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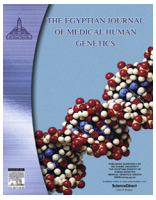

Review

Current strides in AAV-derived vectors and SIN channels further relieves the limitations of gene therapy

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1. Introduction

The tools of molecular biology and biotechnology has appeared more under the more general nomenclature of gene therapy, and other subsidiary nomenclature including gene editing, gene replacement, gene insertion, gene targeting and the likes. However, molecular biology and biotechnology are indeed, bigger than these nomenclatures; fortified with a broader scope. The clear understanding of the cellular and molecular mechanisms is at the heart of this form of therapy involving modification of the blueprint of life. It is about three decades ago from now that the idea of gene therapy was carried to the clinic to be tried as an alternative method of approaching diseases in a way that pharmacotherapy couldn't prove efficient, with about 3000 clinical trials in record. This was not without associated side effects or adverse reactions though. Highly sophisticated gene transfer tools vastly equipped with improved therapeutic efficacy as well as taking safety into consideration has been developed to address the basic challenges that face this therapeutic method. Such sophisticated tools may include viral and non-viral vectors, naked oligonucleotides as well as plasmids. With the particular improvement, a series of clinical trials have been conducted with recorded successes with overtly no side effects, with cancer gene therapy accounting now for the majority of clinical trials worldwide (http://www.wiley.com/legacy/wileychi/genmed/clinical/). A notable instance is Gendicine®, an adenovirus-p53 based gene therapeutic that gained approval into the market for head and neck squamous cell carcinoma treatment, depicting an early evidence of molecular Biology relieving pharmacotherapy of burden [1]. It remarkably showed desired results without overt side effects even after a record of treatment of over 10,000 patients. The gene therapy area of medicine has focused more on the correction of inherited diseases. Similarly, trailing the fate of Gendicine® in 2012, is Glybera® (tiparvovec) an alipogene, which also gained a commercial approval for the treatment of familial lipoprotein lipase deficiency (LPLD) [2]. These are obvious milestones in clinical medicine. Gene therapy generally involves the introduction of new therapeutic genes (Table 1) encoded by nucleic acids (DNA), alteration/modification of existing genes or the introduction of RNA into cells, for the prevention, treatment or cure of disorders and diseases, in order to restore or add gene expression. Most commonly, a faulty or missing gene is substituted with a copy of DNA encoding the desired function. Orphan diseases with damaging monogenetic defects, including primary immunodeficiencies (PID), were the early focus of gene therapy, but this has greatly expanded to cut across a number of diverse disorders and diseases which include Parkinson's disease, diabetes, heart failure, cancers and other neurodegenerative or metabolic disorders. [3]. There are however, a lot of hurdles that gene based therapeutics have to go through before they get to final commercial use. Some major challenges such as the recognition of the protein on the viral capsid by the host immune system has been managed by the development of

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Table 1
Gene types transferred in Gene therapy clinical trials. Data Sourced from: The Journal of Gene Medicine (www.wiley.co.uk/genmed/clinical) on 2nd June 2017.

Gene type	Gene Therapy Clinical Trials	
	Number	Percentage (%)
Growth factor	176	7.1
Antigen	474	19.2
Antisense	17	0.7
Cell cycle	10	0.4
Adhesion molecule	12	0.5
Cytokine	375	15.2
Deficiency	206	8.4
Hormone	10	0.4
Marker	55	2.2
Oncogene regulator	12	0.5
Oncolytic virus	52	2.1
siRNA	11	0.4
Cell protection/Drug resistance	20	0.8
Receptor	259	10.5
Replication inhibitor	94	3.8
Ribozyme	6	0.2
Tumor suppressor	181	7.3
Suicide	173	7
Transcription factor	35	1.4
Porins, ion channels, transporters	23	0.9
Others	199	8.1
Unknown	63	2.6
Total	2463	

