpha-1-antitrypsin deficiency.

### Principles of gene therapy

The concept behind gene therapy is rather straightforward, where the idea of diseases arising from a number of mutations occurring at the molecular level or the gene itself, can thus be corrected by replacing the mutations to either prevent or treat diseases. The types of 'faults' or mutations that can cause a disease range from either deletion, addition or many others as for example gene deletion in alpha thalassemia.

The potential targets for gene therapy include genetic disorders as well as acquired diseases. Genetic disorders such as Duchenne muscular dystrophy, cystic fibrosis, familial hypercholesterolemia, haemophilia, albinism,

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Gaucher's disease, phenylketonuria and acquired diseases such as cancer, neurologic disorders, cardiovascular diseases, rheumatoid arthritis, diabetes mellitus are all potential targets for gene therapy.

### Potential approaches for gene therapy

In order to alter a cell at a molecular level, there are several approaches that can be done.

One approach is corrective gene therapy where a mutated non-functional gene that causes diseases is replaced by a healthy copy of the gene. This technique is usually suitable for dominant disorders and is particularly useful when the defective gene produces a disease-causing protein or an interfering substance.

Gene augmentation therapy is another approach which involves the addition of a normal gene to replace the function of a defective gene. Here, the defective gene is not replaced. This is suitable for recessive disorders. But this approach is not useful when the defective gene produces a disease-causing protein. Inactivating or knocking out a mutated gene that is functioning improperly is also an option.

Introducing a new gene into the body to help fight a disease, repairing the abnormal gene, which returns the gene to its normal function, and altering the regulation (the degree to which a gene is turned off or on) of a particular gene are also other approaches.

### Types of gene therapy

The new genes can be introduced either into a somatic cell or a germline cell. In somatic cell therapy, there is the insertion of a therapeutic gene into somatic cells such as fibroblasts, myoblasts, epithelial cells, nerve cells, and glial cells. It can correct the genetic defect in the patient. But the transgene can affect only the targeted cells of the patient and cannot be passed on to the offspring. Somatic cell therapy is acceptable for cystic fibrosis, cancer and muscular dystrophy.

On the other hand, in germline gene therapy, the normal version of a gene is introduced into germ cells such as sperm, ovum or fertilised ovum. A zygote produced as a result of germline gene therapy will have the correct version of the defective gene and will continue passing it on to the offspring. This will result in the expression of modified genes in both somatic as well as germ cells of the offspring. Nevertheless, it is not advocated in humans as many ethical problems are yet to be answered.

### Steps in gene therapy

There are several prerequisites to be fulfilled before the initiation of gene therapy. These include identification of the disease and defective gene, identification of the gene mutation, establish the relation of mutation to pathophysiology, cloning of the normal healthy gene, identification of target cell or tissue or organ, gene transfer efficacy and safety testing systems and finally the introduction of a biologically pure reagent. Once the prerequisites are fulfilled, the next methods involved are 1) introduction of functional genes into appropriate cells, insertion of a normal functional gene into the host DNA, ensure that the transferred gene (transgene) encodes and produces protein and also that the proteins encoded by the transgene correct the disorder.

The question that will come to mind next would be, how do we alter the defective gene inside the nucleus? There are two methods, *ex vivo* and *in vivo*.<sup>2</sup> Both methods require a vector, viral or non-viral.

In *ex vivo* technique (Fig. 1), patient cells are cultured in the lab followed by the introduction of new genes into the cells, and then the cells with the new genes are administered back into the patient.

While in *in vivo* technique, the transfer of new genes occurs in the body of the patient by means of a vector. This technique can be achieved by using either a systemic infusion of DNA liposome, or tissue injection of a recombinant virus, or using biolistic gene gun to implant a plasmid DNA into the patient's cells.<sup>3</sup>

### What are vectors

Since the genetic material has to be transferred across the cell membrane and preferably into the cell nucleus, different carrier systems are used for gene therapy. These carrier systems are called vectors. Vectors can be viral vectors or non-viral vectors.

Vectors used in gene transfer serve as the messenger due to their capability to infect a human cell and integrate their genetic material into the host cell's genome and later utilise the host cell's organelle machinery to produce the protein they code. The common viral vectors used are retrovirus, adenovirus, adeno-associated virus (AAV), lentivirus and herpes simplex virus.<sup>4</sup> Each of the viruses has its own specificity that can cater to the type of gene to be transferred or the type of cells it needs to infect. For example, adenovirus has a natural tropism for both dividing

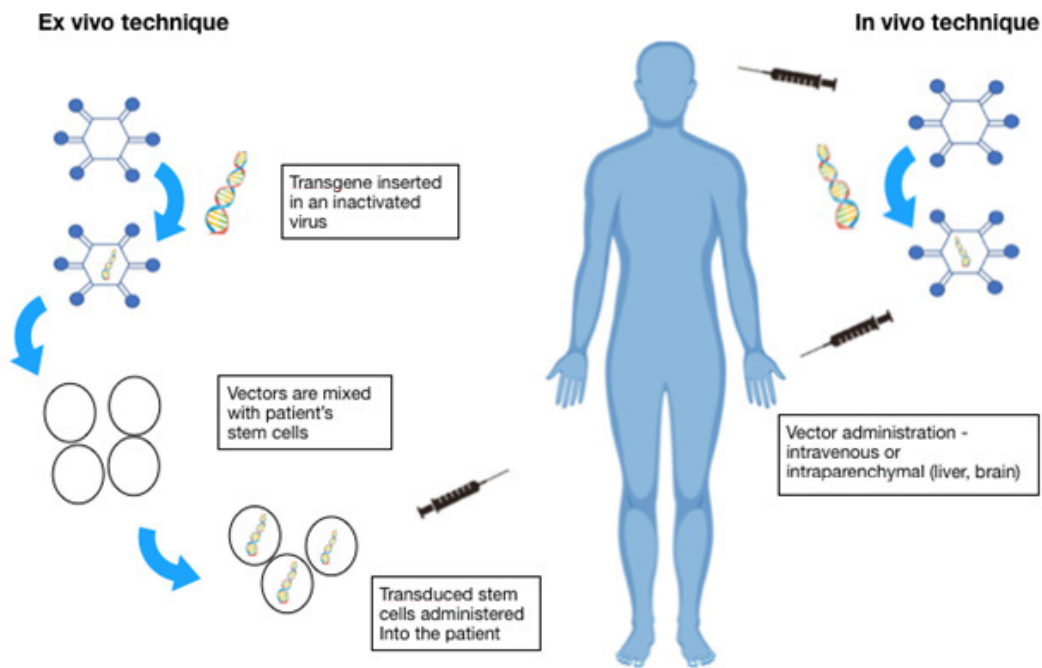


FIG. 1: Difference between *ex vivo* and *in vivo* methods.

