

REVIEW

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Gene therapy for polygenic or complex diseases

Tingting Wu^{1,2}, Yu Hu^{1,2*} and Liang V. Tang^{1,2*}

Abstract

Gene therapy utilizes nucleic acid drugs to treat diseases, encompassing gene supplementation, gene replacement, gene silencing, and gene editing. It represents a distinct therapeutic approach from traditional medications and introduces novel strategies for genetic disorders. Over the past two decades, significant advancements have been made in the field of gene therapy, leading to the approval of various gene therapy drugs. Gene therapy was initially employed for treating genetic diseases and cancers, particularly monogenic conditions classified as orphan diseases due to their low prevalence rates; however, polygenic or complex diseases exhibit higher incidence rates within populations. Extensive research on the etiology of polygenic diseases has unveiled new therapeutic targets that offer fresh opportunities for their treatment. Building upon the progress achieved in gene therapy for monogenic diseases and cancers, extending its application to polygenic or complex diseases would enable targeting a broader range of patient populations. This review aims to discuss the strategies of gene therapy, methods of gene editing (mainly CRISPR-CAS9), and carriers utilized in gene therapy, and highlight the applications of gene therapy in polygenic or complex diseases focused on applications that have either entered clinical stages or are currently undergoing clinical trials.

Keywords Gene therapy, Polygenic diseases, Complex diseases, CRISPR-CAS9

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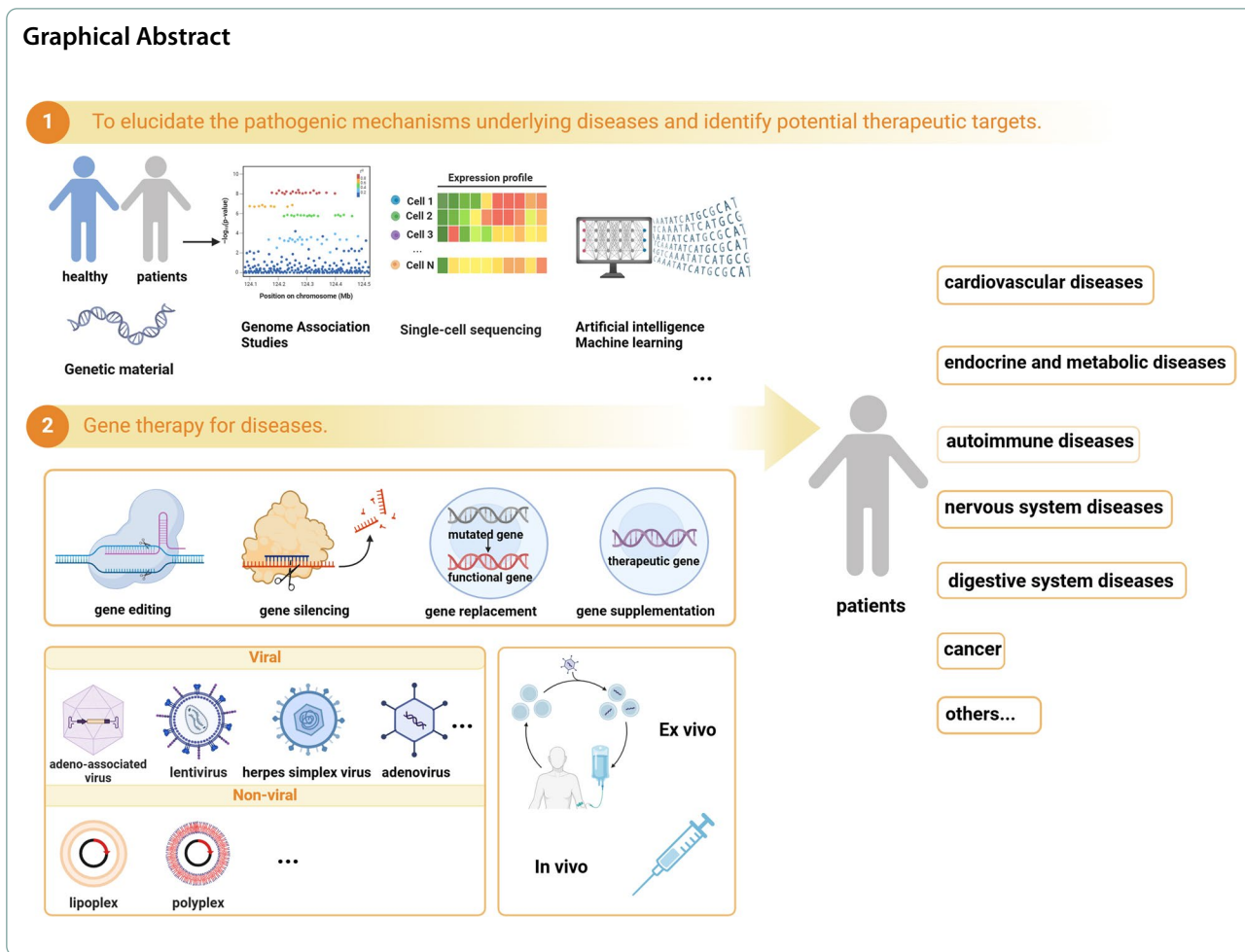
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Introduction

Gene therapy employs nucleic acid-based therapeutics to address human diseases encompassing gene replacement for defective genes in patients, silencing deleterious mutated genes within cells (using siRNA, miRNA or antisense oligonucleotides), overexpressing genes via gene supplementation, and rectifying the patient's genome through gene editing, thereby achieving the goal of disease treatment [1–5]. Gene therapy has emerged as a promising alternative for diseases unresponsive to conventional pharmaceutical interventions, showcasing remarkable potential in the treatment of various ailments, particularly those with hereditary origins. In contrast to traditional drugs, gene therapy represents a "causative therapeutic approach," aiming not only to provide transient symptom relief but also to achieve sustained expression of therapeutic genes, ultimately striving for long-term prevention, treatment, and even eradication of diseases. The first successful gene therapy, which is considered a milestone achievement, employs ex vivo gene therapy to treat severe combined immunodeficiency

(SCID) caused by adenosine deaminase (ADA) deficiency. This therapeutic approach involves the infusion of autologous T cells that have been genetically modified using a recombinant retrovirus carrying the ADA gene, aiming to restore the patient's immune function [6]. The initial focus of gene therapy was primarily directed towards monogenic diseases and cancer. Significant breakthroughs have been accomplished in the field of monogenic diseases, leading to successive approvals and market availability of gene therapy products. These involve a wide range of disorders affecting multiple systems, including hematological conditions (hemophilia A and B [7, 8], sickle cell anemia, β -thalassemia [9]), neurological disorders (spinal muscular atrophy [10], duchenne muscular dystrophy [11], aromatic L-amino acid decarboxylase deficiency [12]), and vision loss [13]. Moreover, with the advancement of gene sequencing technology, more associations between diseases and genes have been revealed. This enhanced understanding and research into the molecular pathological mechanisms underlying diseases have established a foundation for personalized gene

therapy. The success of gene therapy has also facilitated its expansion into the field of non-monogenic or complex diseases.

This review aims to discuss the strategies of gene therapy, methods of gene editing, and carriers utilized in gene therapy. Additionally, it will explore the application of gene therapy in non-monogenic or complex disorders such as cardiovascular diseases, neurodegenerative diseases, autoimmune diseases, and endocrine/metabolic disorders. The primary focus will be on applications that have advanced to clinical trials or are presently implemented within clinical settings.

Basic gene therapy strategies

Gene therapy involves two fundamental strategies [14]. Firstly, by integrating the drug gene into stem cells or precursor cells, it can be transmitted to the daughter cells after division. Secondly, through the utilization of non-integrating vectors, the drug gene can be transferred to quiescent or slowly dividing cells, thereby achieving sustained expression of the drug-gene throughout the cellular lifespan. The non-integrating approach becomes more applicable when the expression of transgenes in post-mitotic cells can serve therapeutic purposes.

Approaches to gene editing

The advancement of gene therapy is intricately linked to the support provided by gene editing technology. Currently, the commonly employed gene editing techniques encompass clustered regularly interspaced short palindromic repeats associated nuclease 9 (CRISPR-CAS9), transcription activator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs). CRISPR-CAS9 was initially discovered within the bacterial natural immune system and exploits the CRISPR-Cas system to precisely cleave the target DNA sequence by pairing Cas9 with specific gRNA. This enables genome modification through the cell's inherent repair mechanism. Notably, CRISPR-CAS9 represents a straightforward, highly efficient, and user-friendly gene editing technique [15, 16]. TALENs constitutes an artificial protein complex comprising transcriptional activation factors and nucleases that facilitate DNA cleavage for genome manipulation via cellular repair mechanisms [17]. ZFNs consist of zinc finger proteins responsible for precise recognition of DNA sequences coupled with nucleases that execute targeted DNA cleavage [18]. Additionally, there exist several innovative approaches to gene editing. The prime editing technique enables precise modifications of DNA sequences, including base substitutions, insertions, and deletions, without requiring double-stranded DNA breaks (DSBs) or donor DNA. This system comprises a Cas9 nickase fused with an engineered reverse

transcriptase and utilizes the prime editing guide RNA (pegRNA) to achieve targeted gene editing [19]. Base editing is a CRISPR-based technology that facilitates precise modifications to specific base pairs on DNA or RNA without inducing DSBs [20]. The Bridge RNAs direct programmable genome-editing system is based on a bispecific non-coding RNA expressed by the IS110 family of mobile genetic elements that enables the precise insertion, excision, or inversion of specific target DNA sequences [21].