elaborate technologies that shields the protein sites from eliciting inflammatory activities, thereby producing excellent safety profile even for *in vivo* gene therapy [4]. There are many gene transfer mechanisms available and could be *in vivo* or *in vitro*. For instance, Glybera® is a recombinant adeno-associated virus-derived vector (AAV) for direct intramuscular injection. Very recent protocols involved in gene therapy involves cell isolation from the beneficiary (patient) followed by genetic modification *in vitro*, thereafter re-introduction (reinfusion) into the beneficiary. One of the key advantages of this protocol outside the body is that, adverse reactions and side-effects are reduced to a feasible minimum. It also gives room for the administration of the desired concentration, since they will be no detoxification load on the liver and the renal system. The pioneering inspiration for the initiation of the gene therapy trial to correct adenosine deaminase (ADA) deficiency using genetically modified T-lymphocytes came from the pioneer work of Rosenberg et al., at NCI. The first application of genes-modified haematopoietic cells was carried out by inserting a gene from bacteria into lymphocytes that are capable of infiltrating tumours in order to track the associated behaviors of melanoma cells after they are administered into the patients [5,6]. They are quite a number of successful clinical trials that buttresses the fact that virtually a lot more can also be done via the modification of T-cells such as the incorporation of chimeric antigen receptors (CAR) to enhance tumour cell recognition aimed at facilitating a fleet of cancer killing cells to the target [7]. One single great achievement in gene therapy advances is the development of inducible suicide genes, inserted into allograft of T-cells; capable of being activated upon GvHD development. This permits patient-specific alloreactivity modulation [8,9]. Similar approaches have also been applied to skin diseases (e.g. epidermolysis bullosa), as well as liver diseases (familial hypercholesterolemia) [10] *in vivo* and *ex vivo* [11]. Therapeutic gene therapy holds better promises for us in treating diseases (including hemoglobinopathies, cancer immunotherapies, haemophilia B, ocular diseases, neurological diseases [12], immunological, metabolic disorders, ocular, neurodegenerative, hematological and other primary immune deficiencies) that have eluded cure, than the more commonly available temporary or suboptimal methods of treatments. We do not yet rule-out completely the occurrence of setbacks, as many limitations are also associated with these breakthroughs; as in the case of later development of leukemia in which some patients died, however, we have recorded the "comeback of gene therapy" [13,14]. Rapid improvements are recorded every year (Fig. 1). Vision of blind patients have been restored, hematopoietic stem cells has been used to terminate incurable blood cancers (though expensive as well as incurring high risks), restoring immunity in patients. Thousands of clinical and experimental trials are in the pipeline towards improving all the factors surrounding the desired success in gene therapy (Table 2). One of the simplest aims of gene therapy is to outperform conventional medications by searching for methods of treating diseases; reassigning functional non-deficient therapeutic nucleic acids to a patient's hosting cell (gene editing) with the target of correcting a defective gene. It therefore intends to redefine diseases that were previously classified untreatable as curable (which include primary immune deficiencies and ocular diseases). Progress in optimized vector systems design for *ex vivo* as well as *in vivo* gene transfer have become a true potential for treating various disease with vastly increased spectrum of medical application beyond genetic disorders; for example blood cancers by means of chimeric antigen receptor (CAR)-modified T-cells [15], that were formally incurable due to setbacks associated with vectors. Clinical gene and cell therapy approach are founded on either *ex vivo* gene incorporation into autologous tissues/cells such as hematopoietic stem cells (HSC) to treat basically hematological as well as other disorders, not excluding immunotherapy via differentiated lineages. For instance T lymphocytes; then an adoptive back-transfer afterwards into the recipient (patient) or *in vivo* gene insertion into post-mitotic target tissues/cells. So far, the adeno-associated virus (AAV) vectors (for instance, Glybera, for treating lipoprotein lipase deficiency) have been established to be of utmost clinical accomplishment for *in vivo* gene delivery in the midst of the range of viral based vector systems [15]. Lentiviral (LVs; having a better preclinical safety report use for non-dividing cells than γ-retroviral) or γ-retroviral-derived retroviral vectors have the ability to integrate their therapeutic genetic modification into the genome (proven in hematopoietic cells) of the target cells [16,17]. The aim of this study therefore is to enlighten on how the tools of molecular biology and biotechnology such as adeno-associated virus (AAV) vectors and Self-inactivating (SIN) Channels therefore relieves Pharmacotherapy of a lot of burdens. This form of rescue for pharmacotherapy has been there for decades, but it's important to point out and get the scientific community updated on the strides recorded in most recent years as it will be useful to all concerned specialists available solutions in the scene of medicine concerning a particular disease.

