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Review

Anti-ageing gene therapy: Not so far away?

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ABSTRACT

Improving healthspan is the main objective of anti-ageing research. Currently, innovative gene therapy-based approaches seem to be among the most promising for preventing and treating chronic polygenic pathologies, including age-related ones. The gene-based therapy allows to modulate the genome architecture using both direct (e.g., by gene editing) and indirect (e.g., by viral or non-viral vectors) approaches. Nevertheless, considering the extraordinary complexity of processes involved in ageing and ageing-related diseases, the effectiveness of these therapeutic options is often unsatisfactory and limited by their side-effects. Thus, clinical implementation of such applications is certainly a long-time process that will require many translation phases for addressing challenges. However, after overcoming these issues, their implementation in clinical practice may obviously provide new possibilities in anti-ageing medicine. Here, we review and discuss recent advances in this rapidly developing research field.

1. Introduction

Over the last decades, life expectancy has risen dramatically and translated into a higher population of elderly people across all developed countries (Skirbekk et al., 2019). This demographic process is evidently accompanied by increasing incidence of age-related pathological conditions, including osteoporosis, cardio-metabolic and neurodegenerative diseases and cancer. Consequently, searching for novel interventional strategies for health span extension is a current priority goal in biomedical research. Traditionally, pharmacological approaches have dominated in this research field. Development of supplements and medications specifically targeted at ageing-related disorders is one of the most rapidly growing fields in modern biogerontology (Vaiserman and Marotta, 2016; Vaiserman and Lushchak, 2017). Despite this, pharmacology-based interventions have provided modest benefits only. Accordingly, great hopes are currently being placed on more innovative (and radical) solutions such as stem cell- and gene-based therapies.

Gene therapy is currently regarded as the most promising treatment option to cure many diseases. The gene therapy concept was developed as early as in 1970s to “replace bad DNA with good DNA” (Friedmann and Roblin, 1972). This therapy is carried out by introducing the functional DNA fragments into the target cells of the patient to correct disease-inducing mutations (Helal et al., 2017). Depending on the type of disease, functional genes can be delivered to replace defective genes or by reducing their products using various biotechnological tools including naked oligonucleotides, as well as viral and non-viral vectors (Kaufmann et al., 2013). Gene therapy-based applications also allow modulating gene expression profiles in specific cells to treat various pathological conditions (Chakraborty et al., 2017; Harries, 2019). Such a modulation may be performed by introducing exogenous nucleic acids such as DNAs, mRNAs, microRNAs (miRNAs), small interfering RNAs (siRNAs) and antisense oligonucleotides (Yin et al., 2014). The gene therapy approaches act in both direct (e.g., by gene editing) and indirect (e.g., by viral or non-viral vectors) modes by modulating the genome architecture and function. The therapeutic approach based on direct *in vivo* gene transfer provides targeted delivery of the gene therapy vectors (i.e. adenoviral vectors, adeno-associated viral (AAV) vectors and non-viral vectors), to specific disease sites of the patient (Nayerossadat et al., 2012). Such a treatment mode is regarded as a preferable therapeutic option in case of difficulty or impossibility of using cell culture-based approaches for particular tissue types, in particular when targeted cells such as neurons cannot be cultured *in vitro* in sufficient numbers or when cultured cells cannot be re-implanted effectively in the patient’s body. In preclinical studies, the use of

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adenoviruses (viral vectors) and plasmids (non-viral expression vectors) represents the most common treatment strategy. The genes delivered in such a way encode predominantly transcription factors, growth factors, anti-inflammatory cytokines and, less frequently, cell signaling proteins, receptors and matrix proteins (Bellavia et al., 2018).

Initially, gene therapy has been considered as a valid approach to treat monogenic inherited diseases (Wirth et al., 2013). However, somatic gene therapy has a much wider potential. With revolutionizing biotechnologies, gene therapy-based therapeutic modalities are increasingly considered as promising tools in treating not only monogenic orphan disorders, but also polygenic complex diseases, including age-related ones. Different gene therapy tools based on integration of foreign DNA into the host genome via gene cloning, transfer vectors, recombinant viruses etc., have been progressively introduced and presently incorporated in the workflow of clinical laboratories worldwide (Naldini, 2015; Ginn et al., 2018). These therapeutic modalities include, among others, targeted disruption (knockout) of genes potentially implicated in certain pathological pathways, complementation of a defective gene product with a normal gene product (gene augmentation), and also genome editing to restore a normal gene product function (Dunbar et al., 2018; Rossor et al., 2018). Antisense oligonucleotides and siRNAs have also attracted substantial attention as potential therapeutics now, involving their use to down-regulate over-expressed genes (Tatiparti et al., 2017). The gene editing approaches are increasingly used in gene therapy now, including transcription activator-like effector nuclease (TALEN) systems and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) systems, that show a major specificity for particular genetic conditions, as they target the site within the gene and type of mutation inheritance. In addition, they offer the possibility to specifically target the mutation (Maeder and Gersbach, 2016; WareJoncas et al., 2018).

Here, we report an overview of applying these therapeutic modalities in anti-ageing medicine, highlighting achievements but also challenges and risks of long-term negative outcomes.

2. Gene therapy technologies

Gene therapy strategies currently include *in vivo* and *ex vivo* approaches (for reviews, see, e.g., Kumar et al., 2016; Glorioso and Lemoine, 2017). The *in vivo* gene therapy is based on the direct injection of specific genetically engineered vectors into a patient's blood stream, in order to transfer the correct gene. In *in situ* gene therapy, genetic material is introduced directly into a localized body area to target specific tissues/organs and to induce there short-term or sustained expression of certain genes. In the *ex vivo* approach, patient's somatic cells are therapeutically modified outside the body in a laboratory setting and then transplanted back again. By this procedure, a normal allele of the defective gene is transferred through viral or non-viral gene delivery vectors to cultured cells. The *ex vivo* gene therapy offers additional advantages including the possibility to analyze and select the modified cells (van Haasteren et al., 2018). Other advantages include higher specificity, safety, and lack of immune response. A schematic diagram presenting these approaches is shown in Fig. 1. Main gene therapy approaches are described in the subsections below in more details.

2.1. Viral vector systems

Since the early years of gene therapy, the virus-mediated gene transfer remains the most used mode of gene delivery. The applicability of viral vector systems is related with the fact that they may be relatively easily manipulated. Moreover, viruses have acquired, through evolution, the ability to effectively transfer their genetic material to the host genomes. For individual therapeutic purposes, the choice of an appropriate viral vector system depends on the nature of the disease (Stone, 2010). Indeed, several therapeutic options require long-term gene transfer, whereas others require transitional or even regulated gene delivery; the widespread gene transfer may be preferred in one cases, and a localized one in others, etc. Among the most commonly used viral vector systems, there are adenoviruses, AAVs, and retroviral and lentiviral vectors. The main advantages and disadvantages of these systems are presented in Table 1.

Among the available gene transfer platforms, recombinant AAVs have currently emerged as vectors of choice in treating complex chronic disorders, including neurodegenerative and cardio-metabolic ones, because of their numerous eligible properties. See also Fig. 2 for schematic presentation.

AAVs are small (25-nm) viruses from the *Parvoviridae* family which are composed of a non-enveloped protein shell containing a linear single-stranded DNA genome. Their beneficial properties include safety, stability, poor immunogenicity, and high efficiency of transduction of a broad range of target tissues (Colella et al., 2017). The number of clinical trials in that AAV vector systems have been applied for *in vivo* gene transfer has steadily increased over the past decade. Initially, these clinical trials were designed to examine inherited blindness, coagulation disorders and neurodegenerative diseases. In recent years, the encouraging results on the efficiency and safety of the AAV gene transfer were also obtained in clinical trials for muscle, brain, retina and liver pathologies (for reviews, see Colella et al., 2017; Naso et al., 2017). In the context of anti-ageing research, it is important to highlight that AAV vectors may transduce both proliferating and non-dividing cells, and therefore maintain long-term (up to several years) expression of the transgene product in tissues with very low proliferation rates, such as the brain tissues (Hampson et al., 2019).

For many years, it has been recognized that host immune response represents a serious concern for clinically applicable gene transfer with AAV vectors. Indeed, antibodies produced by prior exposure to natural viruses or vectors may bind to an AAV vector, thereby preventing it from entering a cell (Barnes et al., 2019). Furthermore, lymphocytes are able to destroy cells infected by AAVs. In order to overcome this issue, various solutions have been proposed, e.g., manipulation of AAV capsid (a protein shell of a virus), by which recombinant vectors lose their ability to interact with neutralizing antibodies against AAVs in the host body (Büning and Srivastava, 2019; Lotfinia et al., 2019). The achievements in optimizing genome design and developing clinically applicable AAV capsids by revolutionary biotech innovations has led to extraordinary progress in the gene therapy field. Recent preclinical and clinical advances in AAV-mediated gene replacement, gene silencing and gene editing have resulted in growing popularity of AAV vector systems, with two AAV-based medications receiving regulatory approval in the United States and in Europe (Wang et al., 2019). To date, AAV is increasingly considered as a vector of choice for neurodegenerative diseases such as Alzheimer's or Parkinson's disease because of its established safety profile and strong neuronal tropism (Hudry and Vandenberghe, 2019).

2.2. Non-viral vectors

Non-viral vectors are plasmids that may be delivered to target cells as naked DNA or in association with specific chemically synthesized vectors, including lipid-based, polymer-based, peptide-based delivery systems, and also inorganic particles (Ramamoorth and Narvekar, 2015). For acute injuries such as myocardial infarction and stroke, injection of plasmid DNA directly into the site of injury can likely be a preferred therapeutic mode because of ease of manufacturing and reduced mutation risk. However, therapeutic efficiency of naked plasmid DNA is sufficiently low owing to its untimely degradation, poor cellular intake, as well as low protein expression (Mason et al., 2015). Non-viral vectors may carry DNA and RNA of therapeutic interest such as microRNA (miRNA) and short interfering RNA (siRNA). The demand to develop such systems has been actualized due to concerns over using

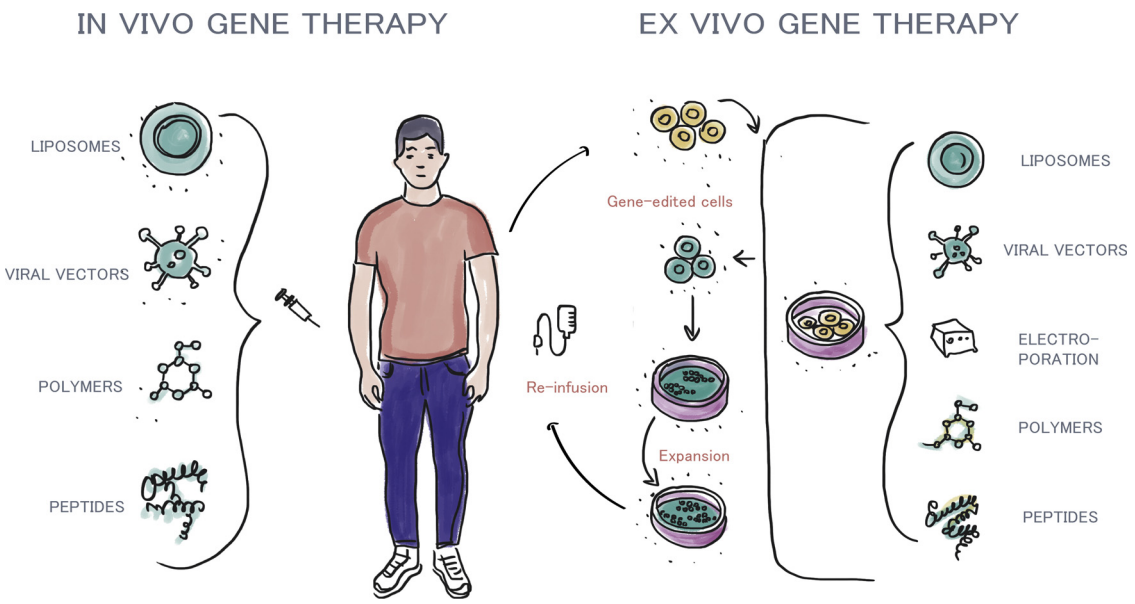

Fig. 1. Schematic representation of *in vivo* and *ex vivo* gene therapy approaches. In the *in vivo* approach, the therapeutic gene may be directly delivered into the target cells of a certain tissue in the patient's body. The *ex vivo* gene therapy involves the genetic modification of cells outside of the body and reintroducing these genetically modified cells back into the patient.

the viral gene transfer systems related to the possibility of immune responses to viral vectors (primarily, adenoviral ones), and also due to possible insertional mutagenesis (ectopic chromosomal integration of viral DNA potentially activating oncogenes or inactivating tumor suppressor genes). The non-viral vector systems offer considerable biosafety advantages, including lesser immunotoxicity, over the viral ones. Their other advantages include low cost and ease of production, and also possibility of chemical modification to fine-tune the physicochemical properties (Mason et al., 2015).