Ex vivo and in vivo gene therapy

Ex vivo

For ex vivo applications, the process typically involves three steps: isolation of target cells from the patient's body, in vitro genetic engineering of these cells, and subsequent autologous transplantation to reintroduce the modified cells back into the patient's body. The modified target cells will continue to express gene of interest, thus achieving the goal of treatment. Due to the minimal patient harm and the knowledge gained from bone marrow transplantation, blood cells have emerged as the primary target cells, including hematopoietic stem cells and mature blood cells [22, 23]. The utilization of T lymphocytes as target cells has become predominant in the study of mature blood cells. In 1990, a clinical trial employed retroviral transduction of the ADA gene into T cells as a therapeutic approach for children with ADA-SCID, restoring their immune responses. Over two years, gene therapy demonstrated sustained expression of the ADA gene [24]. Over the past decade, chimeric antigen receptor (CAR)-T cell therapy has emerged as a highly promising immunotherapeutic approach for combating cancer. Engineered immune cells express antigen receptors capable of recognizing and eliminating tumor cells. Genetically modified immune cells are redirected towards tumor cells via chimeric antigen receptors (CARs), which reprogram the patient's T cells to effectively eradicate malignant neoplastic growth [25]. Although CAR is categorized as a form of cell therapy, it exhibits overlapping characteristics with gene therapy.

The American Food and Drug Administration (FDA) approved two gene therapies, Casgevy (exagamglogene autotemcel) and Lyfgenia (lovotibeglogene autoemcel), for the treatment of sickle cell disease (SCD) on December 8th, 2023 [26, 27]. This milestone represents the inaugural authorization of cell-based gene therapy for patients aged 12 and above with sickle cell disease in the United States. Notably, Casgevy is the first therapeutic intervention utilizing CRISPR-Cas9 gene editing technology to receive FDA approval (Fig. 1). SCD is caused by mutations in the β -globin chain gene of hemoglobin, affecting a global population exceeding

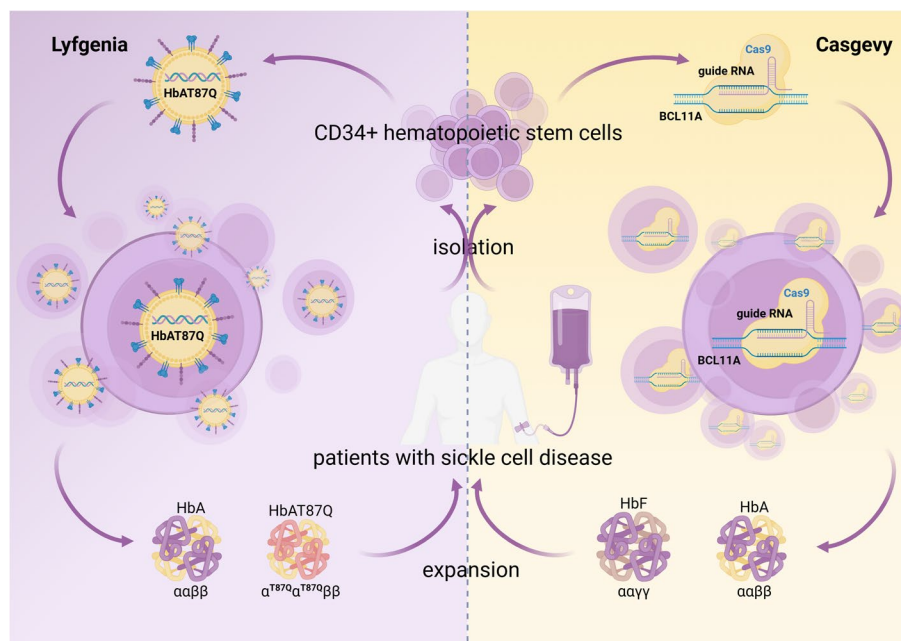


Fig. 1 To illustrate the ex vivo gene therapy approach, we employ sickle cell anemia as a paradigm for gene therapy. Casgevy: Initially, CD34+ hematopoietic stem and progenitor cells were isolated from the patient. Precise gene editing was performed on the patient's hematopoietic stem cells by specifically targeting the erythroid enhancer region of BCL11A using a single-guide RNA molecule (sgRNA). Subsequently, the edited cells were reintroduced into the patient's body with the aim of combating sickle cell anemia through increased expression of HbF. Lyfgenia: The BB305 lentiviral vector was employed to transduce modified β -globin genes into hematopoietic stem cells, resulting in the production of HbAT87Q—a hemoglobin variant that is resistant to disease and capable of inhibiting sickle hemoglobin polymerization. The remaining steps remain consistent with those described by Casgevy. Created with BioRender.com

three million individuals [28]. BCL11A is a transcription factor responsible for the repression of fetal hemoglobin (HbF) expression. After mobilization of the bone marrow, CD34+ hematopoietic stem cells and progenitor cells were harvested from the patient. The erythroid enhancer region of BCL11A was precisely targeted using a single-guide RNA molecule (sgRNA) for precise gene editing on the patient's hematopoietic stem cells. After undergoing a series of myeloablative treatments, the edited target cells were reinfused, resulting in increasing production of HbF [9]. According to the currently available clinical trial data (NCT03745287 and NCT04208529), the proportion of HbF in total hemoglobin (Hb) after administration of Casgevy treatment was 43.9% at the sixth month, and this level was sustained throughout the observation period (at least 24 months). The gene therapy approach employed by Lyfgenia, which also falls within the ex vivo pathway, differs in its treatment strategy (Fig. 1). It utilizes the BB305 lentiviral vector to transduce modified β -globin genes into hematopoietic stem cells, resulting in the production of HbAT87Q, a disease-resistant hemoglobin that can counteract sickle hemoglobin polymerization [29]. As of February 13, 2023, data from the Phase 1/2 HGB-206 Group C (NCT02140554) and

Phase 3 HGB-210 studies (NCT04293185) revealed that complete resolution of severe vaso-occlusive events (sVOE) and VOE was observed in a significant proportion of evaluable patients, with rates reaching 94% (32/34) and 88% (30/34), respectively, during the five-year follow-up period (median duration: 35.5 months; range: 0.3–61 months), encompassing a total of 47 patients.

In addition to the readily obtainable blood cells, the combination of induced pluripotent stem cells (iPSCs) and gene editing technology offers an alternative approach for ex vivo gene therapy. The advent of iPSCs and their derivatives, which emerged 16 years ago, has provided a valuable source of human cells for diverse applications encompassing drug discovery, toxicity assessment, disease modeling, and personalized cell therapy [30]. The synergistic utilization of iPSCs in conjunction with gene editing technology has established a solid groundwork for tailored cellular therapeutics [31]. By isolating peripheral blood mononuclear cells (PBMCs) from patients and subjecting them to gene reprogramming, it is possible to induce their conversion into pluripotent stem cells. Subsequently, these pluripotent stem cells can be genetically modified to rectify the patient's defective genes. Through controlled differentiation conditions, the

pluripotent stem cells can be directed toward specific cell lineages and subsequently reintroduced into the patient's body, thereby accomplishing therapeutic objectives [32].

In vivo

The *in vivo* approach, as opposed to the *ex vivo* gene therapy delivery method, bears a greater resemblance to the conventional administration of pharmaceutical agents. These include peripheral intravenous infusion (systemic administration) or local infusion (such as portal vein, renal vein, intrathecal injection), targeted tissue administration (local injection into specific brain regions, subretinal injections), and peripheral intramuscular injection [33–35]. The research objective of *in vivo* gene therapy is to enhance the precision, efficacy, and long-term effectiveness of gene therapy. Presently, the majority of approved gene therapy drugs predominantly employ *in vivo* administration.

Vectors for the delivery of gene therapy

The critical step in gene therapy entails the delivery of therapeutic genes to target cells and tissues. The carriers utilized for gene delivery include viral vector platforms as well as non-viral vector platforms (Table 1).

Viral vectors

The structural components of viral vectors for gene therapy consist of three essential elements. Firstly, the protein capsid or envelope determines the specific recognition and affinity towards target tissue cells. Secondly, the gene of interest, when expressed within the cell, achieves the desired therapeutic effect. Lastly, the regulatory cassette comprising enhancers, promoters, and other regulatory elements is responsible for precisely regulating either stable or transient expression of the gene of interest. Presently, the most widely accepted and extensively employed viral vector platforms primarily revolve around adenoviruses, lentiviruses, adeno-associated viruses (AAV), and herpes simplex virus (HSV).

Adenoviruses is a non-enveloped, linear, double-stranded DNA virus. The adenoviruses utilized in contemporary gene therapy are derived from human adenovirus serotypes 2 and 5. Through genetic engineering and modification of the wild-type adenovirus genome, specific structures are substituted with transgenes, resulting in a series of adenovirus-based gene therapy vectors. Adenovirus, as a gene therapy vector, possesses significant advantages owing to its robust packaging capacity reaching 36 Kb in a number of modifications. Moreover, adenovirus-based vectors exhibit high transduction efficiency in both quiescent and dividing cells, displaying a wide tropism for different tissue types. Importantly, they remain non-integrative within the host genome.