and non-dividing cells of respiratory epithelium,<sup>4</sup> and its genome usually does not integrate with that of the host cell's genome and the expression of its transgene is transient. This is unlike a retrovirus vector where a retrovirus vector can only infect a dividing cell and it integrates its genome onto the host cell's genome and the expression of the transgene is long-lasting.

#### Non-viral vector methods of gene delivery in gene therapy

The introduction of a new gene into a cell using non-viral vectors usually involve physical or chemical methods. Physical methods involve the usage of either direct delivery, gene gun, electroporation, magnetofection or sonoporation technique.

Direct delivery of naked DNA plasmid by intramuscular injection is the simplest and safe method of non-viral transfection utilising the endocytosis capability of host cells such as in muscles, skin, cardiac muscles and even in solid tumour cells. However, the low transfection rate limits the expression of the transgene.

Electroporation uses an electrical field to increase the permeability of the host cell membrane by means of a pore to allow foreign DNA transfer. Hence, it is also called electropermeabilisation. It is not a novelty since its first usage has been dated as far back as 30 years ago in the 1980s in the field of vaccine

production. Apart from that, electroporation was hailed as one of the few non-viral transfections methods that shows an excellent transfection rate as high as 100 to 1000-fold compared to the other non-viral methods which elevate its efficacy similar to that of viral method.<sup>5</sup> This technique is usually employed on skin or muscle tissues with good efficiency although it is generally known that this method works on almost all cell types, and it is reproducible with reduced chances of immune reaction and inflammation. The downside is, it can cause tissue damage if inappropriate electrical pulses are used.<sup>5,6</sup>

Gene gun or biolistics is a high-pressure device used to deliver elemental particles like gold or tungsten coated with exogenous DNA which is usually a plasmid DNA (pDNA) or transgene into cells.<sup>7</sup> It can be used on any type of cell including plants and its organelles.<sup>8</sup> It is flexible, has a low toxicity with good efficiency and good depth of penetration of up to 100  $\mu\text{m}$  in one ex-vivo study conducted on porcine skin. Another phase 1 clinical study showed that biolistic ejections were safe with mild local skin reaction that resolved within 28 days.<sup>9</sup> One of the drawbacks is the possibility of inserting a multi-copy of a transgene that can cause transgene silencing and thus failure.

Magnetofection is another method which uses a magnetic field to concentrate particles containing the nucleic acid to be endocytosed

or pinocytosed by the host cell. This method is usually employed on primary cells or cells that are difficult to transfect by other methods. Magnetofection is flexible, inexpensive, requires low vector dose with low toxicity and highly effective but transfection is transient.

Sonoporation uses sound to alter the permeability of the cell membrane. It can be used on cells of the brain, cornea, kidney, peritoneum, muscle, heart and vascular cells. It is safe and flexible but has a low transfection rate.

Common chemical method of transfection is by using lipoplexes where a DNA (plasmid) is covered in lipid to prevent degradation and thereby improving its transfection rate. This technique is usually done for respiratory epithelial cells, endothelial cells, hepatocytes or myocytes. However, the major risk is its low safety, potential cytotoxicity and immunogenicity although its efficiency is between low to moderate.

#### **Limitations and challenges of gene therapy**

One of the major limitations of gene therapy is the lack of knowledge about diseases at the DNA (molecular) level. Many approaches have been shown to have limited reproducible outcome with an even more doubtful safety prospect. The fact that most gene therapies, although not all, rely on viral transgenesis, still harbours certain uncertainties even among its staunch supporters. Viral transgenesis is still doubted to be hundred percent safe and side effects are yet to be predicted leaving behind all this, a void so big that nobody dares to gauge. Viral vectors can cause mild to severe immunologic reactions which can trigger full-blown anaphylactic reaction leading to organ failure and even death. Some viral vectors could also trigger activation of oncogenes leading to cancers.

Many of the diseases that can benefit from gene therapy are considered rare diseases, for example, CF, SCID and adrenoleukodystrophy. This brings about the issue of funding and profit which are important since most of the prominent pursuers of gene therapy are from pharmaceutical companies which rely heavily on profits. If the disease is rare, the demand will consequentially be lesser, less markets and less profits and hence less research and advancements.

Gene delivery requires accurate targeting of target cells and also in large numbers. Targeting wrong cells can cause other health problems in the patient or in the offspring while targeting only a small number of cells won't produce the

expected effect. For example, in gene therapy of cystic fibrosis (CF), the vector adenovirus has difficulty to reach the basolateral membrane area where the AECs are mostly present, and thus the transfection rate may be minimal. Also, vectors are different in terms of cell tropism and thus, their efficiency in causing the transfection. The different tropism among species of animals the vector is exposed to (example, primate, canine or rodent), can affect the success of preclinical development. Once delivered, the new gene needs to be activated and once activated, it needs to remain activated. This is a challenge because the human cells have a habit of shutting down genes that are too active or exhibiting unusual behaviours, causing the gene therapy to be short-lived.