The features of non-viral vector systems make them more suitable for short term, transient expression of particular genes in certain pathological conditions. For example, in angiopathies, the transient expression of angiogenic factors in a modest number of modified cells may be sufficient to obtain desired phenotypic effects (Chaanine et al., 2010). Certain disorders such as heart failure require, however, sustained and widespread expression of transgenes in order to achieve desired therapeutic effects. In such pathological states, viral vectors are obviously more suitable, since they provide a high effectiveness of gene transfer and long-term transgene expression. The major limitations of viral-mediated gene transfer systems in such trials include their restricted packaging capacity, inconsistency in purity and bioactivity between different vector stocks, viral-induced immune responses and also biosafety risks (Chaanine et al., 2010). From a safety point of view, treatment with non-viral approaches is considered to be the most attractive in *in vivo* gene therapies of muscular dystrophies, Parkinson and Alzheimer diseases, and also heart failure (Ishikawa et al., 2018).

The application of non-viral gene transfer systems was limited for a long time due to their poor transfection efficiency and transient expression of delivered transgenes due to their intracellular degradation (Glover et al., 2005). In addition, a significant drawback of non-viral carriers is that 2–3 orders of magnitude more DNA is required than that for viral carriers to achieve similar efficiency (Ragusa et al., 2007). Recent advances in developing such vector systems characterized by better specificity and safety, as well as longer duration of transgene expression, have led to an enhancing number of non-viral vector products that enter clinical trials.

2.3. Gene editing

Gene editing represents a group of biotechnological approaches that directly modify DNA. These technologies allow changing, adding or removing the genetic material at precise locations in the genome. To date, among them, three classes of engineered chimeric proteins, namely, the zinc finger nucleases (ZFNs), the TALENs, and also the CRISPR–Cas9 gene editing system, are the most widely applied due to their ability to target nearly any DNA sequence with nucleotide-level precision (Gaj et al., 2013; WareJoncas et al., 2018).

In recent years, the CRISPR/Cas9 system has gained widespread acceptance as a most powerful tool for precisely targeted gene editing. In this technology, a site-specific RNA guide targets the site of interest where the Cas9 protein performs a double stranded DNA cut. Thereafter, target genes may be repaired via the host cell repair machinery either by non-homologous end joining (NHEJ) or by homology-directed repair (HDR) pathways, thereby causing modification or permanent replacement of the target DNA sequence (Singh et al., 2017). The benefits of this DNA-free, mRNA-based molecular tool over the plasmid DNA delivery include improved specificity, reduced toxicity, better on-target efficiency and no danger of unpremeditated DNA insertion (Lino et al., 2018). Moreover, compared to other techniques like ZFN and TALEN, the advantages of CRISPR/Cas9 technique include its cost-effectiveness, flexibility, and being easy-to-use (Karimian et al., 2019). Although CRISPR–Cas9 is regarded as a highly precise technique for genome editing, several undesirable outcomes may nonetheless occur. Indeed, Cas9 proteins can sometimes mistakenly cut off-target sequences with high sequence homology to on-target sites (Hussain et al., 2019). Therefore, great efforts are now being made aimed at improvement of CRISPR/Cas9 technology to increase its efficiency and specificity (Khadempar et al., 2019).

3. Gene therapy in anti-ageing research

Gene therapy-based interventions are increasingly being used to target ageing phenotypes and ageing-related diseases in preclinical studies. Major findings from these studies will be described in more details in subsections below.

3.1. Telomerase gene therapy

Transfer of the gene encoding the telomerase reverse transcriptase (hTERT) by specific plasmid vectors has been used most frequently in

Table 1
Basic characteristics of the most commonly-used viral gene delivery systems.

Viral vector	Nucleic acid	Insert size, kb	Vector genome form	Transgene expression	Advantages	Disadvantages	Ref.
Adenovirus	dsDNA	8-36	Episomal	Transient	High titers; Efficient transduction of most cell types	Potential inflammatory response	Lee et al., 2017
AAV	ssDNA	5	Episomal	Transient or stable	Safe transgene delivery; Non-inflammatory; Non-pathogenic	Small packaging capacity; Depends upon helper adenovirus for replication; Difficult to produce pure viral stocks	Colella et al., 2017; Naso et al., 2017
Retrovirus	ssRNA	7-12	Integrated	Stable	Persistent gene transfer in dividing cells	Only transduces dividing cells; Integration might induce oncogenesis	Vargas et al., 2016
Lentivirus	ssRNA	8	Integrated	Stable	Persistent gene transfer in most tissues	Integration might induce oncogenesis	Wong et al., 2006

ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.

the field of anti-ageing research. Telomere biology is one of the crucial contributory factors in ageing and ageing-related disease risks, because telomeres progressively shorten during each round of mitosis in most human somatic cells, eventually leading to chromosomal instability and cell senescence (Shay and Wright, 2019; Turner et al., 2019). Telomeres may be elongated through action of telomerase, a specific enzyme that adds telomere repeats to the ends of chromosomes and thereby elongates telomeres. Telomerase is a ribonucleoprotein complex composed of hTERT gene and RNA template (hTR) (Leão et al., 2018). The level of the telomerase activity in the cell is limited primarily by the level of hTERT expression. The hTERT is known to be expressed only in germ line cells, proliferative stem cells of renewal tissues, as well as in cancer cells. The hTERT expression in normal cells leads to reconstitute telomerase activity and to prevent the induction of cellular senescence which likely contributes in specific tissues and locations to impairment of physiological functions and development of chronic pathological conditions with increasing age (Boccardi and Boccardi, 2019). However, whereas expressing hTERT results in the maintenance of telomere length in certain cell types and is assumed to be a promising prolongevity option, the blocking of replicative senescence would likely enhance the risk of developing cancer. However, the transient rejuvenation of cells might be advantageous in many cases. Ectopic expression of hTERT (i.e., the expression in cells in which the gene is not usually expressed) has been demonstrated to extend replicative cell life span in many cell types including human skin fibroblasts, keratinocytes, muscle satellite (stem) cells, retinal-pigmented, myometrial, vascular endothelial and breast epithelial cells *in vitro* (Shay and Wright, 2005; Kang and Park, 2007), and also to increase both the health span and life span *in vivo* (Mendelsohn and Larrick, 2012).

In pioneering research in this area, Bodnar and co-workers transduced telomerase-negative human foreskin fibroblasts and retinal pigment epithelial cells with hTERT carried by the myeloproliferative sarcoma virus or by the plasmid vector (Bodnar et al., 1998). The telomerase-expressing clones exhibited signs of slowed aging, and had elongated telomeres compared to the telomerase-negative control clones. Furthermore, the telomerase-expressing clones had normal karyotypes and exceeded their normal replicative life span by at least twenty doublings. Subsequently, high levels of telomerase activity and extended replicative potential were also observed in other cell types such as human osteoblasts transduced with hTERT by the vector plasmid (Yudoh et al., 2001) and in naïve CD8(+) T lymphocytes transduced with hTERT by the retroviral vectors (Rufer et al., 2001). The potential of the telomerase gene therapy in anti-ageing medicine was also confirmed in *in vivo* studies (see Fig. 3 for illustration).

In the study by Bernardes de Jesus et al. (2012), AAV9 vector was used to constitutive expression of the mouse TERT (mTERT) catalytic subunit in a variety of tissues of one- and two-year-old mice. This treatment resulted in a long-lasting (up to eight months) 5- to 15-fold increase of the level of mTERT expression and about a 5-fold increase of telomerase activity. These changes were expectedly associated with increased average telomere lengths in treated mice compared with controls. Treatment of both one- and two-year old mice with AAV9-mTERT had significant beneficial effects on several age-related health parameters such as insulin sensitivity, neuromuscular coordination, osteoporosis, and on some molecular biomarkers of ageing. Levels of insulin-like growth factor-1 (IGF-1), known to be gradually decreased with ageing, were restored in AAV9-mTERT-treated two-year-old mice to levels characteristic of one-year-old animals. In addition, the median life spans of mice treated with AAV9-mTERT at one and two years old were increased by 24 % and 13 %, respectively. Importantly, AAV9-mTERT-treated mice did not develop more tumors than the untreated littermates. Based on these findings, authors suggested that a cancer-promoting activity of telomerase may be substantially reduced when expressed in adult to old organisms using AAV vectors. However, since high-level TERT expression has been shown to be associated with oncogenic transformation in many other studies, considerable caution is

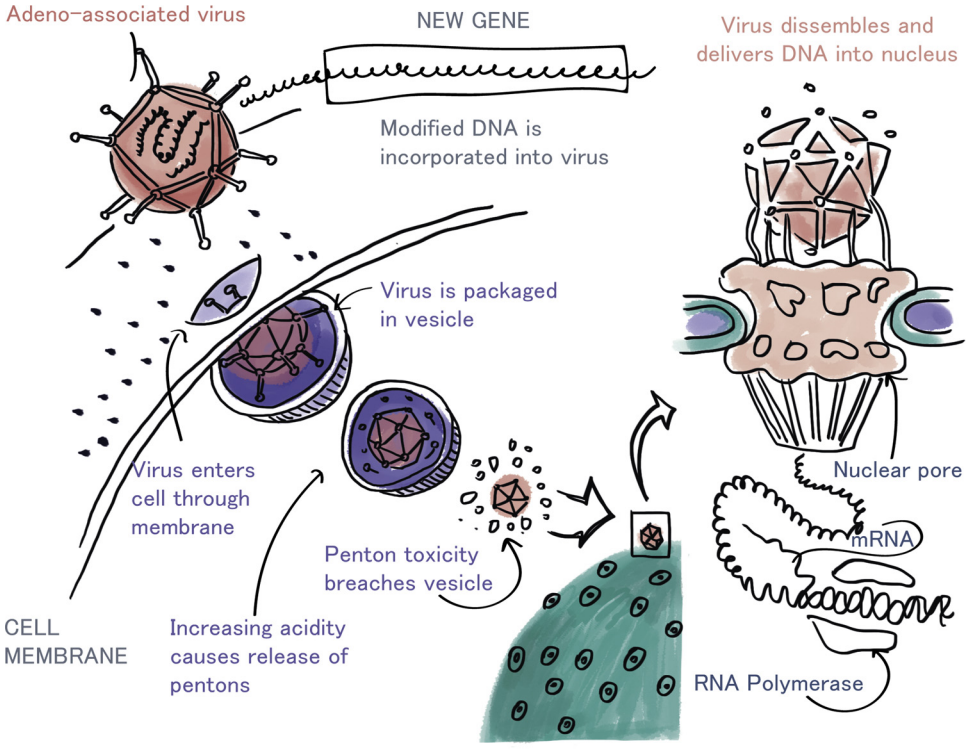

Fig. 2. Schematic representation of gene therapy with adeno-associated viral vector. In this therapeutic modality, genetic material of "the gene of interest" is biochemically engineered into the adenoviral DNA. After injecting into the host, virus utilizes the host vesicle trafficking machinery to deliver this genetic material to the cell nucleus for the desired protein production.

needed in the clinical implementation of such a therapeutic option (Larrick and Mendelsohn, 2015).