However, the widely pre-existing viral immunity in the population and potent immunogenicity of adenovirus impose limitations on its applicability in gene therapy. Presently, it is predominantly employed in innovative vaccine development and cancer treatment [36, 37].

AAV is a single-stranded DNA virus that harbors four known open reading frames. It is currently acknowledged as non-pathogenic and has not been linked to any diseases [38]. Its packaging capacity is approximately 4.7 kb. Due to its limited packaging capacity, a dual-vector system has been developed to facilitate efficient genome packaging [39]. AAV demonstrates a diverse range of tissue and cell tropism, attributed to the existing AAV serotypes and modifications in the viral capsid. Additionally, the incorporation of tissue-specific promoters has significantly enhanced its tropism [40]. In comparison to other viral vectors, AAV is acknowledged for its minimal immunogenicity. Numerous clinical trials based on this platform have proliferated rapidly.





Lentivirus is classified as a single-stranded RNA retrovirus, characterized by its long-term integrated vector and packaging capacity of approximately 9 kb. It serves as an efficient platform for gene-modified cell therapy, allowing the expression of multiple genes using a single vector. *In vitro* gene modification heavily relies on the utilization of lentivirus [41].

Naturally, the HSV primarily spreads through direct contact, predominantly in the perioral region. It typically manifests as a relatively benign ailment; however, in rare instances, it can invade the central nervous system and cornea, precipitating grave consequences such as encephalitis and visual impairment. Engineered HSV-1 has emerged as a promising vehicle for gene therapy due to its neurotropic properties, rendering it potentially valuable in addressing neurological disorders [42]. Furthermore, its robust packaging capacity confers an advantageous attribute. In May 2023, Vyjuvek received approval in the United States. It employs the HSV-1 vector to deliver the COL7A1 gene encoding collagen type VII, which is employed for treating dystrophic epidermolysis bullosa. Moreover, it represents a breakthrough as the first FDA-approved gene therapy product for repeated administration, administered topically in gel form [43].

Non-viral vector

Currently, approximately 70% of clinical trials employ viral vectors. However, the utilization of viral vectors is accompanied by certain limitations such as transgenic insertional mutations, immunogenicity, and intricate preparation procedures, which impose restrictions on their applications [44–46]. Consequently, the exploration of non-viral vectors has exhibited remarkable potential

Table 1 Advantages and disadvantages of viral and non-viral vectors

Viral platforms			
	Advantages	Disadvantages	Approved drugs Drugs/targets/diseases
 <p>Adenovirus</p>	<ul style="list-style-type: none"> • Large cargo capacities (~36 kb) • High transduction efficiency in both quiescent and dividing cells • Non-integrative within the host genome 	<ul style="list-style-type: none"> • Transient expression • Widespread neutralizing antibodies • Stronger immunological response 	<ul style="list-style-type: none"> • Gencicine/ p53 gene/ head and neck cancer • Adstiladrin/ Interferon alfa-2b / NMIBC • Oncorine/ oncolytic / e.g. head and neck cancer; hepatocarcinoma; cervical cancer
 <p>AAV</p>	<ul style="list-style-type: none"> • Minimal immunogenicity • Longer transgene expression • Transduce multiple tissue/cell types 	<ul style="list-style-type: none"> • Small cargo capacities (~4.7 kb) 	<ul style="list-style-type: none"> • Luxturna/ RPE65 / IRD • Glybera/ LPL / LPLD • Zolgensma/ SMN1 / SMA • Upstaza/ AADC/AADC deficiency • Roctavian/ B domain deleted hFVIII/HA • Elevidys/ micro-dystrophin protein / DMD • Hemgenix/ FIX-Padua /HB
 <p>Lentivirus</p>	<ul style="list-style-type: none"> • Long-term transgene expression • Medium cargo capacities (~8 kb) • Powerful vector for in vitro genetic modification 	<ul style="list-style-type: none"> • Integrative within the host genome 	<ul style="list-style-type: none"> • Strimvelis/ autologous CD34 + HSPCs / ADA-SCID • Zynteglo/ autologous CD34 + HSPCs /β-Thalassemia • Libmeldy/ autologous CD34 + HSPCs / MLD • Skysona/ autologous CD34 + HSPCs/ CALD • Kymriah/ autologous T cells/ ALL;DLBCL
 <p>HSV</p>	<ul style="list-style-type: none"> • Large cargo capacities (~30 kb) • Safer • Neurotropic 	<ul style="list-style-type: none"> • Low titers • Complex production process 	<ul style="list-style-type: none"> • Lmlygic/ GM-CSF / melanoma • Delytact/ oncolytic/ glioblastoma • Vyjuvek/ COL7A1 / DEB
Non-viral platforms			
	Advantages	Disadvantages	Investigational drugs Vectors/ targets/diseases
e.g Nanoparticles Cationic- polymers Liposomes	<ul style="list-style-type: none"> • Wide raw materials • Non-pathogenic agents • Easy to modify • High nucleic acid loading • Safety • Lower toxicity and cost 	<ul style="list-style-type: none"> • Lower delivery efficacies • Transient expression 	<ul style="list-style-type: none"> • PEI/ DTA-H19/ Bladder cancer(NCT00595088) • Lipid nanoparticle/ VEGF and KSP/ advanced solid tumors with liver involvement(NCT00882180) • PEG Nanocomplex CALAA-01/ M2 subunit of ribonucleotide reductase / Solid tumor cancers(NCT00689065) • Cationic lipids DOTAP-cholesterol/ FUS1/ Non-small cell lung cancer(NCT00059605)

NMIBC Non-muscle-invasive bladder cancer, AAV Adeno-associated virus, RPE65 Retinal pigment epithelium 65 kDa protein, IRD Inherited retinal degeneration, LPL Lipoprotein lipase, LPLD Lipoprotein lipase deficiency, SMN1 Survival motor neuron 1, SMA Spinal muscular atrophy, AADC Aromatic amino acid decarboxylase, hFVIII Human coagulation factor VIII, HA Hemophilia A, DMD Duchenne muscular dystrophy, FIX Coagulation factor IX, HB Hemophilia B, HSPCs Hematopoietic stem and progenitor cells, ADA-SCID Adenosine deaminase-severe combined immunodeficiency, MLD Metachromatic leukodystrophy, CALD Cerebral adrenoleukodystrophy, ALL Acute lymphoblastic leukemia, DLBCL Diffuse large B-cell lymphomas, HSV Herpes simplex virus, GM-CSF Granulocyte-macrophage colony-stimulating factor, COL7A1 The gene encoding the anchoring fibril component, collagen VII (C7), DEB Dystrophic epidermolysis bullosa, PEI Polyethylenimine, VEGF Vascular endothelial growth factor, KSP Kinesin spindle protein, PEG Polyethylene glycol, DOTAP N-[1-(2,3-Dioleoyloxy) propyl]-N,N,N-trimethylammonium methyl-sulfate, FUS1 Tumor suppressor

and necessity. Compared to viral vectors, non-viral vectors exhibit superior nucleic acid loading capacity and safety profiles, as well as offering greater flexibility in terms of chemical composition and a broader range of raw material options. Non-viral vectors encompass a diverse range of delivery systems, including lipid nanoparticles (LNPs), exosomes, cationic polymers such as (PAE), single-chain cyclic polymer (SCKPs), polyethyleneimine (PEI), poly(amidoamine) (PAMAM), polydimethylaminoethyl methacrylate (PDMAEMA), chitosan (CS), and cyclodextrin (CD), inorganic nanoparticles, and intelligent hydrogels. Notably, LNPs and cationic polymers have gained significant traction in various applications [47, 48]. Recently, Zhang Feng's team has discovered a programmable protein delivery method that utilizes bacterial contractile injection systems (eCISs). These eCISs are natural protein delivery systems similar to an "injector" found in bacteria. The tail fibers of the eCISs have been modified to enable them to target specific cells and carry various proteins such as Cas9 and base editor proteins. Furthermore, modifications to other components of this system could potentially allow for the delivery of RNA or DNA [49]. This system shows promise as a safe and efficient tool for gene therapy in the future.

Gene therapy for non-monogenic disorders/complex diseases

Over the past two decades, there has been a growing ease in identifying the causal genes for highly penetrant, Mendelian (monogenic) human diseases. These diseases, characterized by their low occurrence rate, are commonly classified as orphan diseases. In contrast to monogenic diseases, polygenic diseases are associated with multiple gene mutations or single nucleotide polymorphisms. The impact of these variants is relatively smaller and the underlying mechanism for complex diseases lies in the accumulation of subtle effects on key genes and regulatory pathways that contribute to disease risk. The advent of Genome-Wide Association Studies (GWAS) has provided optimism in identifying single polymorphic variants with discernible functional impacts on complex traits. Polygenic risk scores serve as indicators of an individual's susceptibility to disease [50, 51]. Polygenic diseases encompass a group of prevalent age-related conditions such as cardiovascular disease, diabetes, and cancer. Additionally, early or middle-aged onset complex polygenic disorders like asthma, Crohn's disease, schizophrenia, systemic lupus erythematosus have emerged as current areas of research interest [52]. Differing from monogenic disorders, management strategies for polygenic diseases not only focus on symptom treatment but also emphasize meaningful early preventive measures to mitigate further damage caused by disease progression.