2. Current redirections in gene therapy

In addition to the advances made in molecular biology and biotechnology as applied to molecular gene therapy aimed at alleviating the setbacks and challenges associated with the more conventional pharmacotherapy, there are yet some recent novel attempts at improving on the existing strategies. The last decade has made a very drastic contribution to this field and a few of such recent contributions are discussed and some of the systematic headings that follow.

2.1. Using AAV-derived vectors as substitutes for integrating vectors

As stated earlier, developments in optimized vector organization designs for *ex vivo* as well as *in vivo* gene transfer is on the rise (Table 3). Retroviral as well as lenti-viral vectors have been effectively used as delivery tools by delivering single-stranded vector

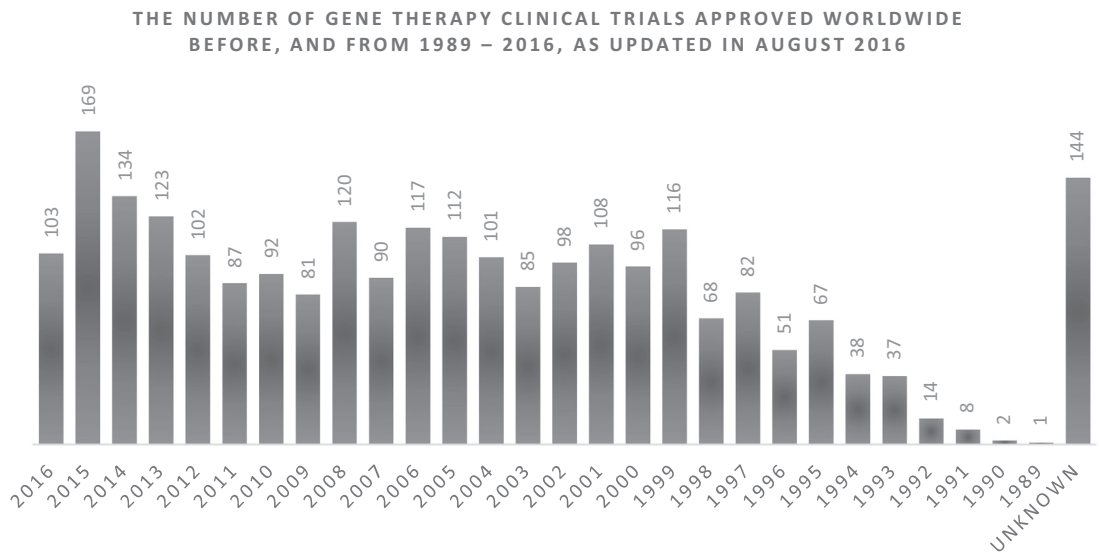

Fig. 1. The number of Gene Therapy Clinical Trials approved worldwide before, and from 1989–2016, as updated in August 2016. Data Sourced from: The Journal of Gene Medicine (www.wiley.co.uk/genmed/clinical) on 2nd June 2017.

Table 2
Phases of Gene Therapy Clinical Trials. Data Sourced from: The Journal of Gene Medicine (www.wiley.co.uk/genmed/clinical) on 2nd June 2017.

Clinical Trial Phase	Number (n)	Percentage (%)
Phase I	1409	57.20%
Phase I/II	500	20.30%
Phase II	429	17.40%
Phase II/III	24	1%
Phase III	93	3.80%
Phase IV	3	0.10%
Single subject	5	0.20%
Total	2463	100%