3.2. Klotho gene therapy

Gene therapy of the Klotho gene is another promising treatment modality in anti-ageing medicine. This gene encodes a single-pass transmembrane protein Klotho, known to play a key role in cellular metabolism and homeostasis (Kim et al., 2015). The deficiency of this protein was repeatedly found to be associated with development of various ageing-related conditions and life shortening (Cheikhi et al., 2019). It was repeatedly found to be associated with development of various ageing-related diseases. Up-regulation of this gene was shown to alleviate the symptoms and prevent the progression of age-related neurodegenerative conditions such as Alzheimer's disease (Paroni et al., 2019). In the Chen et al. (2018) study, targeting the Klotho promoter region by the CRISPR-dCas9 complex resulted in an increased Klotho expression at both gene and protein levels in human neuronal and kidney cell lines, suggesting a therapeutic potential for enhancement of cognitive functions and treating ageing-related demyelinating and

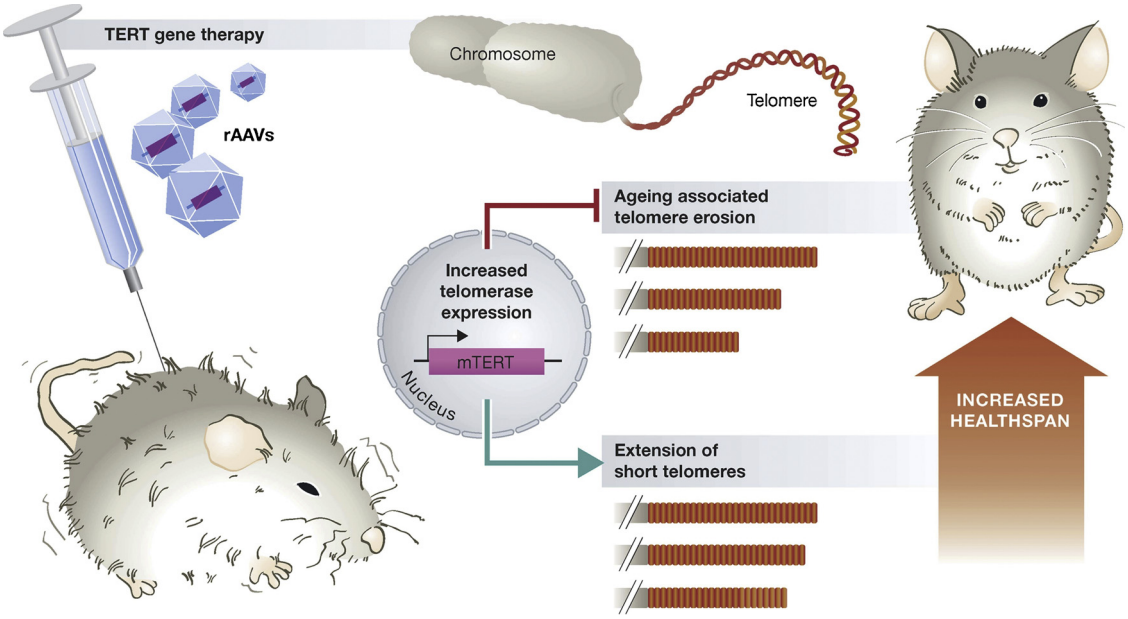

Fig. 3. Promoting healthspan in mice using a telomerase gene therapy. Delivery of telomerase (TERT) using rAAV suppresses aging associated telomere erosion and extends short telomeres in a variety of mouse tissues. Consequently, animals display improved healthspan and extended lifespan. Fig. 3 and its legend are reproduced from Boccardi and Herbig, 2012 with permission from John Wiley and Sons Publisher.

neurodegenerative disorders. The potential therapeutic effects of up-regulation of Klotho expression through its transduction by employing a lentiviral vectors has been also revealed in senescence-accelerated mouse prone-8 (SAMP8) model (Zhou et al., 2018). In 10-month-old SAMP8 mice, the expression of Klotho in the brain was significantly increased three months after injection of the Klotho-carrying lentiviral construct. Moreover, the reduction in neuronal loss, synaptic damage and memory deficits, and increased levels of mitochondrial manganese-superoxide dismutase and catalase expression, suggestive of decreased oxidative stress, were obtained in treated mice.

3.3. Gene therapy for ageing-related diseases

In the following subsections, pre-clinical studies and clinical trials aimed at investigating the clinical potential of gene therapy-based technologies for treating ageing-related diseases are summarized and discussed.

3.3.1. Metabolic disorders

Age-related metabolic alterations are well-known to be critically involved in ageing processes (López-Otín et al., 2016). Obesity and type 2 diabetes are well known to be associated with signs of premature cellular senescence and ageing (Burton and Faragher, 2018). Remarkably, the most common antidiabetic medication, metformin, has been recently proposed as potential anti-ageing drug, simultaneously reducing the risk of various ageing-associated disorders, including cardio-metabolic and neurodegenerative ones, and also cancer (Konopka and Miller, 2019; Piskovatska et al., 2019). The anti-ageing potential of metformin is planned to be explored in a large-scale clinical trial, Targeting Aging with METformin (TAME) (Justice et al., 2018).

Recently, gene therapy-based approaches have demonstrated the potential to counter age-associated metabolic impairments. Treating the streptozotocin-induced diabetic mice with insulin and glucokinase using AAV1 vectors resulted in restoring and maintaining normoglycemic status in skeletal muscle for more than four months after the streptozotocin administration (Mas et al., 2006). Moreover, these animals exhibited normalization of different metabolic parameters, including glucose tolerance as well as food and fluid intake. In the same model, over-expression of a transcription factor, known to increase insulin sensitivity by activating insulin-signaling pathways (TCFE3) mediated by an adenovirus or AAV serotype 2 (AAV2) vector, ameliorated hyperglycemia by glucokinase up-regulation in the mice liver (Kim et al., 2013). The remission of diabetes was also demonstrated in diabetic mice administered with a recombinant adenovirus expressing furin-cleavable rat insulin under control of the hepatocyte-specific and glucose-responsive synthetic promoter (Han et al., 2011), with a single-stranded serotype 8 pseudo-typed AAV (ssAAV2/8) vector encoding the human pro-insulin gene under a constitutive liver specific promoter (Gan et al., 2016), and also with AAV8 carrying a metabolically responsive insulin transgene to the liver (You *et al.*, 2015). The adenoviral vector-based delivery of a pancreatic transcription factor such as neurogenin-3 before the induction of diabetes in mice was also shown to protect from developing hyperglycemia (Phillips and Kay, 2014). Similar evidence for alleviating diabetic symptoms has been obtained in diabetic rat models, where hyperglycemia was reversed through hepatic insulin expression via lentiviral transduction (Elsner et al., 2012). The blood glucose levels were normalized in the treated rats; this effect persisted over the year after the single injection.

In a large-animal (dog) model of diabetes, a single injection with AAV1 encoding for insulin and glucokinase led to normalization of fasting glycemia, normalized disposal of glucose after oral challenge and reduced glycosylated plasma proteins levels for more than four years after the treatment (Callejas et al., 2013). These beneficial outcomes were accompanied by recovery of body weight and improved long-term survival without any secondary complications. More recently, in a long-term (~8 years) follow-up after a single administration of these therapeutic vectors, the persistence of vector genomes and therapeutic transgene expression were revealed over years after the vector delivery in multiple samples from treated dog muscles (Jaén et al., 2017). Moreover, the signs of metabolic correction, including normalization of serum levels of cholesterol, triglycerides and fructosamine and substantial improvement in the response to oral glucose load were observed in treated diabetic animals. Importantly, the successful multi-year control of glycemia was achieved in this study without any need of exogenous insulin administration.

3.3.2. Cardiovascular diseases

Currently, gene therapy represents one of the most popular applications for cardiovascular diseases (Scimia et al., 2014). It is increasingly considered as a promising therapeutic option for treating atherosclerosis, an age-associated condition commonly characterized by systemic oxidative stress and low-grade chronic inflammation. Gene therapy strategies for atherosclerosis fall into two main categories: targeting genes associated with premature atherosclerosis or targeting genes to stabilize vulnerable plaques or inflammation (Misra, 2018). These genes include ApoB, ApoC, LDL receptor, proprotein convertase subtilisin/kexin type 9, matrix metalloproteinase, monocyte chemotactic protein-1 and IL-10. In animal models, suppression of atherogenesis was achieved by delivery of genes encoding regulators of redox sensitive transcriptional factors (e.g., NF-kappa B, Nrf2, AP-1 etc.), antioxidant defense enzymes (e.g., superoxide dismutase, glutathione peroxidase, catalase and heme oxygenase-1) or endothelial nitric oxide synthase (Van-Assche et al., 2011). The evidence for efficiency of gene therapy in treating atherosclerosis has come mostly from rabbit models. For instance, expression of apolipoprotein A-I (apo A-I) in the artery wall by a helper-dependent adenoviral (HDAd) vector was shown to be effective in preventing atherosclerosis in hyperlipidemic rabbits (Flynn et al., 2011; Wacker et al., 2017, 2018). However, despite promising results from animal models, the clinical applicability of such therapeutic modalities has proven to be questionable because of the inability of sustained local expression of these genes in target tissues and the development of immune responses and other side effects (Van-Assche et al., 2011).