Selective clinical trials for gene therapy in non-monogenic diseases are summarized in Table 2.

Cardiovascular diseases

Coronary heart disease

Coronary artery disease (CAD) is primarily attributed to the progressive narrowing of the coronary arteries caused by atherosclerosis, consequently leading to ischemic heart disease. Over the past two decades, there have been several endeavors to apply gene therapy in the treatment of CAD. The therapeutic approaches employed in clinical trials for CAD primarily revolve around promoting therapeutic angiogenesis (Fig. 2). This involves utilizing a vector to deliver the cDNA of specific cell factors that stimulate angiogenesis, targeting the affected myocardial region through direct intramyocardial or intra-arterial injection. Commonly utilized factors include vascular endothelial growth factor (VEGF), fibroblast growth factor family (FGF), and hepatocyte growth factor (HGF), stromal cell-derived factor 1 (SDF-1) with VEGF being extensively investigated. Compared to the direct utilization of protein preparations, the gene therapy-mediated protein generation pathway holds the potential to enhance therapeutic protein levels in a more sustained and stable manner. The initial clinical trial for CAD was conducted in 1998 by Douglas W. Losordo et al. [121]. In this study, the researchers administered a direct injection of a naked plasmid (pVEGF165) encoding VEGF into the ischemic myocardium. Following treatment, a significant reduction in angina incidence was observed among patients, which was further confirmed by single-photon emission computed tomography demonstrating decreased myocardial ischemia. Subsequently, a series of clinical trials were conducted employing Ad or naked plasmid vectors (PI), including KAT301 (Ad, VEGF-D^{ΔNAC}), ASPIRE (Ad, FGF-4), Haoyu Meng et al. (Ad, HGF), AFFIRM (Ad, FGF-4) (NCT02928094), the VEGF-Neupogen trial (PI, VEGF-A165-hGCSF), VIF-CAD (PI, VEGF-A165/bFGF), and NORTHERN (PI, VEGF-A165) [53, 65, 70, 122–124]. Based on completed or ongoing clinical trials, although some have yielded positive results, demonstrating the safety and effectiveness of gene therapy based on therapeutic angiogenesis in the treatment of CAD, overall, the treatment outcomes remain unsatisfactory. The limited transduction efficiency of the vector in myocardial tissue and the transient duration of transgene expression may be key factors impeding therapeutic efficacy. Furthermore, methodological considerations such as stronger placebo effects in experimental design and a lack of sensitive efficacy evaluation indicators also pose challenges to the advancement of gene therapy for CAD [125]. Currently, there are no drugs that have received market approval.

Table 2 Selective clinical trials for gene therapy in non-monogenetic diseases

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
Cardiovascular system									
CAD									
VIF-CAD	plasmid	VEGF-A165/bFGF	NOGA-guided i. my. injections	phase 2	52	median, 133 months	safety	safe	[53]
Symes JF et al.	plasmid	VEGF165	i. my. injections	phase 1	20	180 days	safety; bioactivity	safe; positive	[54]
Kolsut P et al.	plasmid	VEGF165	i. my. injections	-	22	-	safety; efficacy	safe; positive	[55]
Tio RA et al.	plasmid	VEGF165	NOGA-guided i. my. injections	-	35	-	appraise the value of PET in the assessment of the effect	positive	[56]
Rosengart TK et al.	adenovirus	VEGF121	i. my. injections	phase 1	31	median, 11.8 years	safety	safe	[57]
Rosengart TK et al.	adenovirus	VEGF121	i. my. injections	phase 1	21	-	safety; efficacy	safe; positive	[58]
Lathi KG et al.	plasmid	VEGF165	i. my. injections	phase 1	30	180 days	clinical changes in cardiovascular function	negative	[59]
the NOVA trial	adenovirus	VEGF121	NOGA-guided i. my. injections	-	17	52 weeks	safety; efficacy	safe; negative	[60]
Hassinen I et al.	adenovirus	VEGF-D ^{ΔNAC}	combined NOGA and PET mapping; NOGA-guided i. my. injections	phase 1/2a	30	12 months	efficacy of combining electromechanical map with PET imaging to target the ischemic myocardium	positive	[61]
Sarkar N et al.	plasmid	VEGF-A165	i. my. injections	Open-labelled study	39	12 months	safety; bioactivity	safe; may be positive	[62]
Stewart DJ et al.	adenovirus	VEGF121	i. my. injections	phase 2	67	26 weeks	efficacy	positive	[63]
Yang ZJ et al.	adenovirus	HGF	over the wire balloon or by diagnostic coronary catheter	phase 1	18	14 months	safety	safe	[64]
Meng H et al.	Adenovirus	HGF	percutaneous i. my. injections	phase 2a	30	6 months	safety; efficacy	safe; potentially efficient in improving LVEF and lowering LVDD	[65]
Losordo DW et al.	plasmid	VEGF	i. my. injections	phase 1	5	-	safety; bioactivity	safe; positive	[66]
Vale PR et al.	plasmid	VEGF165	i. my. injections	-	13	-	efficacy	positive	[67]

Table 2 (continued)

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
KAT	Adenovirus, plasmid	VEGF	catheter-based local intracoronary gene transfer	phase 2	103	6 months	safety; feasibility	no differences in clinical restenosis rate or minimal lumen diameter; a significant increase was detected in myocardial perfusion in the VEGF-Adv-treated patients	[68]
Euroinject One	plasmid	VEGF165	NOGA-guided i. my. injections	phase 2	80	3 months	efficacy	not significantly improve stress-induced myocardial perfusion abnormalities; improved regional wall motion	[69]
NORTHERN	plasmid	VEGF165	NOGA-guided i. my. injections	-	93	6 months	efficacy	negative	[70]
Ripa RS et al.	plasmid	VEGF-A165	i. my. injections	-	16	3 months	safety; effects	negative	[71]
AGENT	adenovirus	FGF-4	percutaneous i. my. injections	-	79	mean, 311 days	safety; effects	safe; positive	[72]
Grines CL et al.	adenovirus	FGF-4	intracoronary injections	-	52	12 months	safety; effects	safe; positive	[73]
PAD									
Kim HJ et al.	plasmid	VEGF165	i. m. injections	phase 1	9	9 months	safety; clinical effects	safe; positive	[74]
Niebuhr A et al.	plasmid	FGF1	i. m. injections	phase 1 and 2	93	3 years	safety	safe	[75]
pUDK-HGF	plasmid	HGF	i. m. injections	phase 2	240	-	safety; efficacy	safe; positive	[76]
Barć P et al.	plasmid	VEGF165/HGF	i. m. injections	-	28	90 days	efficacy	positive	[77]
Rajagopalan S et al.	adenovirus	VEGF	i. m. injections	phase 2	105	26 weeks	efficacy	negative	[78]
Makino H et al.	plasmid	HGF	i. m. injections	phase 1/2a	22	2 years	efficacy	positive	[79]
Kusumanto YH et al.	plasmid	VEGF165	i. m. injections	-	54	100 days	efficacy	negative	[80]
STOP-PAD	plasmid	SDF-1	i. m. injections	phase 2b	109	3 months	safety; efficacy	safe; negative	[81]
Deev R et al.	plasmid	VEGF165	i. m. injections	-	36	5 years	safety; efficacy	safe; positive	[82]

Table 2 (continued)

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
Rajagopalan S et al.	adenovirus	VEGF121.10	i. m. injections	phase 1	6	30 days	safety; efficacy	safe; positive	[83]
Mohler ER 3rd et al.	adenovirus	VEGF121.10	i. m. injections	phase 1	15	360 days	safety	safe	[84]
NL003	plasmid	HGF	i. m. injections	phase 2	200	6 months	safety; efficacy	safe; positive	[85]
DVC1-0101	rSeV	FGF-2	i. m. injections	phase 1/2a	12	6 months	safety; efficacy	safe; positive	[86]
Cui S et al.	plasmid	HGF	i. m. injections	phase 1	21	3 months	safety; preliminary efficacy	safe; positive	[87]
VM202	plasmid	HGF	i. m. injections	phase 1	12	12 months	safety	safe	[88]
pCK-HGF-X7	plasmid	HGF	i. m. injections	phase 1	21	3 months	safety; preliminary efficacy	safe; positive	[89]
CI-1023	adenovirus	VEGF121.10	i. m. injections	phase 1	18	1 year	safety; efficacy	safe; encouraging trends in ABI at rest and peak walking time	[90]
HF									
SERCA-LVAD trial	AAV1	SERCA2a	intracoronary infusion	phase 2a	5	3 years	safety; feasibility	safe; negative	[91]
STOP-HF	plasmid	SDF-1	endomyocardial injections	phase 2	93	12 months	safety; efficacy	safe; negative	[92]
CUPID	AAV1	SERCA2a	intracoronary infusion	phase 2	39	12 months	safety; efficacy	safe; positive	[93]
CUPID 1	AAV1	SERCA2a	intracoronary infusion	phase 1/ 2	39	3 years	clinical effects	positive	[94]
CUPID 2	AAV1	SERCA2a	intracoronary infusion	phase 2b	250	median 17.5 months	efficacy	negative	[95]
Hammond HK et al.	adenovirus 5	AC6	intracoronary infusion	phase 2	56	1 year	safety; efficacy	safe; positive	[96]
AGENT-HF	AAV1	SERCA2a	intracoronary infusion	phase 2	9	6 months	safety; efficacy	safe; negative	[97]
Hyperlipidemia									
ORION-10 ORION-11	siRNA-based drug	PCSK9	s. c. injections	phase 3	1561;1617	540 days	reductions in LDL cholesterol levels (in patients with atherosclerotic cardiovascular disease and or an atherosclerotic cardiovascular disease risk equivalent)	approximately 50% reductions	[98]