genomes in gene therapy. However, currently, in post mitotic tissue AAV vectors are being considered as the better alternative delivery tool. The remarkable advantage of the alternative method is that AAV vectors are equipped with a protein capsid that is non-enveloped as well as a DNA genome that could be a single-stranded DNA in its native conformation form or as a self-complementary DNA in its artificial conformation [18]. The artificial conformation provides the ability to fold into a double-stranded conformation. Upon release from the viral capsid [19], the double-stranded conformation is made possible by the intramolecular base pairing of the aligning complementing bases producing a significantly improved onset of transgene expression at the expense of the lessening of the coding capacity from approximately 5 to 2.5 kb (50%) [20,21]. This parent AAV vectors are gutless, non-pathogenic (deficient of all viral open reading frames, therefore, they remain principally in an episomal structure) with the inability to self-replicate, however, with the help of the inverted terminal repeats (ITRs) they retain the ability to integrate their genome in the human genome, specifically on a specific site (AAV integration site 1, 19q13.3-qter, AAVS1) on chromosome 19 [20]. The AAV vector genomes episomal nature, not withstanding, they can be integrated into the genome of the target cells with frequency that could be as high as of 10^{-4}–10^{-5}. One great advantage with this is that there is no preference for any specific genomic loci [22,23]. This remarkable adaptability of these AAV vectors has made them clinically applicable in diverse therapy including central nervous system, liver, retina, cardiac muscle and skeletal muscles as specific targets [19,24–28]. This characteristic property of AAV pronounces it a non-integrating vector system, which brings to light the fact that long term correction is only regimented to post-mitotic tissue. For instance, Leber's congenital amaurosis (LCA) (an early onset retinal dystrophy), characterized by a malfunction and deterioration of photoreceptors is caused by mutations in retinal pigment epithelium-specific protein 65 kDa (RPE65) gene [29–31]. This gene codes for a retinoid isomerase; a key factor in retinol function in the visual cycle [28,31,32], by using specific hRPE65 (resulting in a lower expression) or ubiquitously promoters (higher expression), after the injection of AAV2 vectors to the subretina, the over-expression of a functional copy of the gene RPE65 could improve vision [30]. As shown from the low immunogenicity, safety, clinical benefit and good tolerability, in three previous clinical trials, the immune system is an important factor that must be considered in all kinds of experimental and clinical research practices [28]. For instance, the reactivation of memory T-cell responses which results in the loss of vector-modified cells, providing the attenuation therapeutic efficacy, as opposed to the muscle- and liver-directed gene therapy applications [18,33], subretinal injection of AAV vectors would mount low humoral immune responses. In attempting to cure RPE65-associated LCA, a lasting improvement in vision is achievable by RPE65 overexpression with a continuous degeneration of the photoreceptor (a setback that must be counterbalanced by other interventions), measurable by taking the dimensions of the outer photoreceptor layer width [32]. Gene therapy has been duly proven to be safe with reported clinical scores. These include Canavan disease, LCA and others yet in progress. Correspondingly, in order to treat patients with advanced Parkinson's disease, locally injected AAV2 vectors unilaterally into subthalamic nucleus [24], designed to express glutamic acid decarboxylase (GAD) into the subthalamic nucleus produces improvements in safe and tolerable clinical achievements with evidences in clinical trials [34]. In Canavan disease, using AAV2 vectors, the aspartocylase gene (ASPA) which codes for the enzyme that is required for the degradation of N-acetyl-aspartate (NAA) is transported to the brain leading to the stabilization of the atrophy of the brain, alongside

Table 3
Vectors used in Gene Therapy Clinical Trials. Data Sourced from: The Journal of Gene Medicine (www.wiley.co.uk/genmed/clinical) on 2nd June 2017.