To date, gene therapy-based approaches are increasingly used in the treatment of myocardial infarction (Scimia et al., 2014). Among these approaches, there is an attempting to rejuvenate the autologous human cardiac stem/progenitor cells (hCPCs). A serious obstacle to the practical usage of such therapeutic option is that most hCPCs are dysfunctional (senescent) in elderly individuals, who are most susceptible to cardiovascular pathology. Rejuvenating hCPCs from elderly patients by gene therapy approaches is believed to be a way to overcome this challenge. Recently, Khatiwala and co-workers used the lentiviral plasmid containing the short hairpin RNA (shRNA, an artificial RNA molecule with a tight hairpin turn which may be applied to silence the target gene expression via RNA interference) to p16INK4A knockdown aimed at rejuvenating the aged human cardiac progenitor cells via an up-regulation of anti-oxidant and NF-kB signal pathways (Khatiwala et al., 2018). Cell proliferation, survivability and antioxidant defense were significantly enhanced in treated hCPCs compared to control hCPCs. The up-regulation of genes associated with cell senescence, NF-κB signal pathway, apoptosis, and antioxidant defense system was also observed in genetically modified hCPCs. In the study by Tafuro et al. (2009), functional angiogenesis (a growth of new blood vessels from the existing vasculature) was promoted in both normally perfused and ischemic mouse skeletal muscles via regulating the expression of vascular endothelial growth factor by AAV. Nakagami and Morishita (2009) also used the naked plasmid of hepatocyte growth factor as an angiogenic factor for peripheral arterial diseases and reported its efficiency and safety in clinical trial. Some growth factors such as hepatocyte growth factor, fibroblast growth factors and vascular endothelial growth factors have already been tested in other gene therapy-based clinical trials. However, apart from demonstration of increased vascularity, very few

results with clinical significance have been obtained due to problems associated with gene transfer efficiency and short duration of transgene expression (Ylä-Herttuala et al., 2017). These issues, including development of better and more specifically targeted delivery systems and search for more optimal growth factors, must be addressed in future studies.

3.3.3. Musculoskeletal disorders

Sarcopenia (a progressive loss of muscle mass and function with age) is one of the most common hallmarks of human ageing (Distefano and Goodpaster, 2018). Therefore, myostatin (a transforming growth factor-β family member acting as a negative regulator of skeletal muscle mass) is currently regarded as a promising therapeutic target in muscle-wasting disorders (Consitt and Clark, 2018). In rodent models, inhibition of myostatin by gene therapy approaches was repeatedly shown to improve the muscle function as well as the muscle glucose and fat metabolism. The improved growth of skeletal muscle, and also lipid metabolism and glucose regulation have been observed through AAV-mediated delivery of the gene encoding myostatin pro-peptide known to bind and inhibit myostatin in transgenic diabetic db/db mice (Jiang et al., 2017) and in C57BL/6 mice consuming high-fat diet (Yan et al., 2019a). Significantly enhanced muscle growth was also observed in mice injected with the naked plasmid DNA encoding a mutant myostatin propeptide, MProD76A (Hu et al., 2010). Moreover, AAV8-mediated myostatin propeptide gene delivery enhanced muscle growth and ameliorated dystrophic phenotypes in dystrophic (dystrophin-deficient) *mdx* mice (Qiao et al., 2008). Similar effects were found in normal and *mdx* mice following the systemic myostatin inhibition via the liver-targeted gene transfer (Morine et al., 2010).

3.3.4. Neurodegenerative disorders

Currently, gene therapy holds great promise in treating ageing-related neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Sudhakar and Richardson, 2019). Alzheimer's disease (AD) characterized by progressive deterioration of cognitive functions (dementia) is the most common ageing-associated neurodegenerative disease now. Progression of this disease is associated with the accumulation of neurofibrillary tangles (tau proteins) and extracellular amyloid-beta (Ab) plaques in the brain (Chen et al., 2019). The present-day treatments for AD are aimed at reducing Ab and tau levels in the brain (Madav et al., 2019). The potential of gene therapy for treating AD is being thoroughly discussed and examined in pre-clinical studies (Cubinková et al., 2017; Ittner et al., 2019). In a mouse model of AD, AAV-mediated knockdown of the *Acat1* gene encoding the acyl-CoA:-cholesterol acyltransferase 1 lead to decreased Ab levels (Murphy et al., 2013). Significantly reduced Ab levels in the brain was also revealed following the delivery of the neprilysin/membrane metallo-endopeptidase (MME) gene by AAV9 vector through either direct injection into a hippocampus or cortex (Carty et al., 2013) or through intracardiac administration (Iwata et al., 2013). In the later study, the reduction of amyloid burden was accompanied by significant improvement of impaired learning and memory functions. In a transgenic mouse model of amyloidosis, inhibition of Ab plaque formation was also achieved via lentiviral transfer of the gene encoding a major Ab degrading enzyme, neprilysin. Moreover, this treatment ameliorated neurodegenerative changes in the hippocampus and frontal cortex of these mice (Marr et al., 2003). In the same model, the long-term (up to 6 months) neprilysin gene transfer resulted in reduced levels of intracellular Ab and improved behavioral performance of transgenic mice (Spencer et al., 2008).

Leptin, an important hypothalamic regulator of appetite and energy balance known to be involved in pathogenesis of AD, also represents a potential therapeutic target for this disease hypothalamic regulator of appetite and energy balance known to be involved in pathogenesis of this disease (McGregor and Harvey, 2018). Lentivirus-mediated transfer of the leptin gene mitigated Ab-related neuronal damages and prevented memory deficits in transgenic mouse model of AD (Pérez-González et al., 2014). Improved learning and memory performance and reduced Ab levels in the brain are also obtained in transgenic AD mice in consequence of the lentiviral vector-mediated over-expression of F-spondin gene playing an important role in synaptic function (Hafez et al., 2012). Similar effects are observed in aluminum-induced AD mice following down-regulating the expression of caspase-3 gene using lentiviral vector-mediated caspase-3 shRNA (Zhang et al., 2014). Lentivirus-mediated over-expression of the glial cell-derived neurotrophic factor (GDNF) gene in hippocampal astrocytes was also shown to preserve learning and memory of AD mice (Revilla et al., 2014). The AAV8-mediated over-expression of insulin-like growth factor 2 (IGF2), known to play an important role in memory consolidation, in the hippocampus of aged wild-type mice promoted spine formation and rescued behavioral deficits in AD mice (Pascual-Lucas et al., 2014). AAV8-mediated intramuscular delivery of the gene encoding the ectodomain of p75 neurotrophin receptor (a protective factor against Ab toxicity) resulted in reduced brain amyloid burden and neuroinflammation and in improved behavioral phenotypes in APP/PS1 AD transgenic mice (Wang et al., 2016). In a rat model of AD, intra-cerebral administration of a recombinant AAV vector expressing the human hypoxi A-I ndducible factor 1 gene decreased the Ab protein-induced hippocampal neuronal apoptosis (Chai et al., 2014).

Recently, gene therapy-based clinical trials have been applied to AD. Specifically, AAV2-mediated delivery of the nerve growth factor (NGF, a neurotrophic-factor protein known to protect degenerating cholinergic neurons in AD) has been shown to be well-tolerated and producing long-term expression of NGF in AD patients (Rafii et al., 2014, 2018). In another phase 1 trial, no long-term adverse effects and improvement in the rate of cognitive decline were observed following *ex vivo* NGF gene delivery by implanting autologous fibroblasts genetically modified to express NGF into the AD patient's forebrain (Tuszynski et al., 2005).

Parkinson's disease (PD) is the second most common neurodegenerative disease and movement disorder of ageing. This disease is characterized by a loss of dopaminergic neurons in the cerebral substantia nigra and by reduced dopamine levels in the striatum. The potential of gene therapy for treating PD has been thoroughly explored in preclinical animal models (for reviews see, e.g., Lin et al., 2017; Axelsen and Woldbye, 2018) and currently reached the stage of clinical trials. Basic strategic directions in these trials include gene therapy approaches aimed at delivery of genes encoding dopamine biosynthetic enzymes or trophic factors known to improve the survival of dopamine neurons utilizing gene trapping vectors stereotaxically delivered to the brain (Nakamori et al., 2019). Down-regulating the expression of α-synuclein, a presynaptic protein associated with PD progression, using modified antisense oligonucleotides, siRNAs, shRNAs and microRNAs is also offered as an alternative strategy.

The efficiency of gene therapy-based approaches in treating PD has been confirmed in several Phase I clinical trials. However, the majority of these trials failed to show any improvements beyond the placebo effect when advanced to randomized controlled Phase II trials (O'Connor and Boulis, 2015). The most evident clinical benefits have been demonstrated in Phase II clinical trial, where PD patients were directly injected into the subthalamic nucleus with AAV2 vector carrying the glutamic acid decarboxylase (GAD) gene (LeWitt et al., 2011). In this trial, patients administered with AAV2-GAD exhibited improvement of PD symptoms compared to untreated PD patients. In a subsequent long-term follow-up, clinical benefits in PD patients which persisted at 12 months after the bilateral delivery of AAV2-GAD were reported, including the formation of novel polysynaptic functional pathways linking the sub-thalamic nucleus to motor cortical regions (Niethammer et al., 2017). Long-term (up to five years) improvement in motor behavior has been also observed in an open-label Phase 1/2 clinical trial where a bilateral intrastriatal lentiviral vector-based gene therapy was applied to restore the dopamine production in PD patients

(Palfi et al., 2014, 2018). More recently, significant improvements in clinical outcomes and quality of life were found in Phase I trial in PD patients administered with AAV2 carrying the aromatic L-amino acid decarboxylase gene and delivered by a magnetic resonance imaging monitoring to optimize the delivery (Christine et al., 2019).

4. Conclusions, challenges and perspectives

Gene therapy has a great potential in preventing and treating various diseases, including those associated to ageing. However, the therapeutic efficiency of gene therapy-based applications is certainly dependent on understanding the targeted pathological processes. In this regard, the major issue is choosing the appropriate genes for therapeutic delivery. It is undoubtedly even more challenging to target polygenic ageing-associated diseases than the monogenic disorders, since the simultaneous targeting numerous genes could trigger cascades of unpredictable events. Thus, current research is focused on the development of means to selectively insert the gene of interest at a “safe harbor” locus of the cell genome to avoid the risk of insertional mutagenesis through the modification of non-targeted genes (Sadelain et al., 2011). An important challenge would be also enhancing the efficiency of transduction to the tissue or organ of interest only and therefore avoiding propagation into surrounding tissues. In the light of this, innovative approaches such as nanotechnologies for delivering therapeutic genes directly to targeted tissues have been developed (Xu et al., 2014). Likewise, gene delivery by viral vectors require improvements, as this strategy is still associated with a poor targeting efficiency, low packaging ability for large DNA sequences, risk of inducing immune responses and random integration into the human genome (Goswami et al., 2019). Besides, serious concerns regarding the safety of these therapies still exist among both the general public and medical professionals, in particular those related to the genotoxic potential of viral vectors to induce a random integration into the host genome and, consequently, up-regulation of proto-oncogenes (David and Doherty, 2017). These safety concerns limit the universal acceptance of such therapeutic strategies.

In the initial years of gene therapy research, the risk of serious side effects was obviously underestimated, but early experience in this field has demonstrated that these fears were not unfounded (Kaemmerer, 2018). In 1999, 18-year-old Jesse Gelsinger, who was an ornithine transcarbamylase deficient patient volunteered to be part of a gene-therapy study conducted at the University of Pennsylvania, has died following systemic inflammatory response caused by adenoviral gene transfer (Raper et al., 2003). This tragic event caused a wide public outcry because it was the first known human victim of a gene therapy (Yarborough and Sharp, 2009). Another example is developing leukemia in consequence of induced insertional mutagenesis in children treated for severe combined immunodeficiency (Hacein-Bey-Abina et al., 2010). In this long-term follow-up, gene therapy (*ex vivo* retrovirus-mediated transfer of gamma chain to autologous CD34+ bone marrow cells) was initially successful at correcting immune dysfunction in eight of the nine patients. However, after a median follow-up period of 9 years, acute leukemia developed in four patients, and one died. Given these long-term risks, the authors concluded that such a gene therapy approach can be an appropriate treatment option for those patients who do not have an HL A-I dentical donor for haematopoietic stem-cell transplantation and for whom the risks may be deemed acceptable (Hacein-Bey-Abina et al., 2010). In principle, such a balanced risk/reward approach may be reasonable for a variety of gene therapy modalities. For example, angiogenesis can be regarded as a reasonable target for gene therapy of age-related cardiovascular diseases, but it can also promote cancer development. Therefore, the risk/reward trade-off should be considered as a rationale to apply pro-angiogenic gene therapies in treating cardiovascular disorders. This highlights the importance of accounting not only for short-term beneficial therapeutic effects of gene therapy, but also for potential long-lasting adverse effects such as neoplastic transformation. It is obviously much more important when such a therapy is applied to children and young adults having a substantially longer potential life expectancy, but should likely be taken into account when applied to older persons as well.