Table 2 (continued)

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
ORION-9	siRNA-based drug	PCSK9	s. c. injections	phase 3	482	540 days	reductions in LDL cholesterol levels (in patients with heterozygous familial hypercholesterolemia)	approximately 39% reductions	[99]
ORION-1	siRNA-based drug	PCSK9	s. c. injections	phase 2	501	240 days	reductions in LDL cholesterol levels	dose-dependent reductions	[100]
ORION-3	siRNA-based drug	PCSK9	s. c. injection	-	497	4 years	results from the 4-year open-label extension of the ORION-1 trial	averaged mean reduction of LDL-C cholesterol was 44.2%	[101]
ORION-5	siRNA-based drug	PCSK9	s. c. injection	phase 3	56	150 days	reductions in LDL cholesterol levels (in patients with homozygous familial hypercholesterolemia)	negative	[102]
OCEAN[ia]-DOSE	siRNA-based drug	Lipoprotein(a)	s. c. injection	-	281	36 weeks	the percent change in the lipoprotein(a) concentration from baseline to week 36	dose-dependent manner; -70.5% with the 10-mg dose, -97.4% with the 75-mg dose, -101.1% with the 225-mg dose administered every 12 weeks, and -100.5% with the 225-mg dose	[103]
Yeang C et al.	oligonucleotide drug	apo(a)	s. c. injection	phase 2b	286	12 months	the percent change in the Lp(a)-C concentration	compared with placebo, pelacarsen resulted in dose-dependent decreases in Lp(a)-C (2% vs -29% to -67%)	[104]

Table 2 (continued)

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
Gaudet D et al.	ASO	ANGPTL3	s. c. injection	phase 2	105	6 months	percent change in fasting triglycerides from baseline at 6 months	reductions in triglycerides of 36%, 53%, 47%, were observed in the 40mgQ4W, 80mgQ4W, and 20mgQW groups, respectively	[105]
TRANSLATE-TIMI 70	ASO	ANGPTL3	s. c. injection	phase 2b	286	36 weeks	placebo-adjusted percentage change from baseline in non-HDL-C at 24 weeks	ranging from 22.0% in the 60 mg every 2 weeks arm to 27.7% in the 80 mg every 2 weeks arm	[106]
The APPROACH trial	ASO	APOC3	s. c. injection	phase 3	66	52 weeks	percentage change in fasting triglyceride levels from baseline to 3 months	77% decrease in mean triglyceride levels	[107]
The COMPASS trial	ASO	APOC3	s. c. injection	phase 3	114	-	percentage change from baseline to 3 months in fasting triglyceride	reduced mean plasma triglyceride concentration by 71.2%	[108]
Hypertension									
The KARDIA-1	siRNA-based drug	hepatic angiotensinogen	s. c. injection	phase 2	394	6 months	between-group difference in least-squares mean (LSM) change from baseline to month 3 in 24-h mean ambulatory SBP	-14.1 mm Hg (150 mg, once every 6 months); -16.7 mm Hg (300 mg, once every 3 months or every 6 months); and -15.7 mm Hg (600 mg, once every 6 months)	[109]

Table 2 (continued)

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
AGMG0201	vaccine	angiotensin II	i. m. injections	phase 1/2a	24	360 days	safety	tolerated	[110]
Neurodegenerative diseases									
Alzheimer's disease									
Rafiq MS et al.	AAV2	NGF	stereotactically guided intracerebral injections into the nucleus basalis of Meynert	phase 2	49	24 months	change from baseline on the Alzheimer's Disease Assessment Scale-cognitive subscale at month 24	negative	[111]
CERE-110	AAV	NGF	stereotactically guided intracerebral injections into the nucleus basalis of Meynert	open-label	10	2 years	safety and tolerability, and initial efficacy	feasible, tolerated, and able to produce long-term, biologically active NGF expression	[112]
LGT	AAV	hTERT	Ongoing, NCT04133454						
UCSD-BDNF1	AAV2	BDNF	Ongoing, NCT05040217						
LX1001-01	AAVrh.10	APOE2	Ongoing, NCT03634007						
Parkinson's disease									
Kaplitt MG et al.	AAV	GAD	unilateral, subthalamic injections	phase 1	12	12 months	safety, tolerability, and potential efficacy	safe; significant improvements in motor UPDRS scores	[113]
CERE-120	AAV2	NTN	bilateral, stereotactic, intraputamenal injections	phase 1	12	1 year	safety, tolerability, and potential efficacy	partially positive	[114]
Marks WJ Jr et al.	AAV2	NTN	bilateral, putamenal injections	phase 2	58	12 months	change of the UPDRS motor score	negative	[115]
Muramatsu S et al.	AAV	AADC	putamenal injections	phase 1	6	6 months	safety, tolerability, and potential efficacy	safe; positive	[116]
Warren Olanow C et al.	AAV2	NTN	bilateral, putamenal and nigral injections	-	51	24 months	change of the UPDRS motor score	negative	[117]
Niethammer M et al.	AAV2	GAD	bilateral, subthalamic injections	-	45	12 months	long-term clinical outcome	positive	[118]
Mittermeyer G et al.	AAV2	AADC	bilateral, putamenal injections	phase 1	10	4 years	long-term clinical outcome	positive	[119]

Table 2 (continued)

Trial	Vector	Target gene	Approach	Design	n	Follow-up time	Primary endpoint	Main result	Reference
Amyotrophic lateral sclerosis									
AL-Spire	ASO-based drug	ATXN2	ongoing, NCT04494256						
Endocrine and metabolic systems									
Diabetes									
GFX-002	AAV	Pdx1/MafA	remains at the preclinical stage						
VCTX210	stem cell therapy with CRISPR-Cas9 gene editing	B2M, PD-L1, HLA-E	undergoing phase 1 clinical trials and administration to the first patient has been completed						
Others									
Osteoarthritis									
GNSC-001	AAV	IL-1Ra	intra-articular injections	ongoing, NCT05835895					
Systemic lupus erythematosus									
Andreas Mackensen et al.	CAR-T	CD19	autologous T cells reinfusion	-	5	-	clinical efficacy	highly effective	[120]
	CAR-NK	CD19	ongoing, NCT06010472						
	CAR-T	CD19 and BCMA	ongoing, NCT05858684						
	CAR-T	CD19 and BCMA	ongoing, NCT05474885						
	CAR-T	CD19	ongoing, NCT06056921						

CAD Coronary artery disease, *i. my.* Intramyocardial, PET Positron emission tomography, LVEF Left ventricular ejection fraction, LVEDd Left ventricular end-diastolic dimension, PAD Peripheral arterial disease, rSeV Recombinant Sendai virus, ABI Ankle brachial index, HF Heart failure, s. c. Subcutaneous, LDL Low density lipoprotein, ASO Antisense oligonucleotide, SBP Systolic blood pressure, UPDAS Unified Parkinson's Disease Rating Scale

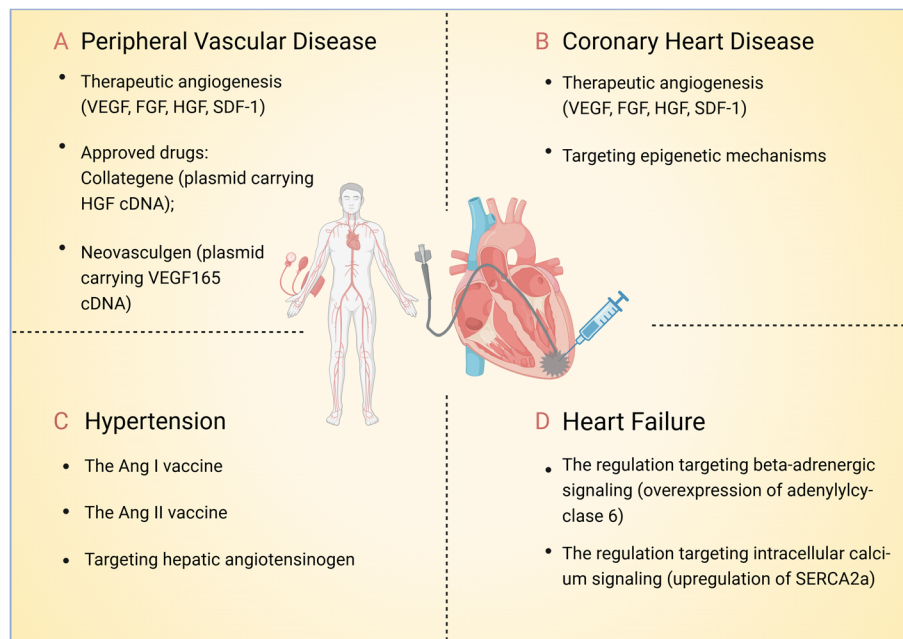


Fig. 2 Gene therapy strategies for cardiovascular diseases. VEGF: Vascular endothelial growth factor; FGF: Fibroblast growth factor family; HGF: Hepatocyte growth factor; SDF-1: Stromal cell-derived factor 1; Ang: Angiotensin; SERCA2a: Sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase. Created with BioRender.com

Recently, studies have demonstrated the pivotal role of epigenetics in cardiovascular diseases. Epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNA, intricately regulate genes associated with disease pathology. Furthermore, therapeutic interventions targeting epigenetic regulatory pathways have been developed to address cardiovascular disorders. Although these drugs are currently in the preclinical or clinical trial stage, this treatment approach offers an additional potential avenue for disease management [126].