Immune response against the viral vectors remains a huge problem although it is not very common due to the vectors being engineered to be non-pathogenic. But in some rare cases, the vectors can still mount an immune response which can cause death or serious illness. This was what happened to Jesse Gelsinger who had a rare disorder of nitrogen metabolism called ornithine transcarbamylase deficiency when he participated in a gene therapy trial but died of complication from an inflammatory response towards the vector, an adenovirus.<sup>10</sup>

Gene integration at the unwanted location throughout the genome can trigger an oncogene leading to cancers. An example of this complication was seen in children with X-linked SCID who participated in gene therapy to restore their gamma c gene. But the gene had been integrated next to a cell-division promoter gene and thus triggered leukaemia.<sup>11</sup>

Commercial availability is another problem. Not only gene therapy is expensive, the diseases it is intended for are usually rare, sometimes as rare as 1 in a million disease and although cancers are common, but most of the time, the patient needs an individualised gene therapy for better efficacy. For example, the patient's own cells need to be taken and modified and then returned to the patient and is much more expensive.

Some disease like diabetes mellitus, hypertension, Alzheimer's and heart disease have been known to be caused by a multitude of gene defects. Multiple gene effect renders the therapy more tedious and laborious as it is expensive.

Ethical issue is one of the more prominent problems in gene therapy faced by scientists worldwide. It is especially pertaining to the

boundary between somatic and germ cells, called Weismann barrier, which is still elusive in the practice of gene therapy, causing anxiety in the public due to its potential of causing serious biosafety issues if breached.

### **Promising success stories of gene therapy**

Despite rapid developments and research on gene therapy, successful interventions are still numbered. However, the miraculous feat achieved by gene therapy in diseases like immune deficiencies, hereditary blindness, haemophilia, blood disease, lipid metabolism disorder, cancer, Parkinson's disease, and adrenoleukodystrophy is worth discussing.

#### *Immune deficiencies*

Adenosine deaminase severe combined immunodeficiency (ADA-SCID) is a life-threatening disease, which results from mutation of the ADA gene leading to the deficiency of adenosine deaminase enzyme which is essential for the immune system. It is typically fatal within the first few years of life, due to lymphopenia leading to severe infections, mostly opportunistic ones, which are otherwise frivolous in nature among children with a healthy immune system. Affected children have signs of failure to thrive, developmental delay and growth retardation<sup>12</sup> as well as skeletal abnormalities, neurological deficits and hepatic.<sup>13</sup> ADA-SCID (severe combined immunodeficiency) was one of the first genetic disorders to be treated successfully with gene therapy, proving that the approach could work where the concept of multiple administrations of lymphocytes transduced with a gamma-retroviral vector via peripheral blood can lead to a sustained engraftment of T cells expressing ADA.<sup>14,15</sup> Despite the tumultuous early years of the treatment for ADA-SCID, the approval of gene therapy for ADA-SCID was finally obtained some 25 years after many human attempts beginning in 1995 and ending with the market approval of Strimvelis in Europe. It was based on data collected from a total of 18 ADA-SCID children treated from 2000 to 2011, with a median follow-up of about 7 years.<sup>16</sup> Survival rate was 100% and majority of patients showed evidence of long-term gene correction in their T lymphocytes population, excellent immune reconstitution, sustained increase in lymphocyte counts, significantly fewer severe infections over time, without showing any sign of hindrance on physical growth. Gene marking was found to be high among T-cell lineage cells with an

average of up to 70% after 1 year of follow up post-treatment which is in agreement with the survival advantage of gene-transduced lymphoid cells. However, the same could not be said on gene marking among myeloid lineage cells, as was seen only 1-2% in CD34+ cells, thereby indicating the limited success of engraftment on transduced haematopoietic stem cells. Overall, genetically modified cells were still detectable in multiple haematopoietic lineages with stable engraftment which persisted throughout long-term follow up, albeit at low levels. This indicated that the correction of haematopoietic stem cells by gene therapy had been achieved.<sup>13</sup> In another study conducted in Necker Children's Hospital in Paris, the success of T cell reconstitution was not only better in patients treated with gene therapy as compared to patients treated with haploidentical HSCT. The former group of patients also showed better thymic output of modified T cells.<sup>17</sup> Comparatively, the overall safety profile of this gene therapy is somewhat similar to those patients receiving pre-emptive chemotherapy conditioning with Busulfan or immune reconstitution with an added bonus of lack of reported leukaemic transformation of the modified cells. Similar success was seen in other gene therapy trials performed employing different gamma-retroviral vectors.<sup>18,19</sup> Ongoing clinical trials on gene therapy employing lentivirus as a vector is aiming not only at ADA-SCID but also on other primary immunodeficiencies such as X-linked SCID (SCID-X1) and Wiscott-Aldrich Syndrome (WAS) with even more promising safety profiles despite the absence of genotoxicity seen in gamma-retroviral vector.<sup>1</sup>

#### *Haemophilia*

Haemophilia B, also known as Christmas disease, is an X-linked bleeding disorder of coagulation factor origin resulting from a defect in the gene encoding coagulation factor IX which is a serine protease necessary in the formation of a stable secondary clot. The severity of the condition depends on the percentage of coagulation factor IX to the normal levels where >5% is mild, 1-5% is moderate and <1% of normal levels is considered severe Haemophilia B. Severe type, more often than not, leads to a difficult childhood filled with recurrent debilitating haematoma from trivial injuries inevitable in the life of a child, especially in the joints, forming arthropathy which impairs the quality of life of the patient, and worse, formation of intracerebral haemorrhage which can lead to an early death.<sup>20,21</sup>