Over the last decade, the interest of pharmaceutical researchers and clinicians to gene therapy modalities is being steadily increasing due to recent advancements in developing novel recombinant viral vectors and gene editing protocols (Goswami et al., 2019). Gene therapy-based drugs for curing some inherited diseases, including hemophilia, eye, neurodegenerative and immune disorders, and also lymphoid cancers have been comprehensively tested for efficacy and safety in preclinical and clinical trials, and approved by the regulatory agencies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Dunbar et al., 2018). Over the last years, several of these gene therapy products have reached the market. Among these products, there are, e.g., Kymria and Yescart, gene therapy-based immunotherapeutic medicines used to treat patients with advanced lymphocytic malignancies. Both these therapies are *ex vivo* treatments, in which transgenes are introduced into the patient's T cells outside their body to produce specific antigen receptors, thereby directing these T cells to attack certain cancer cells (Dushenkov and Jungsuwadee, 2019). Another example is *in vivo* gene therapy, Luxturna, approved by FDA at the end of 2017 to treat a blinding disease such as biallelic RPE65 mutation–associated retinal dystrophy (Rodrigues et al., 2018). In this treatment, the delivery of the therapeutic transgene is carried out by intraocular injection. In May 2019, AAV vector-based gene therapy (Zolgensma) designed to deliver a functional copy of the human survival motor neuron gene to the motor neuron cells of patients with spinal muscular atrophy), was approved in the USA for the treatment of children under the age of 2 with spinal muscular atrophy and bi-allelic mutations in the SMN1 gene encoding the survival motor neuron protein (Hoy, 2019).

Currently, innovative non-inflammatory vectors capable to effectively propagate through tissues are actively developed. Using nanoparticles which are relatively small and do not incorporate into genome can be considered as a promising future strategy for gene transfer (Jeevanandam et al., 2019; Yan et al., 2019b). Recently, mitochondrion modified to be capable to secrete therapeutic proteins has been suggested to be a suitable gene delivery vector for anti-ageing applications (at least, for post-mitotic cells) (Renteln, 2018). Extracellular vesicles (natural lipid particles released by many cell types), such as exosomes and microvesicles, which may mediate cell-to-cell communication via transferred contents including proteins, nucleic acids, and metabolites, have been also proposed as an innovative methodology to improve gene transfer with AAV vectors (György and Maguire, 2018).

Based on the above research findings and considerations, we can conclude that gene therapy can be a promising treatment option in anti-ageing medicine. However, caution is obviously needed before translating results from preclinical studies into clinical applications. The dynamics of approval/cancellation of gene therapy-based medications by regulatory agencies across the world indicates the complexity and ambiguity of issues in the field, including limited efficacy, safety risks, manufacturing hurdles and ethical problems (Carvalho et al., 2017, 2019). Thus, clinical translation of gene therapy-based treatment modalities will be a long-term process involving many iterative cycles from bench to bedside. However, after overcoming challenges discussed above, the implementation of innovative gene therapy-based approaches in biogerontological research may obviously open new horizons in combating aging and age-related diseases.

Declaration of Competing Interest

The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Axelsen, T.M., Woldbye, D.P.D., 2018. Gene therapy for Parkinson's disease, an update. J. Parkinsons Dis. 8, 195–215. https://doi.org/10.3233/JPD-181331.

Barnes, C., Scheideler, O., Schaffer, D., 2019. Engineering the AAV capsid to evade immune responses. Curr. Opin. Biotechnol. 60, 99–103. https://doi.org/10.1016/j.copbio.2019.01.002.

Bellavia, D., Veronesi, F., Carina, V., Costa, V., Raimondi, L., De Luca, A., Alessandro, R., Fini, M., Giavaresi, G., 2018. Gene therapy for chondral and osteochondral regeneration: is the future now? Cell. Mol. Life Sci. 75, 649–667. https://doi.org/10.1007/s00018–017–2637–3.

Bernardes de Jesus, B., Vera, E., Schneeberger, K., Tejera, A.M., Ayuso, E., Bosch, F., Blasco, M.A., 2012. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Mol. Med. 4, 691–704. https://doi.org/10.1002/emmm.201200245.

Boccardi, M., Boccardi, V., 2019. Psychological wellbeing and healthy aging: focus on telomeres. Geriatrics (Basel) 4https://doi.org/10.3390/geriatrics4010025. pii: E25.

Boccardi, V., Herbig, U., 2012. Telomerase gene therapy: a novel approach to combat aging. EMBO Mol. Med. 4, 685–687. https://doi.org/10.1002/emmm.201200246.

Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., Wright, W.E., 1998. Extension of life-span by introduction of telomerase into normal human cells. Science 279, 349–352. https://doi.org/10.1126/science.279.5349.349.

Büning, H., Srivastava, A., 2019. Capsid modifications for targeting and improving the efficacy of AAV vectors. Mol. Ther. Methods Clin. Dev. 12, 248–265. https://doi.org/10.1016/j.omtm.2019.01.008.

Burton, D.G.A., Faragher, R.G.A., 2018. Obesity and type–2 diabetes as inducers of premature cellular senescence and ageing. Biogerontology 19, 447–459. https://doi.org/10.1007/s10522-018-9763-7.

Callejas, D., Mann, C.J., Ayuso, E., Lage, R., Grifoll, I., Roca, C., Andaluz, A., Ruiz–de Gopegui, R., Montané, J., Muñoz, S., Ferre, T., Haurigot, V., Zhou, S., Ruberte, J., Mingozzi, F., High, K.A., Garcia, F., Bosch, F., 2013. Treatment of diabetes and long–term survival after insulin and glucokinase gene therapy. Diabetes 62, 1718–1729. https://doi.org/10.2337/db12–1113.

Carty, N., Nash, K.R., Brownlow, M., Cruite, D., Wilcock, D., Selenica, M.L., Lee, D.C., Gordon, M.N., Morgan, D., 2013. Intracranial injection of AAV expressing NEP but not IDE reduces amyloid pathology in APP + PS1 transgenic mice. PLoS One 8, e59626. https://doi.org/10.1371/journal.pone.0059626.

Carvalho, M., Martins, A.P., Sepodes, B., 2019. Hurdles in gene therapy regulatory approval: a retrospective analysis of European Marketing Authorization Applications. Drug Discov. Today 24, 823–828. https://doi.org/10.1016/j.drudis.2018.12.007.

Carvalho, M., Sepodes, B., Martins, A.P., 2017. Regulatory and scientific advancements in gene therapy: state–of–the–art of clinical applications and of the supporting european regulatory framework. Front. Med. (Lausanne) 4, 182. https://doi.org/10.3389/fmed.2017.00182.

Chaanine, A.H., Kalman, J., Hajjar, R.J., 2010. Cardiac gene therapy. Semin. Thorac. Cardiovasc. Surg. 22, 127–139. https://doi.org/10.1053/j.semtcvs.2010.09.009.

Chai, X., Kong, W., Liu, L., Yu, W., Zhang, Z., Sun, Y., 2014. A viral vector expressing hypoxia–inducible factor 1 alpha inhibits hippocampal neuronal apoptosis. Neural Regen. Res. 9, 1145–1153. https://doi.org/10.4103/1673–5374.135317.

Chakraborty, C., Sharma, A.R., Sharma, G., Doss, C.G.P., Lee, S.S., 2017. Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine. Mol. Ther. Nucleic Acids 8, 132–143. https://doi.org/10.1016/j.omtn.2017.06.005.

Cheikhi, A., Barchowsky, A., Sahu, A., Shinde, S.N., Pius, A., Clemens, Z.J., Li, H., Kennedy, C.A., Hoeck, J.D., Franti, M., Ambrosio, F., 2019. Klotho: an elephant in aging research. J. Gerontol. A Biol. Sci. Med. Sci. 74, 1031–1042. https://doi.org/10.1093/gerona/glz061.

Chen, C.D., Zeldich, E., Li, Y., Yuste, A., Abraham, C.R., 2018. Activation of the anti–aging and cognition–enhancing gene Klotho by CRISPR-dCas9 transcriptional effector complex. J. Mol. Neurosci. 64, 175–184. https://doi.org/10.1007/s12031-017-1011-0.

Chen, Y., Fu, A.K.Y., Ip, N.Y., 2019. Synaptic dysfunction in Alzheimer's disease: mechanisms and therapeutic strategies. Pharmacol. Ther. 195, 186–198. https://doi.org/10.1016/j.pharmthera.2018.11.006.

Christine, C.W., Bankiewicz, K.S., Van Laar, A.D., Richardson, R.M., Ravina, B., Kells, A.P., Boot, B., Martin, A.J., Nutt, J., Thompson, M.E., Larson, P.S., 2019. Magnetic resonance imaging–guided phase 1 trial of putaminal AADC gene therapy for Parkinson's disease. Ann. Neurol. 85, 704–714. https://doi.org/10.1002/ana.25450.

Colella, P., Ronzitti, G., Mingozzi, F., 2017. Emerging issues in AAV–mediated in vivo gene therapy. Mol. Ther. Methods Clin. Dev. 8, 87–104. https://doi.org/10.1016/j.omtm.2017.11.007.

Consitt, L.A., Clark, B.C., 2018. The vicious cycle of myostatin signaling in sarcopenic obesity: myostatin role in skeletal muscle growth, insulin signaling and implications for clinical trials. J. Frailty Aging 7, 21–27. https://doi.org/10.14283/jfa.2017.33.

Cubinková, V., Valachová, B., Brezováková, V., Szabó, R., Zimová, I., Kostecká, Z., Jadhav, S., 2017. Next generation tau models in Alzheimer's disease research – virus based gene delivery systems. Acta Virol. 61, 13–21. https://doi.org/10.4149/av_2017_01_13.

David, R.M., Doherty, A.T., 2017. Viral vectors: the road to reducing genotoxicity. Toxicol. Sci. 155, 315–325. https://doi.org/10.1093/toxsci/kfw220.

Distefano, G., Goodpaster, B.H., 2018. Effects of exercise and aging on skeletal muscle. Cold Spring Harb. Perspect. Med. 8https://doi.org/10.1101/cshperspect.a029785. pii: a029785.

Dunbar, C.E., High, K.A., Joung, J.K., Kohn, D.B., Ozawa, K., Sadelain, M., 2018. Gene therapy comes of age. Science 359 pii: eaan4672. https://doi.org/0.1126/science.aan4672.

Dushenkov, A., Jungsuwadee, P., 2019. Chimeric antigen receptor T-cell therapy: foundational science and clinical knowledge for pharmacy practice. J. Oncol. Pharm. Pract. 25, 1217–1225. https://doi.org/10.1177/1078155219836480.

Elsner, M., Terbish, T., Jörns, A., Naujok, O., Wedekind, D., Hedrich, H.J., Lenzen, S., 2012. Reversal of diabetes through gene therapy of diabetic rats by hepatic insulin expression via lentiviral transduction. Mol. Ther. 20, 918–926. https://doi.org/10.1038/mt.2012.8.