Peripheral vascular disease

Peripheral arterial disease (PAD) is caused by the occlusion of major arteries in the lower extremities as a result of atherosclerosis, leading to limb ischemia and a range of clinical manifestations including pain, claudication, and ulcers. Similar to the treatment of CAD, therapeutic angiogenesis emerges as a key treatment strategy for PAD. FGF has been investigated in this context through trials such as Comerota et al. [127], TALISMAN [128], and TAMARIS [129], utilizing plasmids as vectors. Morishita et al. [130], HGF-STAT [131], and Powell et al. [132] employed plasmid vectors as well; however, their distinction lies in the specific targeting of HGF. RAVE [133], Deev, et al. [134], and other experiments used adenovirus as a vector carrying VEGF cDNA. It is noteworthy that two treatment drugs have received approval

for the management of PAD. Collategene [135], a gene therapy product carrying the HGF gene on a plasmid, was granted approval by the Japan Ministry of Health, Labour and Welfare in 2019. This therapeutic approach targets occlusive arteriosclerosis and thromboangiitis obliterans cases that do not respond to standard drug therapy. Furthermore, Neovasculgen [136], based on plasmid-VEGF165, obtained regulatory clearance in Russia back in 2011.

Heart failure

With advancements in the understanding of heart failure pathogenesis, several crucial targets implicated in disease onset and progression have been identified. These targets exhibit a characteristic resistance to conventional pharmacological interventions; however, gene therapy has emerged as a promising approach. Various transgenic strategies primarily focus on three key areas: angiogenesis, intracellular calcium signaling regulation, and β -adrenergic signaling. Sarcoplasmic/endoplasmic reticulum Ca²⁺ATPase (SERCA2a) serves as the primary calcium pump in cardiomyocytes, facilitating the recycling of calcium ions within the sarcoplasmic reticulum. Its pivotal role in maintaining a stable intracellular calcium ion concentration is indispensable. The upregulation of SERCA2a has shown promising potential in enhancing cardiac function, rendering it an attractive target gene for heart failure therapy [137]. The CUPID

2 trial enrolled a cohort of 250 patients diagnosed with heart failure, who underwent a single coronary artery infusion of AAV1-SERCA2. Regrettably, the administration of AAV1-SERCA2 in this clinical trial did not yield any discernible improvement in the overall clinical trajectory of patients suffering from heart failure [138]. Stromal cell-derived factor-1 (SDF-1) is a chemotactic protein that binds to the CXCR-4 receptor, thereby promoting angiogenesis and facilitating tissue regeneration. The STOP-HF trial administered a naked DNA plasmid encoding human SDF-1 (pSDF-1) to patients with heart failure via endocardial injection. Although the efficacy of pSDF-1 was not established by the STOP-HF trial, its data substantiated the safety profile of this therapeutic approach [92]. Adenylyl cyclase (AC) catalyzes the conversion of adenosine triphosphate into cyclic adenosine monophosphate (cAMP). AC6, an isoform found in cardiac muscle cells, plays a crucial role. Dysfunction of the β -adrenergic signaling system is a significant pathological mechanism contributing to heart failure. Targeting overexpression of AC to elevate cAMP levels may present a promising therapeutic approach for treating heart failure. This Phase 2 clinical trial aims to investigate the efficacy of Ad5 encoding adenylyl cyclase 6 (Ad5.hAC6). At week 4, the treatment group exhibited a significant improvement in ejection fraction (EF) (+6.0 [1.7] EF units; $n=21$; $P<0.004$); however, by week 6, the results demonstrated a lack of sustained effect [139]. These unsatisfactory findings suggest that gene therapy for heart failure has encountered an impasse.

Hypertension

DNA or RNA-based vaccines initially emerged in the field of infectious diseases. In 2023, scientists Katalin Karikó and Drew Weissman were honored with the Nobel Prize in Physiology or Medicine for their groundbreaking discoveries in nucleotide modification, which paved the way for the development of highly effective mRNA vaccines against COVID-19. The modification of nucleotide bases can prevent the body from recognizing synthesized mRNA as foreign, thereby significantly expediting the clinical application of mRNA vaccines. During the COVID-19 pandemic, BNT162b2 (Comirnaty) and mRNA-1273 (Spikevax) are two representative mRNA-based vaccines [140]. DNA vaccines are constructed using recombinant plasmids that encode viral antigens. Upon transduction into host cells, these plasmids facilitate the production of proteins or peptides through transcription and translation processes. The concept of developing a vaccine against hypertension involves substituting the encoding sequence for the viral antigen with that of the self-antigen. Firstly, angiotensin I (Ang I) emerges as the primary target for self-directed

intervention. In this randomized double-blind placebo-controlled clinical trial, the Ang I vaccine PMD3117 was administered to hypertensive patients; however, the findings did not demonstrate a significant reduction in blood pressure [141]. Given the potential involvement of the renin and Ang II feedback pathway in its failure, researchers have redirected their attention towards Ang II. The Ang II vaccine has demonstrated promising results in preclinical studies [142]. The AGMG0201 trial, a phase I/IIa trial, aims to assess the safety, tolerability, and immunological response of the modified angiotensin II DNA vaccine (AGMG0201). Notably, patients in the AGMG0201 group exhibited detectable levels of antibodies against angiotensin II, particularly among those receiving higher doses [110]. However, further clinical efficacy needs to be substantiated through additional rigorous clinical trials. Zilebesiran, an RNA interference therapeutic targeting hepatic angiotensinogen synthesis, demonstrated significant reduction in 24-h mean ambulatory systolic blood pressure (SBP) at month 3 in adults with mild to moderate hypertension during the phase 2 study, when administered at various doses and intervals of either 3 or 6 months (NCT04936035).

Dyslipidemia

Hyperlipidemia is a well-established risk factor for CAD, with low-density lipoprotein (LDL) being closely related to the development of coronary atherosclerosis. Consequently, reducing LDL levels has emerged as the primary objective of lipid-lowering therapy. In recent years, advancements in genetic analyses have unveiled novel targets, including proprotein convertase subtilisin/kexin Type 9 (PCSK9), angiopoietin-like protein (ANGPTL3), apolipoprotein C-III (ApoC-III), and Lipoprotein(a) (Lp(a)) (Fig. 3). The objective of lipid-lowering therapies is to achieve optimal efficacy and long-term durability. The development of lipid-lowering treatment drugs is progressing from traditional oral medications (administered daily) to monoclonal antibody drugs (given monthly or semi-monthly), oligonucleotide drugs (administered weekly or monthly), siRNA drugs (administered semi-annually), and beyond, with extended durations being explored [143]. Antisense oligonucleotides (ASOs) are chemically modified fragments of single-stranded DNA or RNA molecules, utilized in the treatment of human diseases through mechanisms such as translational inhibition, mRNA degradation, and regulation of splicing. The RNA interference pathway, mediated by small double-stranded RNA molecules (dsRNA), promotes mRNA degradation and downregulates gene expression, inducing gene-silencing effects [144]. PCSK9, acting as a partner of the LDL receptor, facilitates the trafficking of the LDL