The current mode of treatment is varied, ranging from desmopressin (DDAVP) which can increase FIX level, and also intravenous injection of recombinant FIX usually two to three times a week, which in itself is a good symptomatic as well as prophylactic treatment, albeit non-curative. Apart from that, it is also expensive and inevitably leads to formation of inhibitors against the recombinant FIX which leads to resistance to the treatment in the future. Somatic gene therapy offers what these modalities cannot provide, which is curative treatment by using transgene transduction of hepatic cells using adeno-associated virus (AAV). The first successful therapy in 2011 by Nathwani and colleagues from Royal Free Hospital, London used an adeno-associated virus (AAV) on six patients. In response to previous lacklustre result using AAV2 where the expression level was transient, Nathwani *et al.* had several ingenious tweaks in their approach to ensure the best safety and functional benefits. The first was the use of self-complementary AAV (scAAV) vectors where each virion contains a codon-optimized FIX (FIXco) expression cassette packaged as a complementary dimer which ensures a better transgene expression among hepatocytes.<sup>22,23</sup> Secondly, they pseudotyped the AAV to 'don' a new capsid from AAV serotype 8 (AAV8) to overcome the attack by humoral immunity since AAV8 seroprevalence is lower than that of AAV2. Besides, by 'donning' a capsid from AAV8, the vector was rendered to have stronger tropism for hepatocytes, which allowed administration of the therapy intravenously thereby lessening the risk of bleeding.<sup>21</sup> This approach proved successful as all of the 6 participants showed a persistent increase of clotting factor level above 1% of normal values with a collective increment in prophylactic FIX infusion interval, reduction in haemorrhagic episodes as well as an absence of obvious signs of rejection of the transgene by the immune system.<sup>21</sup> This success story was succeeded by another groundbreaking success in 2017 utilising a liver-specific AAV vector containing the transgene with Padua mutation. The trial showed even more encouraging results where the FIX levels in all participants were as high as 33% of normal levels as well as excellent safety profiles.<sup>24</sup>

Haemophilia A is the more common sister of Haemophilia B but instead of coagulation factor IX, it is coagulation factor VIII that is reduced in concentration. The clinical pictures and the management are almost identical to Haemophilia

B. However, in the perspective of gene therapy, the feat is a daunting task due to the big size of the gene coding region and this has hindered the wanted progressive outcome in the prospect. Needless to say, this hurdle remained true until the prolific success of gene therapy was achieved for Haemophilia A in 2017. The therapy utilised an AAV5 vector carrying a B-domain-deleted factor VIII (AAV5-hFVIII-SQ). The nine participants were sorted into three cohorts depending on the dose, low-dose, intermediate-dose and high-dose and were followed up for a year (52 weeks). The outcome showed a generally encouraging outcome, more so among the latter group where 6 of the participants had a normal level of factor VIII (>50 IU/dL) even after a year as well as reduction in the number of episodes of bleeding tendencies in a year from 16 to 1.<sup>25</sup>

#### *Beta haemoglobinopathies*

Beta thalassemia is a disease of the beta globin chain, a constituent of haemoglobin whose function, in combination with alpha globin chain is to transport oxygen throughout the body for aerobic metabolism. The disease is inherited in an autosomal recessive fashion where it manifests in a spectrum of severity depending on the amount of deficiency of the beta-globin chain. The clinical features are mostly due to ineffective haematopoiesis, which in turn leads to endocrine, cardiovascular, and bone abnormalities. The standard treatment involves blood transfusion which is only symptomatic. Transfusion also inevitably leads to another complication of this disease which is iron overload with its own sets of problems to the patient. Apart from blood transfusions, the only curative option for beta thalassaemic patients is haematopoietic stem cell transplantation (HSCT) which is becoming increasingly difficult and rare owing to the necessity of finding a perfectly HLA-matched donor. However, thanks to gene therapy, the curative option for beta-thalassemia is becoming less bleak and more hopeful. Many trials have been conducted over the years and steady improvements have been observed. The first beta thalassemia patient to become transfusion independent was a patient with an extremely low levels of functional haemoglobin in his body, subsequently treated with a transgene-induced HSCT. He became transfusion independent one year after the therapy with maintenance of stable haemoglobin range between 8 g/dL to 9 g/dL as well as adequate gene marking.<sup>26</sup> The recent progress in the field is focused mainly on

enhancing the transgene expression by means of an improved vector (lentivirus) with promising result.<sup>27</sup>

Sickle cell anaemia is another monogenic disease of the haemoglobin where the mutation is a simple missense change of amino acid from Valine to Leucine. This mutation renders the haemoglobin prone to sickling in times of stress, leading to cellular oxidative stress which manifests in the patient in a spectrum of severity, from mild anaemic symptoms such as fatigue and shortness of breath to a more ominous symptom such as sickling crisis where there is blockage of small blood vessels causing thrombotic events like chest pain, priapism and dactylitis. The burden of this disease is most evident in the African continent, Mediterranean, and even as far east to the South East Asia where the disease is usually encountered, as a form of protective mechanism to its sufferers against another debilitating infectious disease, Malaria. The current management of this disease involves a holistic approach combining effective screening, prevention by avoiding sickling triggers, as well as medications for the acute symptoms, which are all only symptomatic but not curative. This is where gene therapy offers solace, and a good one at that because of the disease's monogenic nature at a molecular level. The first patient to be treated using gene therapy for sickle cell disease was a boy from France in 2014. He was diagnosed with homozygous sickle cell disease and had had multiple vaso-occlusive episodes throughout his life inevitably leading to splenectomy and cholecystectomy. He was also frequently given hydroxyurea, but resistance ensued so much so that he had to have red cell transfusion to relieve him of his painful symptoms. The gene therapy started with the invention of the vector, LentiGlobin BB305, eponymously named after its developer, BLueBird Bio. This vector carries a transgene coding for beta-globin chain with an extra twist, and it is able to prevent HbS polymerisation. A win-win situation considering that having a normally functioning HbA would still be impeded by the presence of HbS polymerization.<sup>28</sup> The patients CD34+ cells were extracted and transduced with the vector *ex vivo*, following which the patient was subjected to myeloablation and conditioning before the infusion of the transduced CD34+ cells. Following the procedure 15 months after, the patient's HbA and HbS ratio improved steadily reaching a peak of almost 1:1, with Hb level at 12g/dL and with no vaso-occlusive

or anaemic or haemolytic symptoms. A number of side effects were noted after the treatment ranging from grade 2 side effects such as limb pain, and elevated transaminases, both of which were transient, and grade 4 side effects such as neutropenia with staphylococcal infection with positive blood culture which also resolved with standard measures.<sup>29</sup> Apart from the phenotypical correction, the therapy also led to stable gene marking, hence genotypical correction was also achieved.<sup>26</sup> Ever since the success of this therapy, other clinical trials have been sprouting all over the western hemisphere especially in the US, employing the same concept on the vector, transgene as well as transduction method although the results are yet to be published.<sup>26</sup>