Flynn, R., Qian, K., Tang, C., Dronadula, N., Buckler, J.M., Jiang, B., Wen, S., Dichek, H.L., Dichek, D.A., 2011. Expression of apolipoprotein A-I in rabbit carotid endothelium protects against atherosclerosis. Mol. Ther. 19, 1833–1841. https://doi.org/10.1038/mt.2011.133.

Friedmann, T., Roblin, R., 1972. Gene therapy for human genetic disease? Science 175, 949–955. https://doi.org/10.1126/science.175.4025.949.

Gaj, T., Gersbach, C.A., Barbas 3rd, C.F., 2013. ZFN, TALEN, and CRISPR/Cas–based methods for genome engineering. Trends Biotechnol. 31, 397–405. https://doi.org/10.1016/j.tibtech.2013.04.004.

Gan, S.U., Notaridou, M., Fu, Z.Y., Lee, K.O., Sia, K.C., Nathwani, A.C., Della Peruta, M., Calne, R.Y., 2016. Correction of murine diabetic hyperglycaemia with a single systemic administration of an AAV2/8 vector containing a novel codon optimized human insulin gene. Curr. Gene Ther. 16, 65–72.

Ginn, S.L., Amaya, A.K., Alexander, I.E., Edelstein, M., Abedi, M.R., 2018. Gene therapy clinical trials worldwide to 2017: an update. J. Gene Med. 20, e3015. https://doi.org/10.1002/jgm.3015.

Glorioso, J.C., Lemoine, N., 2017. Gene therapy–from small beginnings to where we are now. Gene Ther. 24, 495–496. https://doi.org/10.1038/gt.2017.23.

Glover, D.J., Lipps, H.J., Jans, D.A., 2005. Towards safe, non–viral therapeutic gene expression in humans. Nat. Rev. Genet. 6, 299–310. https://doi.org/10.1038/nrg1577.

Goswami, R., Subramanian, G., Silayeva, L., Newkirk, I., Doctor, D., Chawla, K., Chattopadhyay, S., Chandra, D., Chilukuri, N., Betapudi, V., 2019. Gene therapy leaves a vicious cycle. Front. Oncol. 9, 297. https://doi.org/10.3389/fonc.2019.00297.

György, B., Maguire, C.A., 2018. Extracellular vesicles: nature's nanoparticles for improving gene transfer with adeno–associated virus vectors. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 10, e1488. https://doi.org/10.1002/wnan.148.

Hacein-Bey-Abina, S., Hauer, J., Lim, A., Picard, C., Wang, G.P., Berry, C.C., Martinache, C., Rieux-Laucat, F., Latour, S., Belohradsky, B.H., Leiva, L., Sorensen, R., Debré, M., Casanova, J.L., Blanche, S., Durandy, A., Bushman, F.D., Fischer, A., Cavazzana-Calvo, M., 2010. Efficacy of gene therapy for X-linked severe combined immunodeficiency. New. Eng. J. Med. 363, 355–364.

Hafez, D.M., Huang, J.Y., Richardson, J.C., Masliah, E., Peterson, D.A., Marr, R.A., 2012. F–spondin gene transfer improves memory performance and reduces amyloid–β levels in mice. Neuroscience. 223, 465–472. https://doi.org/10.1016/j.neuroscience.2012.07.038.

Hampson, D.R., Hooper, A.W.M., Niibori, Y., 2019. The application of adeno–associated viral vector gene therapy to the treatment of fragile X syndrome. Brain Sci. 9https://doi.org/10.3390/brainsci9020032. pii: E32.

Han, J., McLane, B., Kim, E.H., Yoon, J.W., Jun, H.S., 2011. Remission of diabetes by insulin gene therapy using a hepatocyte–specific and glucose–responsive synthetic promoter. Mol. Ther. 19, 470–478. https://doi.org/10.1038/mt.2010.255.

Harries, L.W., 2019. RNA Biology provides new therapeutic targets for human disease. Front. Genet. 10, 205. https://doi.org/10.3389/fgene.2019.00205.

Helal, N.A., Osami, A., Helmy, A., McDonald, T., Shaaban, L.A., Nounou, M.I., 2017. Non–viral gene delivery systems: hurdles for bench–to–bedside transformation. Pharmazie. 72, 627–693. https://doi.org/10.1691/ph.2017.7092.

Hoy, S.M., 2019. Onasemnogene abeparvovec: first global approval. Drugs 79, 1255–1262. https://doi.org/10.1007/s40265-019-01162-5.

Hu, S., Chen, C., Sheng, J., Sun, Y., Cao, X., Qiao, J., 2010. Enhanced muscle growth by plasmid–mediated delivery of myostatin propeptide. J. Biomed. Biotechnol. 862591. https://doi.org/10.1155/2010/862591.

Hudry, E., Vandenberghe, L.H., 2019. Therapeutic AAV gene transfer to the nervous system: a clinical reality. Neuron 101, 839–862. https://doi.org/10.1016/j.neuron.2019.02.017.

Hussain, W., Mahmood, T., Hussain, J., Ali, N., Shah, T., Qayyum, S., Khan, I., 2019. CRISPR/Cas system: a game changing genome editing technology, to treat human genetic diseases. Gene. 685, 70–75. https://doi.org/10.1016/j.gene.2018.10.072.

Ishikawa, K., Weber, T., Hajjar, R.J., 2018. Human cardiac gene therapy. Circ. Res. 123, 601–613. https://doi.org/10.1161/CIRCRESAHA.118.311587.

Ittner, L.M., Klugmann, M., Ke, Y.D., 2019. Adeno–associated virus–based Alzheimer's disease mouse models and potential new therapeutic avenues. Br. J. Pharmacol. 176, 3649–3665. https://doi.org/10.1111/bph.14637.

Iwata, N., Sekiguchi, M., Hattori, Y., Takahashi, A., Asai, M., Ji, B., Higuchi, M., Staufenbiel, M., Muramatsu, S., Saido, T.C., 2013. Global brain delivery of neprilysin gene by intravascular administration of AAV vector in mice. Sci. Rep. 3, 1472. https://doi.org/10.1038/srep01472.

Jaén, M.L., Vilà, L., Elias, I., Jimenez, V., Rodó, J., Maggioni, L., Ruiz–de Gopegui, R., Garcia, M., Muñoz, S., Callejas, D., Ayuso, E., Ferré, T., Grifoll, I., Andaluz, A., Ruberte, J., Haurigot, V., Bosch, F., 2017. Long-term efficacy and safety of insulin and glucokinase gene therapy for diabetes: 8-year follow–up in dogs. Mol. Ther. Methods Clin. Dev. 6, 1–7. https://doi.org/10.1016/j.omtm.2017.03.008.

Jeevanandam, J., Pal, K., Danquah, M.K., 2019. Virus-like nanoparticles as a novel delivery tool in gene therapy. Biochimie 157, 38–47. https://doi.org/10.1016/j.biochi.2018.11.001.

Jiang, J.G., Shen, G.F., Li, J., Qiao, C., Xiao, B., Yan, H., Wang, D.W., Xiao, X., 2017. Adeno-associated virus-mediated expression of myostatin propeptide improves the growth of skeletal muscle and attenuates hyperglycemia in db/db mice. Gene Ther.