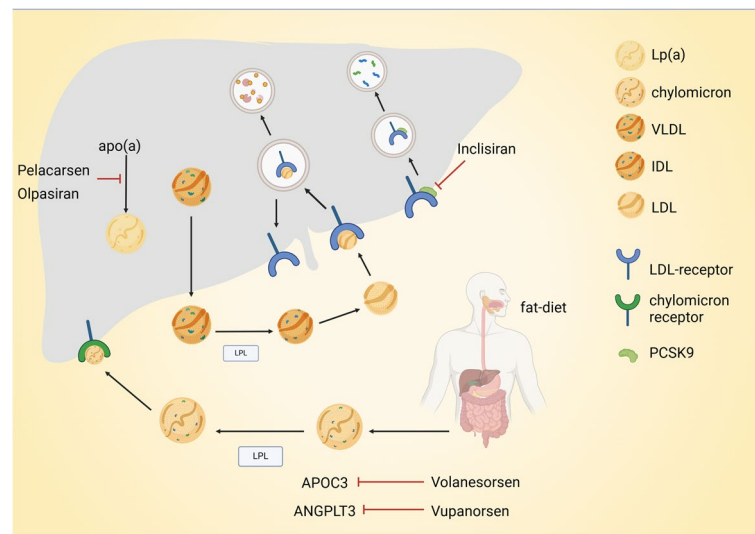


Fig. 3 The targets for gene therapy in the treatment of hyperlipidemia. PCSK9, acting as a partner of the LDL receptor, facilitates the trafficking of the LDL receptor to the lysosome, thereby enhancing its degradation. Inclisiran is a siRNA-based drug targeting PCSK9, it hampers the degradation process of the LDL receptor and promotes its recycling instead, ultimately leading to reduced levels of LDL. Olpasiran is a siRNA-based agent that effectively impedes the hepatic assembly of Lp(a) by downregulating its expression in hepatocytes, Pelacarsen is an oligonucleotide drug that inhibits the synthesis of apo(a), resulting in a dose-dependent reduction in Lp(a) levels. Vupanorsen and Volanesorsen are ASO drugs targeting ANGPTL3 and ApoC-III respectively. Lp(a): Lipoprotein(a); VLDL: Very low-density lipoprotein; IDL: Intermediate-density lipoprotein; LDL: Low-density lipoprotein; PCSK9: Proprotein convertase subtilisin/kexin type 9; APOC3: Apolipoprotein C-III; ANGPTL3: Angiopoietin-like protein; ASO: Antisense oligonucleotides; LPL: Lipoprotein lipase. Created with BioRender.com

receptor to the lysosome, thereby enhancing its degradation. In cases where PCSK9 function is impaired, it hampers the degradation process of the LDL receptor and promotes its recycling instead, ultimately leading to reduced levels of LDL. Inclisiran is a siRNA-based drug targeting PCSK9. The ORION-3 study is a four-year open-label extension conducted in five countries, demonstrating an average reduction of 44.2% (95% CI: 47.1 ~ 41.4) and 62% ~ 77.8% in LDL-C levels and PCSK9 levels respectively over the four-year observation period. The incidence of possibly treatment-related serious adverse reactions was found to be 1%. These findings provide robust evidence supporting the sustained efficacy and favorable tolerability profile of biannual Inclisiran injections for LDL-C lowering over a prolonged duration [101]. Previously, the ORION-9 [99], ORION-10 [98], and ORION-11 [145] trials elucidated the lipid-lowering efficacy and tolerability of Inclisiran over a duration of up to 18 months. Olpasiran is also a siRNA-based agent that effectively impedes the hepatic assembly of Lp(a) by downregulating its expression in hepatocytes, thereby resulting in reduced levels of Lp(a). The phase 2 OCEAN[a]-DOSE trial demonstrates that a subcutaneous injection of Olpasiran at a dosage of 75 mg or higher every 12 weeks leads to a remarkable reduction in Lp(a) levels exceeding 95% after a follow-up period of 36 weeks. Notably, all patients (100%)

receiving either the 75 mg or the 225 mg dose of Olpasiran every 12 weeks achieved normal Lp(a) levels [103]. Pelacarsen (AKCEA-APO(a)-LRx, IONIS-APO(a)-LRx, TQJ230) is an oligonucleotide drug that inhibits the synthesis of apo(a), resulting in a dose-dependent reduction in Lp(a) levels up to 80% [146]. Currently, phase III clinical trials are ongoing (NCT04023552). Additionally, Vupanorsen and Volanesorsen have demonstrated promising results as ASO drugs targeting ANGPTL3 and ApoC-III respectively [105, 107, 108]. Furthermore, in vivo, gene editing of PCSK9 in primates has shown potential for long-term lipid reduction by lowering LDL for at least 8 months [147].

Central nervous system diseases

Alzheimer's disease

Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder that affects more than 50 million individuals globally, predominantly the elderly population [148]. AD is characterized by the abnormal accumulation of amyloid-beta ($A\beta$) in the cerebral cortex and hippocampus as well as the aberrant formation of neurofibrillary tangles, leading to progressive memory loss and cognitive impairment. Currently, the underlying pathological mechanisms of AD remain incompletely elucidated. Hypotheses regarding its pathogenesis encompass elevated levels of tau proteins, diminished

antioxidant capacity, impaired cholinergic activity, and neural inflammation, among others. Moreover, through advancements in GWAS and epidemiological investigations, numerous genes potentially implicated in AD have been identified, including APOE, TREM2, FERMT2, APP, etc.; these genes are involved in a diverse array of proteins directly or indirectly associated with AD [148]. Human apolipoprotein E (APOE) is considered the most significant genetic factor associated with late-onset AD, with APOE having three alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. Among these alleles, $\epsilon 4$ encodes apoE4, which confers a lifetime risk estimate of developing AD by the age of 85 at approximately 30% in apoE4 heterozygotes and approximately 65% in apoE4 homozygotes. However, APOE encoded by $\epsilon 2$ is believed to exert a protective effect. Under physiological conditions, APOE primarily facilitates lipid and cholesterol transport and metabolism while also playing a crucial role in neuronal maintenance and repair. ApoE4 promotes A β production while impeding its degradation, induces abnormal hyperphosphorylation of tau protein, and affects neuroinflammatory cell function and activation, ultimately contributing to the pathogenesis of AD [149]. This ongoing phase 1/2 open-label study (NCT03634007) aims to employ intrathecal administration of serotype AAVrh.10 gene transfer vector expressing the cDNA encoding human apolipoprotein E2 (APOE2) to evaluate the therapeutic efficacy of this intervention in individuals with AD who are homozygous for APOE4. Preclinical safety assessments have been conducted using non-human primates [150]. The Nerve Growth Factor (NGF), an endogenous neurotrophic factor with neuroprotective properties, represents another target for AD gene therapy. AAV2-NGF was administered via stereotactic intracerebral injection into the basal ganglia in a study (NCT00876863). However, regrettably, no significant difference was observed between the treatment and placebo groups based on the Alzheimer Disease Assessment Scale–cognitive subscale [151]. Given the association between late-onset AD and aging, this ongoing study (NCT04133454) aims to employ Libella Gene Therapy (LGT), specifically utilizing AAV vectors carrying active telomerase (hTERT), to attenuate neuronal senescence. However, the current progress of this research remains undisclosed. In 2022, a novel clinical trial (NCT05040217) was initiated targeting brain-derived neurotrophic factor (BDNF), employing AAV2 as the vector. BDNF exerts regulatory control over neuronal function within the crucial memory circuit of the brain; however, no reports on its outcomes have been published yet.

Currently, the application of CRISPR-Cas9 in AD is primarily in the stage of animal models, encompassing the

establishment of more phenotypically relevant models for studying disease pathogenesis and exploring potential treatments [152].

Parkinson's disease

Parkinson's disease (PD) is a heterogeneous disease influenced by multiple factors. To date, more than 100 genes or gene loci associated with susceptibility to PD have been identified (e.g., SNCA, LRRK2, GBA). The majority of cases arise from the combined effect of numerous common or rare genetic mutations. PD is characterized by basal ganglia neuronal dysfunction, where the degeneration of dopamine neurons in the substantia nigra impairs the signal pathway between the substantia nigra and the striatum. The susceptibility genes of PD have complex associations with synaptic, lysosomal, mitochondrial dysfunctions, and immune responses [153]. Based on these revealed potential susceptibility genes, gene therapies targeting these points have been applied to clinical trials. The treatment strategies mainly include protecting neurons, promoting dopamine production, and enhancing the γ -Aminobutyric acid (GABA) signaling pathway.

Neurturin (NTN) and glial cell line-derived neurotrophic factor (GDNF) are neurotrophic factors that confer neuronal protection. The upregulation of NTN and GDNF, promoting neuronal regeneration, represents a therapeutic target for PD. In the phase 2 randomized trial conducted by Marks WJ Jr and colleagues, patients with PD underwent bilateral putamen injections of AAV2-NTN. The findings revealed no statistically significant difference in the primary endpoints between the treatment group and the control group that received sham surgery [115]. In another clinical trial, researchers demonstrated the feasibility and safety of bilateral stereotactic substantia nigra and putamen injection with AAV2-neurturin (CERE-120) (Class IV evidence) [154]. Clinical trials targeting GDNF include NCT01621581 and NCT04167540, both of which utilize AAV2 as the vector.

Aromatic L-amino acid decarboxylase (AADC) is a crucial enzyme essential for dopamine biosynthesis. Tyrosine hydroxylase (TH) facilitates the conversion of L-tyrosine into the precursor molecule dopamine. The VY-AADC01 trial employed bilateral putamen delivery guided by magnetic resonance imaging (using gadoteridol and AAV2 carrying AADC cDNA). The three dose cohorts exhibited respective increases in enzyme activity of 13% (total dose, $\leq 7.5 \times 10^{11}$ vg), 56% (total dose, $\leq 1.5 \times 10^{12}$ vg), and 79% (total dose, $\leq 4.7 \times 10^{12}$ vg), with enzyme activity assessed via PET [155]. In another trial, a lentivirus vector carrying the gene encoding ADCC, TH, and cyclohydrolase 1 (ProSavin) was employed. Throughout the 12-month follow-up period, no significant adverse effects were observed. A notable

improvement in the average UPDRS part III motor scores was evident in the 6th month (mean score 38 [SD 9] vs. 26, $n=15$, $p=0.0001$). Furthermore, a sustained enhancement in UPDRS part III motor scores was also noted in the 12th month (38 vs. 27; $n=15$; $p=0.0001$) [156]. The clinical trials, namely NCT03562494 (AAV2-DDC, VY-AADC02) and NCT03733496 (AAV2-DDC, VY-AADC01), are currently being conducted.