Vector	Gene Therapy Clinical Trials	
	Number	%
Alphavirus (VEE) Replicon Vaccine	1	0
E. coli	2	0.1
Bifidobacterium longum	1	0
CRISPR-Cas9	7	0.3
Adenovirus + Sendai virus	1	0
Adenovirus + Vaccinia virus	8	0.3
Antisense oligonucleotide	6	0.2
Vaccinia virus	125	5.1
Venezuelan equine encephalitis virus replicon	3	0.1
Vesicular stomatitis virus	3	0.1
Vibrio cholerae	1	0
Flavivirus	8	0.3
Gene gun	5	0.2
Herpes simplex virus	90	3.7
Lactococcus lactis	6	0.2
Lentivirus	158	6.4
Lipofection	116	4.7
Adeno-associated virus	183	7.4
Adenovirus	509	20.7
Adenovirus + Modified vaccinia Ankara virus (MVA)	11	0.4
Adenovirus + Retrovirus	3	0.1
Measles virus	10	0.4
Modified Vaccinia Ankara virus (MVA)	8	0.3
mRNA Electroporation	5	0.2
Naked/Plasmid DNA	419	17
Naked/Plasmid DNA + Adenovirus	3	0.1
Naked/Plasmid DNA + Modified Vaccinia Ankara virus (MVA)	2	0.1
Non-viral	1	0
Naked/Plasmid DNA + Vesicular stomatitis virus	3	0.1
Newcastle disease virus	1	0
Listeria monocytogenes	25	1
Poliovirus	3	0.1
Poxvirus	70	2.8
Poxvirus + Vaccinia virus	36	1.5
Retrovirus	459	18.6
RNA transfer	48	1.9
Naked/Plasmid DNA + Vaccinia virus	3	0.1
RNA transfer + Naked/Plasmid DNA	1	0
RNA virus	5	0.2
Saccharomyces cerevisiae	9	0.4
Salmonella typhimurium	4	0.2
Semliki forest virus	2	0.1
Sendai virus	4	0.2
Shigella dysenteriae	1	0
Simian virus	2	0.1
siRNA	5	0.2
Sleeping Beauty transposon	10	0.4
Streptococcus mutans	1	0
Unknown	76	3.1
Total	2463	

the decrease of N-acetyl-aspartate in the brain [26]. Muscle tissue-targeted delivery of AAV vectors has shown a lot of advantages over time, such as lower risk of vector distribution when compared with liver delivery. There is the finding that, pre-existing humoral immunity against AAV is a commonly encountered challenge in liver delivery as well as blood delivery, however, this challenge is absent in the case of muscle tissues, without the blockage of transduction during gene therapy. This method was employed in the early treatment of haemophilia B (with the restriction of haemophilia B patients with missense mutations) through gene therapy, demonstrating the safety and applicability of muscle-directed gene therapy. It is however, not without its own limitations though, pointing to the risk of triggering immune responses by the pre-defined vector dose directed at the transgene product [19,35]. The liver is a better option when this point is considered [36]. For cardiovascular diseases, heart muscle are a great target for gene therapy with pre-clinical research on designing optimized AAV vectors for that purpose still ongoing [37]. Calcium up-regulation by percutaneous administration of gene therapy in cardiac disease (CUPID) which was launched in 2008 has proven AAV1 vectors (same serotype as for LPLD) to be successful in safely transducing human cardiac tissue following antegrade epicardial coronary artery infusion in treating advanced heart failure [27,38]. The strategy in this case take advantage of the over-expression potential of the sarcoplasmic reticulum calcium ATPase pump which is coded by SERCA2a and making it the target. During heart failure, the protein expression of SERCA2a which is essential for calcium (Ca^{2+}) homeostasis is reduced and as a result produces elevated end-diastolic and systolic Ca^{2+} levels which eventually contributes to prolonging Ca^{2+} re-uptake [27]. The success of this approach has been proven with a Phase I and Phase II trials [25,36,38]. Though a relatively high vector doses are needed to achieve therapeutic effect, the use of AAV-mediated *in vivo* gene therapy discloses outstanding safety and efficiency [39,40]. With strategies geared towards improving cell specificity as well as transgene expression restriction, a danger shared by all the serotypes in the intrinsic potential for off-target transduction resulting from the broad tropism nature is obvious [39,41]. These challenges notwithstanding, in addition to the AAV transcriptional and post-transcriptional strategies that are in the pipeline to provide solutions, engineered capsids modified to surmount these spotted complications holds the promise for the use of AAV vectors to treat patients with even a pre-existing anti-AAV humoral immunity [20,41].