24, 167–175. https://doi.org/10.1038/gt.2016.85.
Justice, J.N., Niedernhofer, L., Robbins, P.D., Aroda, V.R., Espeland, M.A., Kritchevsky, S.B., Kuchel, G.A., Barzilai, N., 2018. Development of clinical trials to extend healthy lifespan. Cardiovasc Endocrinol Metab. 7, 80–83. https://doi.org/10.1097/XCE.0000000000000159.
Kaemmerer, W.F., 2018. How will the field of gene therapy survive its success? Bioeng. Transl. Med. 3, 166–177. https://doi.org/10.1002/btm2.10090.
Kang, M.K., Park, N.H., 2007. Extension of cell life span using exogenous telomerase. Methods Mol. Biol. 371, 151–165. https://doi.org/10.1007/978–1–59745–361–5_12.
Karimian, A., Azizian, K., Parsian, H., Rafieian, S., Shafiei-Irannejad, V., Kheyrollah, M., Yousefi, M., Majidinia, M., Yousefi, B., 2019. CRISPR/Cas9 technology as a potent molecular tool for gene therapy. J. Cell. Physiol. 234, 12267–12277. https://doi.org/10.1002/jcp.27972.
Kaufmann, K.B., Büning, H., Galy, A., Schambach, A., Grez, M., 2013. Gene therapy on the move. EMBO Mol. Med. 5, 1642–1661. https://doi.org/10.1002/emmm.201202287.
Khadempar, S., Familghadakchi, S., Motlagh, R.A., Farahani, N., Dashtiahangar, M., Rezaei, H., Gheibi Hayat, S.M., 2019. CRISPR–Cas9 in genome editing: its function and medical applications. J. Cell. Physiol. 234, 5751–5761. https://doi.org/10.1002/jcp.27476.
Khatiwala, R.V., Zhang, S., Li, X., Devejian, N., Bennett, E., Cai, C., 2018. Inhibition of p16INK4A to rejuvenate aging human cardiac progenitor cells via the upregulation of anti–oxidant and NFkB signal pathways. Stem Cell Rev. 14, 612–625. https://doi.org/10.1007/s12015–018–815–z.
Kim, J.H., Hwang, K.H., Park, K.S., Kong, I.D., Cha, S.K., 2015. Biological role of anti–aging protein Klotho. J. Lifestyle Med. 5, 1–6. https://doi.org/10.15280/jlm.2015.5.1.1.
Kim, M.Y., Jo, S.H., Park, J.M., Kim, T.H., Im, S.S., Ahn, Y.H., 2013. Adenovirus–mediated overexpression of Tcfe3 ameliorates hyperglycaemia in a mouse model of diabetes by upregulating glucokinase in the liver. Diabetologia 56, 635–643. https://doi.org/10.1007/s00125–012–2807–7.
Konopka, A.R., Miller, B.F., 2019. Taming expectations of metformin as a treatment to extend healthspan. Geroscience 41, 101–108. https://doi.org/10.1007/s11357-019-00057-3.
Kumar, S.R., Markusic, D.M., Biswas, M., High, K.A., Herzog, R.W., 2016. Clinical development of gene therapy: results and lessons from recent successes. Mol. Ther. Methods Clin. Dev. 3, 16034. https://doi.org/10.1038/mtm.2016.34.
Larrick, J.W., Mendelsohn, A.R., 2015. Telomerase redux: ready for prime time? Rejuvenation Res. 18, 185–187. https://doi.org/10.1089/rej.2015.1695.
Leão, R., Apolónio, J.D., Lee, D., Figueiredo, A., Tabori, U., Castelo–Branco, P., 2018. Mechanisms of human telomerase reverse transcriptase (hTERT) regulation: clinical impacts in cancer. J. Biomed. Sci. 25, 22. https://doi.org/10.1186/s12929–018–0422–8.
Lee, C.S., Bishop, E.S., Zhang, R., Yu, X., Farina, E.M., Yan, S., Zhao, C., Zheng, Z., Shu, Y., Wu, X., Lei, J., Li, Y., Zhang, W., Yang, C., Wu, K., Wu, Y., Ho, S., Athiviraham, A., Lee, M.J., Wolf, J.M., Reid, R.R., He, T.C., 2017. Adenovirus–mediated gene delivery: potential applications for gene and cell–based therapies in the new era of personalized medicine. Genes Dis. 4, 43–63. https://doi.org/10.1016/j.gendis.2017.04.001.
LeWitt, P.A., Rezai, A.R., Leehey, M.A., Ojemann, S.G., Flaherty, A.W., Eskandar, E.N., Kostyk, S.K., Thomas, K., Sarkar, A., Siddiqui, M.S., Tatter, S.B., Schwalb, J.M., Poston, K.L., Henderson, J.M., Kurlan, R.M., Richard, I.H., Van Meter, L., Sapan, C.V., During, M.J., Kaplitt, M.G., Feigin, A., 2011. AAV2–GAD gene therapy for advanced Parkinson's disease: a double–blind, sham–surgery controlled, randomised trial. Lancet Neurol. 10, 309–319. https://doi.org/10.1016/S1474–4422(11)70039–4.
Lin, J.Y., Xie, C.L., Zhang, S.F., Yuan, W., Liu, Z.G., 2017. Current experimental studies of gene therapy in Parkinson's disease. Front. Aging Neurosci. 9, 126. https://doi.org/10.3389/fnagi.2017.00126.
Lino, C.A., Harper, J.C., Carney, J.P., Timlin, J.A., 2018. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 25, 1234–1257. https://doi.org/10.1080/10717544.2018.1474964.
López-Otín, C., Galluzzi, L., Freije, J.M.P., Madeo, F., Kroemer, G., 2016. Metabolic control of longevity. Cell 166, 802–821. https://doi.org/10.1016/j.cell.2016.07.031.
Lotfinia, M., Abdollahpour–Alitappeh, M., Hatami, B., Zali, M.R., Karimipoor, M., 2019. Adeno–associated virus as a gene therapy vector: strategies to neutralize the neutralizing antibodies. Clin. Exp. Med. 19, 289–298. https://doi.org/10.1007/s10238–019–00557–8.
Madav, Y., Wairkar, S., Prabhakar, B., 2019. Recent therapeutic strategies targeting beta amyloid and tauopathies in Alzheimer's disease. Brain Res. Bull. 146, 171–184. https://doi.org/10.1016/j.brainresbull.2019.01.004.
Maeder, M.L., Gersbach, C.A., 2016. Genome–editing technologies for gene and cell therapy. Mol. Ther. 24, 430–446. https://doi.org/10.1038/mt.2016.10.
Marr, R.A., Rockenstein, E., Mukherjee, A., Kindy, M.S., Hersh, L.B., Gage, F.H., Verma, I.M., Masliah, E., 2003. Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. J. Neurosci. 23, 1992–1996.
Mas, A., Montané, J., Anguela, X.M., Muñoz, S., Douar, A.M., Riu, E., Otaegui, P., Bosch, F., 2006. Reversal of type 1 diabetes by engineering a glucose sensor in skeletal muscle. Diabetes 55, 1546–1553. https://doi.org/10.2337/db05–1615.
Mason, D., Chen, Y.Z., Krishnan, H.V., Sant, S., 2015. Cardiac gene therapy: recent advances and future directions. J. Control. Release 215, 101–111. https://doi.org/10.1016/j.jconrel.2015.08.001.
McGregor, G., Harvey, J., 2018. Food for thought: leptin regulation of hippocampal function and its role in Alzheimer's disease. Neuropharmacology 136, 298–306. https://doi.org/10.1016/j.neuropharm.2017.09.038.
Mendelsohn, A.R., Larrick, J.W., 2012. Ectopic expression of telomerase safely increases health span and life span. Rejuvenation Res. 15, 435–438. https://doi.org/10.1089/rej.2012.1359.
Misra, A., 2018. Recent gene delivery patents for treatment of atherosclerosis. Pharm. Pat. Anal. 7, 103–105. https://doi.org/10.4155/ppa–2018–0006.
Morine, K.J., Bish, L.T., Pendrak, K., Sleeper, M.M., Barton, E.R., Sweeney, H.L., 2010. Systemic myostatin inhibition via liver–targeted gene transfer in normal and dystrophic mice. PLoS One 5, e9176. https://doi.org/10.1371/journal.pone.0009176.
Murphy, S.R., Chang, C.C., Dogbevia, G., Bryleva, E.Y., Bowen, Z., Hasan, M.T., Chang, T.Y., 2013. Acat1 knockdown gene therapy decreases amyloid–β in a mouse model of Alzheimer's disease. Mol. Ther. 21, 1497–1506. https://doi.org/10.1038/mt.2013.118.
Nakagami, H., Morishita, R., 2009. Gene therapy for anti–aging medicine. Nihon Rinsho. 67, 1407–1411.
Nakamori, M., Junn, E., Mochizuki, H., Mouradian, M.M., 2019. Nucleic acid–based therapeutics for Parkinson's disease. Neurotherapeutics 16, 287–298. https://doi.org/10.1007/s13311–019–00714–7.
Naldini, L., 2015. Gene therapy returns to centre stage. Nature 526, 351–360. https://doi.org/10.1038/nature15818.
Naso, M.F., Tomkowicz, B., Perry 3rd, W.L., Strohl, W.R., 2017. Adeno–associated virus (AAV) as a vector for gene therapy. BioDrugs 31, 317–334. https://doi.org/10.1007/s40259–017–0234–5.
Nayerossadat, N., Maedeh, T., Ali, P.A., 2012. Viral and nonviral delivery systems for gene delivery. Adv. Biomed. Res. 1, 27. https://doi.org/10.4103/2277–9175.98152.
Niethammer, M., Tang, C.C., LeWitt, P.A., Rezai, A.R., Leehey, M.A., Ojemann, S.G., Flaherty, A.W., Eskandar, E.N., Kostyk, S.K., Sarkar, A., Siddiqui, M.S., Tatter, S.B., Schwalb, J.M., Poston, K.L., Henderson, J.M., Kurlan, R.M., Richard, I.H., Sapan, C.V., Eidelberg, D., During, M.J., Kaplitt, M.G., Feigin, A., 2017. Long–term follow–up of a randomized AAV2–GAD gene therapy trial for Parkinson's disease. JCI Insight 2, e90133. https://doi.org/10.1172/jci.insight.90133.
O'Connor, D.M., Boulis, N.M., 2015. Gene therapy for neurodegenerative diseases. Trends Mol. Med. 21, 504–512. https://doi.org/10.1016/j.molmed.2015.06.001.
Palfi, S., Gurruchaga, J.M., Lepetit, H., Howard, K., Ralph, G.S., Mason, S., Gouello, G., Domenech, P., Buttery, P.C., Hantraye, P., Tuckwell, N.J., Barker, R.A., Mitrophanous, K.A., 2018. Long–term follow–up of a phase I/II study of ProSavin, a lentiviral vector gene therapy for Parkinson's disease. Hum. Gene Ther. Clin. Dev. 29, 148–155. https://doi.org/10.1089/humc.2018.081.
Palfi, S., Gurruchaga, J.M., Ralph, G.S., Lepetit, H., Lavisse, S., Buttery, P.C., Watts, C., Miskin, J., Kelleher, M., Deeley, S., Iwamuro, H., Lefaucheur, J.P., Thiriez, C., Fenelon, G., Lucas, C., Brugières, P., Gabriel, I., Abhay, K., Drouot, X., Tani, N., Kas, A., Ghaleb, B., Le Corvoisier, P., Dolphin, P., Breen, D.P., Mason, S., Guzman, N.V., Mazarakis, N.D., Radcliffe, P.A., Harrop, R., Kingsman, S.M., Rascol, O., Naylor, S., Barker, R.A., Hantraye, P., Remy, P., Cesaro, P., Mitrophanous, K.A., 2014. Long–term safety and tolerability of ProSavin, a lentiviral vector–based gene therapy for Parkinson's disease: a dose escalation, open–label, phase 1/2 trial. Lancet. 383, 1138–1146. https://doi.org/10.1016/S0140–6736(13)61939–X.
Paroni, G., Panza, F., De Cosmo, S., Greco, A., Seripa, D., Mazzoccoli, G., 2019. Klotho at the edge of Alzheimer's disease and senile depression. Mol. Neurobiol. 56, 1908–1920. https://doi.org/10.1007/s12035–018–1200–z.
Pascual-Lucas, M., Viana da Silva, S., Di Scala, M., Garcia–Barroso, C., González–Aseguinolaza, G., Mulle, C., Alberini, C.M., Cuadrado–Tejedor, M., Garcia–Osta, A., 2014. Insulin–like growth factor 2 reverses memory and synaptic deficits in APP transgenic mice. EMBO Mol. Med. 6, 1246–1262. https://doi.org/10.15252/emmm.201404228.
Pérez–González, R., Alvira–Botero, M.X., Robayo, O., Antequera, D., Garzón, M., Martín–Moreno, A.M., Brera, B., de Ceballos, M.L., Carro, E., 2014. Leptin gene therapy attenuates neuronal damages evoked by amyloid–β and rescues memory deficits in APP/PS1 mice. Gene Ther. 21, 298–308. https://doi.org/10.1038/gt.2013.85.
Phillips, N., Kay, M.A., 2014. Characterization of vector–based delivery of neurogenin–3 in murine diabetes. Hum. Gene Ther. 25, 651–661. https://doi.org/10.1089/hum.2013.206.
Piskovatska, V., Stefanyshyn, N., Storey, K.B., Vaiserman, A.M., Lushchak, O., 2019. Metformin as a geroprotector: experimental and clinical evidence. Biogerontology 20, 33–48. https://doi.org/10.1007/s10522-018-9773-5.
Qiao, C., Li, J., Jiang, J., Zhu, X., Wang, B., Li, J., Xiao, X., 2008. Myostatin propeptide gene delivery by adeno–associated virus serotype 8 vectors enhances muscle growth and ameliorates dystrophic phenotypes in mdx mice. Hum. Gene Ther. 19, 241–254. https://doi.org/10.1089/hum.2007.159.
Rafii, M.S., Baumann, T.L., Bakay, R.A., Ostrove, J.M., Siffert, J., Fleisher, A.S., Herzog, C.D., Barba, D., Pay, M., Salmon, D.P., Chu, Y., Kordower, J.H., Bishop, K., Keator, D., Potkin, S., Bartus, R.T., 2014. A phase1 study of stereotactic gene delivery of AAV2–NGF for Alzheimer's disease. Alzheimers Dement. 10, 571–581. https://doi.org/10.1016/j.jalz.2013.09.004.
Rafii, M.S., Tuszynski, M.H., Thomas, R.G., Barba, D., Brewer, J.B., Rissman, R.A., Siffert, J., Aisen, P.S., 2018. AAV2–NGF Study Team. Adeno–associated viral vector (serotype 2)–nerve growth factor for patients with Alzheimer disease: a randomized clinical trial. JAMA Neurol. 75, 834–841. https://doi.org/10.1001/jamaneurol.2018.0233.
Ragusa, A., García, I., Penadés, S., 2007. Nanoparticles as nonviral gene delivery vectors. IEEE Trans. Nanobiosci. 6, 319–330.
Ramamoorth, M., Narvekar, A., 2015. Non viral vectors in gene therapy – an overview. J. Clin. Diagn. Res. 9, GE01–6. https://doi.org/10.7860/JCDR/2015/10443.5394.
Raper, S.E., Chirmule, N., Lee, F.S., Wivel, N.A., Bagg, A., Gao, G.P., Wilson, J.M., Batshaw, M.L., 2003. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol. Genet. Metab. 80, 148–158.
Renteln, M., 2018. A synthetic mitochondrial–based vector for therapeutic purposes. Med. Hypotheses 117, 28–30. https://doi.org/10.1016/j.mehy.2018.05.014.