#### *Lipid metabolism disorders*

Lipid metabolism disorders include a wide spectrum of diseases involving many different pathways of lipid metabolism and different types of lipoproteins. Most of the inherited lipid metabolic disorder has an autosomal dominant inheritance pattern with gene dosage leading to a wide spectrum of presentation, ranging from a mild form of hyperlipidemia with no obvious clinical symptoms, to a severe form of hyperlipidemia where the LDL cholesterol level, being the 'bad' cholesterol, rises up to 30 mmol/L which is notoriously high. Most of the unfortunate patients die in the early adulthood due to cardiovascular or cerebral complications. Although there are many genes involved in the disease, based on the knowledge of which lipoprotein is mostly increased, a known set of genes was purviewed helping the personalised target of that gene possible. In 2012, the first gene therapy for the extremely rare disease of lipoprotein lipase (LPL) deficiency hyperlipidemia had a prolific breakthrough with the approval of Glybera. Although the glitz and glamour persisted only for a few years, the hard-work, the ingenuity behind all those involved and the magnitude of the success shall not be offset by the discontinuation of the therapy due to its staggering cost of \$1 million, considering the disease targeted is very rare. The therapy used an AAV with a working copy of the LPL gene, injected intramuscularly into the patient in several doses with an estimated number of virions infused closer to trillions of copies. The result was a stark difference in the plasma colour from a milky creamy white, telling of a high triglyceride level in the blood, to a normal amber plasma. Besides that, patients also had

fewer episodes of pancreatitis which used to be almost a daily occurrence in their lives.<sup>30</sup>

### Cancer

Perhaps one of the strongest driving forces behind the propulsion of gene therapy into the centre stage of personalised medicine is cancer, a terrible disease whose prevalence is as unfortunate as its prognosis. Cancer is a multifaceted disease, both in its pathogenesis as well as presentation. It can happen due to hereditary mutated genes or it can occur sporadically in people with no family history of cancer, usually secondary to a multitude of factors ranging from lifestyle choices such as alcohol consumption, cigarette smoking, sexual behaviour, or as a part of occupational hazard or exposure to ionizing radiation. Though the molecular pathogenesis of cancer involves several factors, the most common notorious players are the disequilibrium between the oncogenes and tumour-suppressor genes as well as several other genes intertwined. Gene therapy for cancer has been in development for past 3 decades<sup>31</sup> targeting many pathways involved in the pathogenesis of cancer such as augmenting immune defence against cancer cells by cytokine gene transfer, drug sensitisation with genes for prodrug delivery, oncogene inactivation or tumour suppressor gene activation.<sup>32</sup> Of all these methods, immunotherapy has been hailed as the frontrunner, leading a number of clinical trials especially in haematological cancer, leukaemia. This technique uses modified T lymphocytes whose surface receptors have been altered to better recognise tumour-associated antigens leading to better clearance of cancer cells. This specialised T lymphocytes are called CAR-T (chimeric antigen receptor-modified T cells). In 2011, a patient went into complete remission from his chronic lymphocytic leukaemia after infusion of CAR-T designed to attack CD19-bearing lymphocytes with specific immune response seen in the bone marrow following the treatment as well as the persistence of remission within 10 months post treatment.<sup>33</sup> This success propelled the drive towards the development of many more improved therapies with better engraftment and fewer side effects, such as the production and an FDA-approved CAR-T cell gene therapy, Kymriah in 2017. The trial was conducted on 63 children and young adults suffering from B-ALL and the remission rate within the first 3 months was an astounding 83 percent. In another story, a modified version of herpes simplex 1 virus (HSV-1) which normally

causes cold sore has been shown to be effective against melanoma. This immunotherapy called T-VEC (Imlygic/Talimogene laherparepvec) uses a herpes virus that has been modified to an extent where it is unable to cause a cold sore. It can only infect cancer cells and not healthy cells and is also able to make signals that attract the patient's own immune cells towards the cancer cells leading to their clearance by means of oncolysis of the tumour cells. This helps immune cells to recognise and fight cancer cells throughout the body, even those distant from the injected sites. The virus is injected directly into the patient's tumours, where it replicates inside the cancer cells until the cells burst, releasing more virions that can infect other cancer cells, reminiscent of a natural life cycle of an otherwise normal herpesvirus.<sup>34</sup> The same principle of treatment was also employed in patients suffering from non-Hodgkin lymphoma (NHL) receiving axicabtagene ciloleucel (axi-cel), marketed as Yescarta by Kite Pharma which has been approved by FDA since October 2017. NHL, a subtype of lymphoma affecting the B lineage of mature lymphocytes, is one of the most common types of haematological malignancies. It is known to be refractory after chemotherapy with an overall poor prognosis.<sup>35</sup> With the advent of Axi-Cel which is an anti CD19 chimeric antigen receptor T cell (CAR-T), the limitations posed to the immune system by cancer cells could be overcome. The lymphocyte-cancer interaction was enhanced leading to an increased clearance of cancer cells.<sup>36</sup> According to a clinical trial conducted in 2017, within 17 days post-infusion of the modified T lymphocytes, 99% of the patients showed success in achieving sufficient level of the lymphocytes, 83% showed a positive response of whom 39% showed complete remission. Perhaps, it will be unwise to talk about these cancer immunotherapies without talking about its predecessor, the origin of gene therapy for cancer, which is Gendicine, a recombinant Adenovirus carrying a wild-type p53 tumour suppressor gene, manufactured in 2003 by Shenzhen Sinobio GeneTech Company for head and neck cancer. Head and neck cancer, especially Nasopharyngeal Carcinoma is one of the most common cancers affecting mostly the Orientals, purportedly due to incense smoking.<sup>37</sup> When it was found that almost 50% of tumours have a mutated p53 gene, it triggered the idea that if an intact unmutated p53 gene could be delivered to the cancer cells, it could lead to either DNA damage repair, apoptosis or senescence of



that particular cancer cells.<sup>38</sup> The outcome of its first successful clinical trials led to an astonishing 90-96% rate of total response (CR+PR) which far exceeded the success rate of standard cancer treatment. Following the success of this therapy, an astounding 30,000 people have benefited from this therapy, of which 10% reside in more than 50 countries outside China.<sup>38</sup> Apart from head and neck cancer, other cancers such as breast cancer, lung cancer and ovarian cancer patients have also been enrolled in the P53 gene therapy for whom the combined effect with standard chemotherapy and radiotherapy response was better than those with only standard cancer treatment.