2.2. Self-inactivating (SIN) Channels

Self-inactivating (SIN) configuration refers to the deletions in the U3 region of the 30 long-terminal repeats (LTR) located in retroviral vector which results in a transcriptionally inactive 50 LTR via reverse transcription. This therefore reduces the transactivation potential of the vector under consideration. This concept has greatly enhanced the safety profile of applicable vectors, confirmed by a very recent approval in Germany for treating CGD. The obvious advantage of this method over the gammaretroviral vectors has made it the preferred method for gene transfer into HSCs [42]. Currently, combining the Self-inactivating (SIN) configuration with physiological promoters is in the clinical trial scene as seen in the clinical trial status of SIN gammaretroviral vector harbouring the elongation factor short (EFS) promoter driving the expression of ILR2G, aimed at treating SCID-X1. Progress is already being recorded as obvious in the reconstitution of T lymphocyte with partial restoration of humoral immunity [43]. The advancement recorded in this method is that the vector consists of a short myeloid-specific promoter which is derived from the human c-FES gene that modulates the expression of a codon-enhanced gp91phox cDNA [44]. Importantly, while retroviruses such as gammaretroviruses largely rely on the dissolution of the nuclear membrane in the process of mitosis to deliver their cargo into the target cell nucleus, the preintegration complex of lentivirus vectors (pseudotypable with envelopes consisting of vesicular stomatitis virus glycoproteins (VSVg)) is translocated actively into the nucleus of the target cell, and as a result its able to facilitate the efficient transduction of even non-dividing cells such as HSCs via SIN-lentiviral vectors. The easy pseudotyping effectively provides for a broad tropism thereby enabling the effective transduction of target cells (including CD34þ HSC). The contributions of molecular biology and biotechnology in these areas did not completely push pharmacotherapy aside, but facilitates the robust manufacturing as well as purification protocols, that contribute to a superior