Revilla, S., Ursulet, S., Álvarez–López, M.J., Castro–Freire, M., Perpiñá, U., García–Mesa, Y., Bortolozzi, A., Giménez–Llort, L., Kaliman, P., Cristòfol, R., Sarkis, C., Sanfeliu, C., 2014. Lenti–GDNF gene therapy protects against Alzheimer's disease–like neuropathology in 3xTg–AD mice and MC65 cells. CNS Neurosci. Ther. 20, 961–972. https://doi.org/10.1111/cns.12312.

Rodrigues, G.A., Shalaev, E., Karami, T.K., Cunningham, J., Slater, N.K.H., Rivers, H.M., 2018. Pharmaceutical development of AAV-based gene therapy products for the eye. Pharm. Res. 36, 29. https://doi.org/10.1007/s11095-018-2554-7.

Rossor, A.M., Reilly, M.M., Sleigh, J.N., 2018. Antisense oligonucleotides and other genetic therapies made simple. Pract. Neurol. 18, 126–131. https://doi.org/10.1136/practneurol–2017–001764.

Rufer, N., Migliaccio, M., Antonchuk, J., Humphries, R.K., Roosnek, E., Lansdorp, P.M., 2001. Transfer of the human telomerase reverse transcriptase (TERT) gene into T lymphocytes results in extension of replicative potential. Blood 98, 597–603. https://doi.org/10.1182/blood.v98.3.597.

Sadelain, M., Papapetrou, E.P., Bushman, F.D., 2011. Safe harbours for the integration of new DNA in the human genome. Nat. Rev. Cancer 12, 51–58. https://doi.org/10.1038/nrc3179.

Scimia, M.C., Gumpert, A.M., Koch, W.J., 2014. Cardiovascular gene therapy for myocardial infarction. Expert Opin. Biol. Ther. 14, 183–195. https://doi.org/10.1517/14712598.2014.866085.

Shay, J.W., Wright, W.E., 2005. Use of telomerase to create bioengineered tissues. Ann. N. Y. Acad. Sci. 1057, 479–491. https://doi.org/10.1196/annals.1356.037.

Shay, J.W., Wright, W.E., 2019. Telomeres and telomerase: three decades of progress. Nat. Rev. Genet. 20, 299–309. https://doi.org/10.1038/s41576–019–0099–1.

Singh, V., Braddick, D., Dhar, P.K., 2017. Exploring the potential of genome editing CRISPR–Cas9 technology. Gene 599, 1–18. https://doi.org/10.1016/j.gene.2016.11.008.

Skirbekk, V.F., Staudinger, U.M., Cohen, J.E., 2019. How to measure population aging? The answer is less than obvious: a review. Gerontology 65, 136–144. https://doi.org/10.1159/000494025.

Spencer, B., Marr, R.A., Rockenstein, E., Crews, L., Adame, A., Potkar, R., Patrick, C., Gage, F.H., Verma, I.M., Masliah, E., 2008. Long–term neprilysin gene transfer is associated with reduced levels of intracellular Abeta and behavioral improvement in APP transgenic mice. BMC Neurosci. 9, 109. https://doi.org/10.1186/1471–2202–9–109.

Stone, D., 2010. Novel viral vector systems for gene therapy. Viruses. 2, 1002–1007. https://doi.org/10.3390/v2041002.

Sudhakar, V., Richardson, R.M., 2019. Gene therapy for neurodegenerative diseases. Neurotherapeutics 16, 166–175. https://doi.org/10.1007/s13311–018–00694–0.

Tafuro, S., Ayuso, E., Zacchigna, S., Zentilin, L., Moimas, S., Dore, F., Giacca, M., 2009. Inducible adeno–associated virus vectors promote functional angiogenesis in adult organisms via regulated vascular endothelial growth factor expression. Cardiovasc. Res. 83, 663–671. https://doi.org/10.1093/cvr/cvp152.

Tatiparti, K., Sau, S., Kashaw, S.K., Iyer, A.K., 2017. siRNA Delivery strategies: a comprehensive review of recent developments. Nanomaterials Basel (Basel) 7. https://doi.org/10.3390/nano7040077.

Turner, K.J., Vasu, V., Griffin, D.K., 2019. Telomere biology and human phenotype. Cells 8https://doi.org/10.3390/cells8010073. pii: E73.

Tuszynski, M.H., Thal, L., Pay, M., Salmon, D.P., U, H.S, Bakay, R., Patel, P., Blesch, A., Vahlsing, H.L., Ho, G., Tong, G., Potkin, S.G., Fallon, J., Hansen, L., Mufson, E.J., Kordower, J.H., Gall, C., Conner, J., 2005. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nat. Med. 11, 551–555. https://doi.org/10.1038/nm1239.

Vaiserman, A., Lushchak, O., 2017. Implementation of longevity–promoting supplements and medications in public health practice: achievements, challenges and future perspectives. J. Transl. Med. 15, 160. https://doi.org/10.1186/s12967–017–1259–8.

Vaiserman, A.M., Marotta, F., 2016. Longevity–promoting pharmaceuticals: is it a time for implementation? Trends Pharmacol. Sci. 37, 331–333. https://doi.org/10.1016/j.tips.2016.02.003.

van Haasteren, J., Hyde, S.C., Gill, D.R., 2018. Lessons learned from lung and liver in–vivo gene therapy: implications for the future. Expert Opin. Biol. Ther. 18, 959–972. https://doi.org/10.1080/14712598.2018.1506761.

Van-Assche, T., Huygelen, V., Crabtree, M.J., Antoniades, C., 2011. Gene therapy targeting inflammation in atherosclerosis. Curr. Pharm. Des. 17, 4210–4223. https://doi.org/10.2174/138161211798764799.

Vargas, J.E., Chicaybam, L., Stein, R.T., Tanuri, A., Delgado-Cañedo, A., Bonamino, M.H., 2016. Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives. J. Transl. Med. 14, 288. https://doi.org/10.1186/s12967-016-1047-x.

Wacker, B.K., Dronadula, N., Bi, L., Stamatikos, A., Dichek, D.A., 2018. Apo A–I (apolipoprotein A–I) vascular gene therapy provides durable protection against atherosclerosis in hyperlipidemic rabbits. Arterioscler. Thromb. Vasc. Biol. 38, 206–217. https://doi.org/10.1161/ATVBAHA.117.309565.

Wacker, B.K., Dronadula, N., Zhang, J., Dichek, D.A., 2017. Local vascular gene therapy with apolipoprotein A–I to promote regression of atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 37, 316–327. https://doi.org/10.1161/ATVBAHA.116.308258.

Wang, D., Tai, P.W.L., Gao, G., 2019. Adeno–associated virus vector as a platform for gene therapy delivery. Nat. Rev. Drug Discov. 18, 358–378. https://doi.org/10.1038/s41573–019–0012–9.

Wang, Q.H., Wang, Y.R., Zhang, T., Jiao, S.S., Liu, Y.H., Zeng, F., Li, J., Yao, X.Q., Zhou, H.D., Zhou, X.F., Wang, Y.J., 2016. Intramuscular delivery of p75NTR ectodomain by an AAV vector attenuates cognitive deficits and Alzheimer's disease–like pathologies in APP/PS1 transgenic mice. J. Neurochem. 138, 163–173. https://doi.org/10.1111/jnc.13616.

WareJoncas, Z., Campbell, J.M., Martínez–Gálvez, G., Gendron, W.A.C., Barry, M.A., Harris, P.C., Sussman, C.R., Ekker, S.C., 2018. Precision gene editing technology and applications in nephrology. Nat. Rev. Nephrol. 14, 663–677. https://doi.org/10.1038/s41581–018–0047–x.

Wirth, T., Parker, N., Ylä–Herttuala, S., 2013. History of gene therapy. Gene 525, 162–169. https://doi.org/10.1016/j.gene.2013.03.137.

Wong, L.F., Goodhead, L., Prat, C., Mitrophanous, K.A., Kingsman, S.M., Mazarakis, N.D., 2006. Lentivirus-mediated gene transfer to the central nervous system: therapeutic and research applications. Hum. Gene Ther. 17, 1–9. https://doi.org/10.1089/hum.2006.17.1.

Xu, H., Li, Z., Si, J., 2014. Nanocarriers in gene therapy: a review. J. Biomed. Nanotechnol. 10, 3483–3507.

Yan, C., Quan, X.J., Feng, Y.M., 2019a. Nanomedicine for gene delivery for the treatment of cardiovascular diseases. Curr. Gene Ther. 19, 20–30. https://doi.org/10.2174/1566523218666181003125308.

Yan, H., Meng, J., Zhang, S., Zhuang, H., Song, Y., Xiao, X., Wang, D.W., Jiang, J., 2019b. Pretreatment of rAAV–mediated expression of myostatin propeptide lowers type 2 diabetes incidence in C57BL/6 mice on high–fat diet. Hum. Gene Ther. 30, 661–671. https://doi.org/10.1089/hum.2018.140.

Yarborough, M., Sharp, R.R., 2009. Public trust and research a decade later: what have we learned since Jesse Gelsinger's death? Mol. Genet. Metab. 97, 4–5. https://doi.org/10.1016/j.ymgme.2009.02.002.

Yin, H., Kanasty, R.L., Eltoukhy, A.A., Vegas, A.J., Dorkin, J.R., Anderson, D.G., 2014. Non–viral vectors for gene–based therapy. Nat. Rev. Genet. 15, 541–555. https://doi.org/10.1038/nrg3763.

Ylä-Herttuala, S., Bridges, C., Katz, M.G., Korpisalo, P., 2017. Angiogenic gene therapy in cardiovascular diseases: dream or vision? Eur. Heart J. 38, 1365–1371. https://doi.org/10.1093/eurheartj/ehw547.

You, S., Anitha, M., deSouza, S.M., Jia, D., Lu, X., Kozlowski, M., Olson, D.E., Srinivasan, S., Thulé, P.M., 2015. Hepatic insulin gene therapy prevents diabetic enteropathy in STZ–treated CD–1 mice. Mol. Ther. Methods Clin. Dev. 2, 15028. https://doi.org/10.1038/mtm.2015.28.

Yudoh, K., Matsuno, H., Nakazawa, F., Katayama, R., Kimura, T., 2001. Reconstituting telomerase activity using the telomerase catalytic subunit prevents the telomere shorting and replicative senescence in human osteoblasts. J. Bone Miner. Res. 16, 1453–1464. https://doi.org/10.1359/jbmr.2001.16.8.1453.

Zhang, Q., Li, N., Jiao, X., Qin, X., Kaur, R., Lu, X., Song, J., Wang, L., Wang, J., Niu, Q., 2014. Caspase–3 short hairpin RNAs: a potential therapeutic agent in neurodegeneration of aluminum–exposed animal model. Curr. Alzheimer Res. 11, 961–970.

Zhou, H.J., Zeng, C.Y., Yang, T.T., Long, F.Y., Kuang, X., Du, J.R., 2018. Lentivirus–mediated Klotho up–regulation improves aging–related memory deficits and oxidative stress in senescence–accelerated mouse prone–8 mice. Life Sci. 200, 56–62. https://doi.org/10.1016/j.lfs.2018.03.027.