#### *Parkinson's disease*

Parkinson's disease is a disease of the central nervous system involved in motor activity. The pathogenesis results from loss of dopaminergic neuron cells in the substantia nigra, a region in the brain called internal capsule whose function is to initiate and maintain movement. As the disease advances, patients lose the ability to control their movements leading to limited mobility and reduced quality of life. The standard treatment for Parkinson's disease is the Levodopa which is a synthetic dopamine, to replace the body's own lack of dopamine. It is a fairly good drug especially in controlling the motor discomfort in patients. However, not only can it lead to drug-induced dyskinesia commonly seen in patients receiving Levodopa for a couple of years, but it is also not curative.<sup>39</sup> Then the idea of gene therapy comes in, armed with the logic that if the neuron cells could be provided with the GAD (glutamic acid decarboxylase) enzyme gene, which in essence, converts the excitatory neurotransmitter glutamate to its inhibitory counterpart GABA, the imbalance between these two can be reversed.<sup>40</sup> Although many clinical trials for the development of this gene therapy had been released for many years now, the one worth mentioning here is the one by a company based in the UK funded by BioMedica called ProSavin. The therapy uses an AAV2 vector carrying a transgene for GAD injected into the putamen bilaterally, on 45 patients, for 12 months and subsequently checking the motor improvement according to Unified Parkinson's Disease Rating Scale (UPDRS) as well as any adverse reactions to the therapy. There was significant muscle tone improvement in all patients which continued at 12 months.<sup>40</sup> The same method of trial<sup>41</sup> was conducted with similar positive results showing improvements in motor function of the patients

even at 12 months post-therapy. Another trial conducted by Voyager Therapeutics based in Cambridge, Massachusetts, developed another technique to counter the dwindling levels of dopamine in internal capsule of patients with Parkinson's disease by means of elevating the converting enzyme, aromatic L-amino acid decarboxylase (AADC) responsible to convert exogenous levodopa to dopamine, thereby aiming to lower the dose of L-dopa in patients. The therapy, called VY-AADC, employing an adeno-associated virus as the vector-transfected with the gene coding for the AADC enzyme was injected into 15 patients who were segregated into 3 cohorts with different dosage of therapy. The result showed that all patients in all 3 different cohorts had a reduction in their L-dopa doses in the first 6 months, had a better quality of life with longer period of time without the side effects of levodopa-induced dyskinesia. However, it was noted that participant in cohort 1 (i.e, those receiving low dose of the gene therapy) still needed higher dose of L-dopa after 12 and 18 months, while those in cohort 3 (high dose of gene therapy) had more levodopa-induced dyskinesia as compared to the other two cohorts suggesting greater fluctuations in motor symptoms due to greater reduction in levodopa dosage. With this finding, the researchers concluded that cohort 2 was the likely "stable dose" for this therapy.<sup>42</sup>

#### *Adrenoleukodystrophy*

This is a progressive neurodegenerative disease and adrenal insufficiency resulting from inactivating mutation of the ABCD1 gene which produces ALD protein that is responsible for the degradation of very long fatty acid chains. This leads to their toxic accumulation and impairment in the production of the myelin sheath of neurons. It is an X-linked disorder commonly affecting boys. Tragically, the affected patients usually have a normal first few years of life followed by slow and progressive regression in mental function, behaviour and eventually will enter into a vegetative state in as early as 10 years after diagnosis. Before the advent of gene therapy, allogeneic stem cell transplantation was the only solace available to at least delay the inevitable debilities in this devastating disease, with variable outcome. Gene therapy for the disease involved *ex vivo* recombinant retrovirus transfer of the ABCD1 coding sequence into autologous patient's cells, specifically the CD34+ cells. There were 17 patients enrolled for the trial, their

CD34+ cells were harvested and transduced with a lentivirus vector (elivaldogene tavalentivec, Lenti-D). After 2 years of follow-up, all patients had a measurable ALD protein level in the blood, 88% (15/17) were alive and free of major complications either from the disease progression or the treatment complications. However, among the remaining two, one had a rapid neurological deterioration and passed away while the other one quit the trial to pursue allogeneic stem cell transplantation but unfortunately succumbed to transplant-associated complications. Also, none of the patients had accidental activation of oncogene after transduction or serious infections or even graft failure or GVHD.<sup>43</sup>

#### *Leber's congenital amaurosis*

Leber's congenital amaurosis (LCA) is a disease of the retina eponymously named after its founder, Theodor Leber. It is the most common cause of inherited blindness in childhood inherited in an autosomal recessive pattern. However, it is a rare type of retinal dystrophy unlike retinitis pigmentosa (RP) with an incidence of 1 in 100,000 live births per year.<sup>44</sup> This degenerating retinal disease is due to the mutation of many genes involved one way or another in the function of the retina in the visual cycle. So far, at least 30 genes have been implicated in its pathogenesis. LCA2 is one of the variants of LCA that occurs in 10% of cases. LCA2 is due to the mutation in the RPE65 gene coding for an enzyme called all-trans retinyl esters isomerase, whose absence leads to the degeneration of retinal cells.<sup>45</sup> Before its first successful gene therapy in 2008, the affected children had vision impairment as early as the first decade of their lives associated with nystagmus and absent pupillary reflex.<sup>46</sup> The breakthrough result from the first successful gene therapy in 2008 used an adenovirus-associated vector (AAV) carrying a transgene hRPE65v2 that promotes the expression of the RPE65 gene.<sup>45</sup> This vector was then surgically inserted into the subretinal place under general anaesthesia. Following the procedure, follow-up of the patients showed overall safety and there was no evidence of systemic dissemination or immune response against the vector, and vector sequence was found only in a tear sample as early as day 1.<sup>45</sup> As for the change in vision, there was a statistically significant improvement in visual acuity, pupillary reflex as well as the nystagmus. All these successes consequently led to the groundbreaking FDA approval of Luxturna (Voretigene neparvovec) in December 2017 with

its first commercial sale occurred in March the following year.