pharmaceutical quality of the vectors under consideration [45]. To emphasize what has been stated earlier, Phase I/II clinical trials mostly in UK, Italy, the US (NCT01380990), Switzerland, Germany and France with a focus on SIN-lentiviral vectors are on the pipeline for more than a few PIDs (including WAS, CGD, ADA-SCID) and for some non-PID using gene modified HSCs (e.g. X-linked adrenoleukodystrophy (X-ALD), b-thalassaemia and metachromatic leukodystrophy (MLD) [46]. About four patients are recorded to have been treated with a lentiviral vector containing the EF1a promoter modulating the expression of the ADA cDNA [47]. For X-CGD, a Phase I/II clinical study using a SIN-lentiviral vector was recently approved in the UK and is currently under regulatory review in Germany and Switzerland. In contrast to the gammaretroviral vector employed in the first WAS gene therapy trial, a typical strategy in this case of WAS is the use of 1.6 kb stretch of the WAS gene's at the upstream of the regulatory region of the SIN-lentiviral vector to propel the transgene expression of WAS in haematopoietic cells [48]. Restoration of the WAS protein expression was the obvious outcome of this strategy in multiple lineages of leukocytes, leading to an increased platelet counts. This ultimately enhances the immune system functions as well as ameliorating the clinical manifestation of the disease [46]. Hsa-miR-223 and MRP8 c-FES genes code for promoters and their expression is a potential target for treating the functional defect in CGD which has a direct effect on myeloid cells at the differentiation phase. This strategy though has limitations *in vivo* could be fortified by fusing the promoter elements with other regulatory sequences (e.g. the strong viral CMV promoter, ubiquitously acting chromatin opening element (UCOE) or Cathepsin G) [49]. Similarly, CathepsinG/c-FES promoter is chosen to drive gp91phox in X-CGD in terminally differentiating myeloid cells. Pre-clinical trials showed this to be within the confines of expectation [49]. The outcome of this could further enhanced microRNA (miR) target sequences inserted into the lentiviral vector [50,51]. Modifying autologous HSC not only helps in managing PIDs alone but offers treatment potential for other monogenic disease such as lysosomal storage disorders or mucopolysaccharide disorders and the leukodystrophies, which could also be cured by HLA-matched allogeneic HSC transplantation. This offers very unique opportunity for treating diverse metabolic disorders including a diseases that entails a defect in an ATP-binding cassette transporter; ALD protein encoded by defected *ABCD1* gene resulting in progressive and irreversible X-ALD (severe cerebral demyelinating disorder) [52,53]. The lentiviral vector was indispensable in these trials. A demyelinating lysosomal storage disorder MLD (metachromatic leukodystrophy) originating from arylsulphatase A (ARSA) deficiency demonstrated in haematopoietic lineages is a viable example [54]. Success in the transplantation of *ex vivo* gene modified HSCs in adult patient buttresses the fact that the application of the SIN-lentiviral vector is paramount, and also evident in the use of mini-globin gene-contained SIN-lentiviral along with its introns as well as its 30 -enhancer region, promoter and a locus managed region. This also included two copies of the 250-bp core element that constitutes the cHS4 chromatin insulator. After about a year, the recipients became independent of blood transfusions; one of the very obvious therapeutic advantages related to the growth of a myeloid-restricted cell clone. The lentiviral vector integration resulted in the induction of a steady, unexpectedly spliced HMGA2 (a tumour suppressor gene) that lead to a clonal growth that is benign [55,56]. Even though there has been a recorded delay of the translation into clinical practice due to the challenges of tissue constrained expression of b-globin gene, this area of gene therapy has also over time been interested in treating one of the most widespread class of inherited disorders; the b-haemoglobinopathies. Against this limitation though, the application of gene therapy to the treatment of b-globin deficiency-caused b-thalassaemia has begun. Lentiviral vectors, apart from their application in *ex vivo* gene therapy, have also been used *in vivo* in the treatment of Parkinson's disease (a CNS pathology) and ocular diseases including Usher syndrome, Stargardt's disease, and the wet variety of macular degeneration type 1B, (http://www.oxfordbiomedica.co.uk/clinical-trials-1/). High repertoire of clones causative of gene marked haematopoiesis in bone marrow CD34þ cells as well as peripheral blood myeloid, B and T cells is usually linked with clinical lentiviral vectors usage (e.g. X-ALD, WAS and MLD trials) in gene therapy [57,58]. The results summarized from these studies were preceded by reasonable follow-up for months, up to 62 months in some cases [59]. Though this time frame of follow-up looks short, evidence for the lack of clonal outgrowth and the lack of transplantation-related side effects or adverse reaction in the therapy or management of these monogenic diseases shows the suitability of gene therapy, found useful in cases where suitable HSC donors are not available. The use of lentiviral vectors *in vivo* are not without limitations. Challenges of off-target location, as well as the need for highly concentrated and purified particles, achievable through the application of cell lines that are stable exists [60]. The remarkable and pointed new and recent development to one of the challenges is the development of engineered lentiviral vectors with envelope pseudotypes [61]. Depending on the expected outcome of the experiments or trials involving gene transfer in post-mitotic tissues such as the insertion of the human RPE65 gene into the retina for the correction of Leber congenital amaurosis, non-integrating vectors can be made by generating integration-deficient lentiviral vectors (IDLV), which on transcription produces extrachromosomal DNA circles [62,63]. This could be very functional in cases where only transient transgene expression (such as the vaccination approaches in delivering transposases or designer nucleases) is needed. [63–65].

3. Conclusion

From the use of stem cell therapy to the science of targeted specific gene correction, the advantages of gene therapy over pharmacotherapy in the art of healing cannot be overstated. The various opportunities like the same individual donor and recipient identity in HSC transplant in order to combat the risk of incompatibility, managing haemoglobin disorders such as Sickle Cell disease and β-Thalassaemia, ocular gene transfer, cancer immunotherapy using gene-modified T cells, and targeted specific gene correction places gene therapy ahead of many other forms of treatment. There is room for so much improvement on gene therapy because the opportunity to improve on the AAV-derived Vectors and Self-inactivating (SIN) Channels. This is in addition to the trending approach and strategy for combating particular diseases via personalising treatments, and there may actually be no better approach than tailoring a cure to a specific disease in a particular individual. Instead of managing diseases and administering drugs which may have diverse side-effects on the body, with gene therapy, the cause of the disease is targeted at the source and dealt with. The argument that the application of gene therapy in treatment has not been with us long enough to see the side effects it may present in subsequent generations does not stand much ground because it cannot be denied that in gene therapy lies current and future answers to treatment of insurmountable diseases that have defied cure. With gene therapy, the mishaps of nature can be corrected and we may well be on our way to reproducing 'super humans'.

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