#### *Cystic fibrosis*

Cystic fibrosis is a debilitating disease of the respiratory system common in the western hemisphere. It is due to the mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene encoding for the CFTR protein. This mutation results in the production of a thick and viscous respiratory secretion which tends to clog airways as opposed to a thin and watery secretion in normal individuals. This leads to the common clinical features seen in such patients such as shortness of breath, fever, recurrent cough, recurrent pneumonia and fungal infection of the lungs. The disease however does not only affect the respiratory system but also other organs such as pancreas, kidney, liver and intestine. In some males, infertility is not uncommon. Treatment usually involves perpetual consumption of cocktail of mucolytics, decongestants and antibiotics. A cure was not possible except lung transplantation. The first attempt of gene therapy for CF employed an adenovirus carrying the CFTR transgene but the transduction was inefficient to provide any significant effect. However, the breakthrough success was found in one randomised, double-blind, placebo-controlled phase 2b trial conducted in the United Kingdom in 2015.<sup>47</sup> The trial used a non-viral method in which they subjected the participants to repeated nebulisation of a gene-liposome, pGM169/GL67A complex every month for a year. The outcome showed an overall improvement in the forced expiratory volume in one second (FEV1), lung function measurements, computerised tomography (CT) scans, and also Cystic Fibrosis Questionnaire (CFQ-R) scores among the study group as compared to the placebo group.

#### *Muscular dystrophies*

Muscular dystrophies are a group of diseases characterised by muscle weakness which can be due to a myriad of causes depending on the stage of impairment of muscle physiology. In spinal muscular atrophy (SMA), there is a defect in SMN1 gene encoding the protein SMN which is an essential protein for lower motor neuron whose deficiency leads to a gradual deterioration of anterior horn cell neurons' innervation of the skeletal muscles leading to paralysis. While in Duchenne Muscular Dystrophy (DMD), it could be either due to a reduced number of SMN as

seen in SMA, or due to an absence of a protein called dystrophin as seen in DMD. This group of diseases is usually severely debilitating, if not fatal early on in life and characterised by delayed motor milestones including difficulty in feeding in the affected infants. However, thanks to the nature of these diseases being a monogenic disease and the progress made in gene therapy, several clinical trials have begun showing tremendously promising outcome. Such success was seen in a trial conducted by researchers from Nationwide Children's Hospital in collaboration with Avexis Inc. and the Ohio State University College of Medicine beginning in early 2017 using the gene therapy model called AVXS-101 to treat SMA type 1. The model used modified adeno-associated virus serotype 9 (AAV9) to deliver SMN gene to 15 patients, 12 out of which were given a single high dose and the remaining 3 received a single low dose. At the end of the study period, all 15 patients were noted to have reported no obvious side effects or complications.<sup>48</sup> Often, by the age of 12 months, affected children show common milestone delay such as the inability to speak and eat properly. However, among all subjects who received high dose of AVXS-101, 11 were able to speak, and eat, achieved head control, able to sit with assistance, 9 were able to roll over, sit for 30 seconds and longer, while 7 did not require mechanical ventilation and 2 could crawl, pull to stand, stand and walk without aid. What this first phase of clinical trial showed was, a significant improvement in the natural history of milestones of patients receiving the treatment as compared to those who did not. The therapy is given intravenously and is able to cross the blood-brain barrier instead of being given intrathecally. The subsequent study from a similar group of authors in 2018, 24 months after the initial dose also showed results that corroborated the first study in which most patients had improved pulmonary support, improved swallowing, improved speech, as well as improvement in motor milestone.<sup>48</sup> Another newcomer gene therapy is also making headlines, Spinraza, which is a brand name for Nusinersen, a gene therapy developed by BioGen and Ionis Pharmaceuticals with collaboration from the University of Massachusetts Medical School. Approval of Spinraza by the FDA has been a holistic achievement for the entire SMA community, and it is at the leading edge of SMA treatment. Spinraza is an exemplary success story of gene therapy for rare genetic disease research. Although intended for the same disease, it has

rather a peculiar concept of 'tweaking'. Instead of delivering a transgene of SMN gene to the patient, it is a type of antisense oligonucleotide (ASO) meant to take advantage of SMN2 gene whose function in a normal setting is usually truncated due to the splicing of exon 7. Due to this ASO, the intronic splicing silencer (ISS-N1) was discovered in Singh Laboratory back in 2004 which paved the way for this alternative.<sup>49</sup> Its use in silencing the splicing of exon 7 of SMN2 gene was thought of as a potential to increase the amount of SMN2 gene protein that could alleviate the loss of SMN1 gene protein and hence be useful for SMA patients. *In vivo* study of Spinraza on a severe SMA model proved its therapeutic efficacy as hypothesized.<sup>50</sup> One of its many clinical trials was called ENDEAR, a randomised, double-blind phase 3 trial conducted in mid-2014 where 12mg of Nusinersen was injected intrathecally and the results demonstrated were all encouraging.<sup>51</sup>

#### **Future hope in gene therapy**

The promising success stories of gene therapy have been increasing since its first conception in the 1980s. Just like any other novelties, its story was not one without challenges and limitations, but those only serve as motivation for scientists all over the world to further the technology to maximise its use while at the same time minimising its limitations and side effects. There are hundreds if not thousands of diseases as of current, that are not only debilitating but can also be fatal as early as during infancy and the burden it poses to the society is tremendous, notwithstanding the emotional sentiment it often perturbs.

Of utmost importance and the rising incidence is acquired immunodeficiency syndrome (AIDS), which has been steadily increasing in trend since its first discovery outside of the African continent in the 1980s. It is caused by a retrovirus, Human Immunodeficiency Virus (HIV) originally isolated from monkeys in Africa. The infection mainly affects the cellular arm of acquired immunity in humans, which is the T-lymphocytes causing gradual yet definitive shutdown of the immune system exposing the patient to opportunistic infections that are otherwise harmless in an immunocompetent patient, which constitute most of the causes for hospitalisations and of course, the eventual demise of affected patients. The treatment and outcome for HIV-infected patients have been improving over the years thanks to highly active

antiretroviral therapy (HAART) that can help in reducing the viral load as well as increasing the CD4 count. However, HAART alone is not able to eradicate the reservoir and the latent infection in view of the high mutation rate of the retrovirus as well as its cunning ability to evade the immune response.<sup>52</sup> Nevertheless, in the study of the pathogenesis of the infection, especially in terms of its first contact or attachment to a T-lymphocyte, it was noted that the surface receptor on the T-lymphocyte plays a role in the susceptibility of getting infection. The study noted that 10% of the Caucasian population has a mutation in the surface receptors, CCR4 or CXCR5 that render them 'resistant' to HIV infection.<sup>53</sup> With this idea in mind, gene therapy can theoretically be incorporated to take advantage of this finding. If a vector could bring a gene replacement into the T-lymphocytes to manufacture a type of resistant surface receptor in place of a susceptible one, this could mean that it could be used as a prevention in high-risk individuals and even in an already infected patient to limit the spread of the virus to other T-lymphocytes which in turn could reduce the chances of having dormant HIV laying around undetected in immune cells that might resurface at times of stress.

Diabetes Mellitus (DM) is one of the most common metabolic diseases that is widespread as it is difficult to treat. It has no racial preponderance although type 1 DM is more commonly seen in the Caucasian population and in developed western countries where autoimmunity has been heralded as one of the possible causes.<sup>54</sup> DM is due to either the absence or deficiency in insulin, an anabolic hormone secreted in fed state to convert glucose to stored energy in the liver and muscle. Its deficiency or absence, seen in type 2 and type 1 respectively, will lead to a diversity of metabolic complications namely metabolic acidosis, kidney injury, hypertension, reduced cholesterol clearance from the blood, heart disease and even stroke, among many others. The current guideline for treatment involves phases starting from lifestyle modification, failure of which (i.e HbA1c level > 6.5%) will prompt the clinician to move to the next phase of treatment by means of hypoglycemic medications such as metformin, a biguanide that enhances insulin's glucose-lowering action. The last choice for treatment, or in case of type 1 DM, the only choice of treatment, is insulin derived from various sources such as bovine or porcine or the

latest one, human recombinant insulin, to lower the blood glucose level. The tedious stages of treatment, along with other variables such as patient's compliance, resources and side effects from subcutaneous injections of insulin, pose a burden to the entire management team. But all hope is not lost yet, gene therapy might be able to offer the solution to this 'sweet' problem. By using a viral vector to insert a working copy of insulin gene into tissues like the heart, liver, muscles and especially the pancreas, insulin level can be 'endogenously' corrected which subsequently will help in stabilizing glucose level.<sup>55</sup> Meanwhile for type 1 DM patients, whose problem is usually due to auto-antibody attacking beta cells in the islet of Langerhans of the pancreas responsible in producing insulin, an immunotherapy of CAR-T reminiscent of the one used in Kymriah to attack the auto-antibodies, preventing the loss of pancreatic cells can be employed.

Another promising success appears to be in muscular dystrophies. Duchenne muscular dystrophy (DMD) is another muscular dystrophy disease that can benefit from gene therapy owing to its monogenic cause where there is mutation in the DMD gene located at locus Xp21. This gene is responsible for the production of dystrophin, a cytoskeleton protein that provides physical stability to muscle fibres. In DMD, there is a disruption in the reading frame of the gene leading to absent expression of the protein dystrophin or reduced expression in the case of Becker's muscular dystrophy. It is inherited in X-linked recessive manner affecting mostly boys and can become apparent as early as the first 4 years of life. The affected children present with gradual muscle weakness starting at the central part of their body, especially the hips and spreading outward to eventually involve all muscles of the body leading to permanent and complete muscle weakness. The average lifespan of affected individuals is usually up to 26 years although a few rare cases, with extremely good care and resources, the affected individuals can last up to their 30s. Despite being a monogenic disease, the treatment is difficult due to the widespread distribution of dystrophin including the heart and vessels. Gene therapy offers the most appropriate solution. The strategies for gene therapy in DMD patient can be either vector-mediated mini- or micro-dystrophin gene delivery, nonsense read-through therapy, or by exon skipping with synthetic antisense oligonucleotide (AOs) or genome editing.<sup>56</sup>

In particular, genome editing using clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR/Cas9) shows a promising outcome. It involves the administration of viral vectors such as AAV8 or AAV9 to deliver the CRISPR/cas9 apparatus into the patient to correct the mutation in the DMD gene in related tissues.<sup>56</sup>

## CONCLUSION

Gene therapy is a novelty worth pursuing in the field of medical treatment. It is precise, personalisable, and effective. The initial drawback of possible insertion or activation of oncogene triggering de novo carcinogenesis has been minimised greatly by the advent of more accurate techniques of genome editing. It is a therapy, despite the hefty price tag, worth investing in because the benefit it offers is not only applicable in treating diseases per se, but also useful in the field of pharmacogenetics and microbiology as we strive to provide a holistic and personalised medicine to people. Following a difficult two decades from the tragic setbacks in clinical trials, the field of gene therapy is now entering into an exciting time. By 2020, genuine progress can be seen in many fronts. Stunning progress in the development of various CRISPR-based technologies has been witnessed in the last few years. The therapeutic applications of CRISPR technologies are exciting. With the development of a better delivery system, and certain other technical modifications, it is hoped that gene therapy will impact the medical field and will be really a boon to humankind in future.

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