Glutamic acid decarboxylase (GAD) serves as the rate-limiting enzyme in GABA production, a crucial inhibitory neurotransmitter within the central nervous system. Dysfunction within the GABA signaling pathway has been implicated in PD. The objective of this Phase 2 randomized clinical trial, which enrolled 66 patients with PD, is to evaluate the safety and therapeutic efficacy of AAV2-GAD administration in the subthalamic nucleus. Over a period of 6 months, they initially verified the safety profile and potential treatment benefits associated with AAV2-GAD [157].

Glucosylceramidase, an enzyme localized in the lysosomes and encoded by the GAB1 gene, plays a crucial role in catalyzing the hydrolysis of glucosylceramide into glucose and ceramide. Genetic defects within the GAB1 gene have been linked to PD [157, 158]. In pre-clinical models, enhancing the expression of GAB1 may hold therapeutic implications for PD [159]. An ongoing clinical trial (NCT04127578) is utilizing AAV9 carrying the GBA1 gene to evaluate its safety and efficacy against GBA1 deficiency-related PD. Additionally, a clinical trial based on ASO gene therapy BIIB094 (NCT03976349) is currently underway for LRRK2 gene-related PD [160].

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the central nervous system, which can be divided into sporadic and familial forms, with sporadic cases accounting for approximately 85% of all instances. In addition to the previously acknowledged monogenic inheritance pattern, polygenic inheritance involving multiple genes also significantly contributes to the onset and progression of ALS. Several ALS-related genes have been identified, including penetrant genes such as C9orf72, TARDBP, SOD1, FUS, and genes that confer susceptibility to the disease but do not directly cause it, such as ANG, ATXN2, and DCTN1 [161]. Notably, mutations in the SOD1 gene account for approximately 15% of familial ALS cases [162]. Clinical trials have utilized ASO drugs targeting SOD1 while gene therapies aimed at other causative genes for familial ALS are currently under investigation [163, 164]. Ataxin-2 (ATXN2) is a multifunctional RNA binding protein, primarily serving as a regulatory factor in stress granule assembly. It has been established that the expansion of

trinucleotide repeats in ATXN2 can result in spinocerebellar ataxia type 2 (SCA2). Intermediate expansions that do not reach the SCA2 threshold are now recognized as a risk factor for ALS [165]. Additionally, ATXN2 modulates TDP-43 activity and the mutated variant of TDP-43 aggregates into insoluble deposits within brain and spinal neurons. Notably, TDP-43 localizes to atxn2-dependent stress granules, representing a common pathological hallmark [166, 167]. Consequently, gene therapy targeting ATXN2 holds promise not only for addressing ATXN2-related ALS but also for treating other prevalent forms of ALS, particularly sporadic non-familial cases. The ongoing clinical trial (NCT04494256), known as the ALSpire Study, aims to investigate the safety, tolerability, and efficacy of BIIB105 in adult patients with ALS. BIIB105 is an ASO-based drug designed to target ATXN2.

Cancer

The underlying molecular mechanisms of cancer are highly intricate and exhibit a significant degree of heterogeneity among diverse individuals. Currently, conventional treatments encompass surgical intervention, radiation therapy, and combined chemotherapy, while gene therapy heralds a new era in the field of cancer treatment. The current strategies for cancer treatment include suicide gene therapy, oncolytic virus gene therapy, activation of tumor suppressor genes, inhibition of oncogene activation, anti-angiogenesis approaches, immunotherapy, and treatments targeting the tumor microenvironment, as well as therapeutic cancer vaccines [168–171] (Fig. 4).

Suicide gene therapy employs a carrier to encode a specific enzyme capable of activating the precursor drug, which is subsequently delivered to the tumor cells. The patient then receives the chemotherapy drug in its precursor form, prompting transcription and translation processes within the cell to generate the enzyme required for activation. Consequently, this mechanism effectively eradicates malignant cells [172]. To date, the herpes simplex virus thymidine kinase/ganciclovir system (HSV-TK/GCV) stands as the most extensively utilized suicide gene therapy system [173].

Tumor suppressor genes, including Rb1 (the pioneering anti-cancer gene identified in retinoblastoma), P53, MLH1, APC, MLH1, BRCA, etc., are associated with the development of various cancers due to their inactivation mechanisms involving post-transcriptional modifications [174]. The first approved gene therapy product, Gendicine [175], is a recombinant human P53 Adenovirus Type 5 injection. It introduces the therapeutic gene P53 into target cells and expresses the tumor suppressor gene P53 in these cells, thereby augmenting the body's cancer

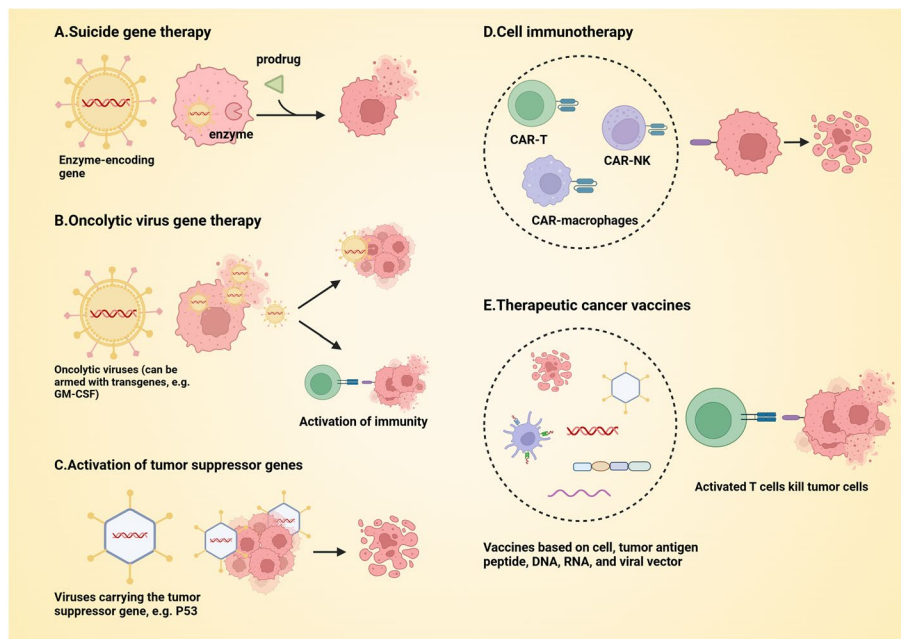


Fig. 4 Strategies for tumor gene therapy. GM-CSF: Granulocyte–macrophage colony-stimulating factor; CAR-T: Chimeric antigen receptor T-cell; NK: Natural killer cell. Created with BioRender.com

inhibition mechanism to effectively eradicate tumors. This treatment is approved for head and neck tumors.

Oncolytic viruses, whether natural or modified, have the ability to selectively infect and eradicate tumor cells while sparing normal cells. Among them, adenoviruses and HSV have been extensively investigated. The mechanisms by which oncolytic viruses eliminate tumor cells include direct lysis of infected cells, induction of damage-associated molecular patterns, and pathogen-associated molecular patterns to trigger immune responses in the body [176]. Furthermore, engineered oncolytic viruses can be armed with transgenes such as granulocyte macrophage colony stimulating factor (GM-CSF) [177], interleukin-12 (IL-12) and decorin co-expression [178], CD40L and 4-1BBL co-expression [179], cytosine deaminase [180] and tumor necrosis factor- α (TNF α) [181] to enhance their efficacy in eradicating tumors. Approved in 2005, Oncorine is the pioneering oncolytic virus product, which is a recombinant adenovirus type 5 injection liquid developed for the treatment of nasopharyngeal cancer [182]. Imlygic, the first oncolytic virus product sanctioned by the U.S. FDA, is an attenuated herpes simplex virus type 1 (HSV1) that has been genetically modified to selectively replicate within tumor cells and express the immune-stimulating protein M-CSF for melanoma therapy [183].

Therapeutic cancer vaccines targeting neoantigens represent a promising avenue for personalized cancer treatment. The majority of cancer patients harbor distinct

mutated antigens, which can arise from single nucleotide mutations, insertions, gene fusions, or splicing alterations. These mutations give rise to neoantigenic epitopes that are recognized by T cells and elicit robust anti-tumor immune responses leading to tumor cell eradication. These antigens may exist as singular entities or as combinations of multiple antigenic sites, and they can be shared across different types of tumors [184]. Currently, despite the longstanding proposal of therapeutic cancer vaccines, most of them remain in preclinical or clinical trial stages, indicating a substantial journey ahead before their clinical application can be realized. For instance, Ugur Sahin and colleagues have developed a lipid nanoparticle-based RNA vaccine encoding four frequently observed tumor-associated antigens (NY-ESO-1, MAGE-A3, tyrosinase, TPTE) for melanoma treatment. In this phase I trial, researchers have provided preliminary evidence supporting the feasibility of this vaccine [185].

Since the approval of the first CAR-based cell immunotherapy, Kymriah, in August 2017, a new era has emerged in cancer treatment [186]. Subsequently, the field of gene modification combined with cell immunotherapy has witnessed rapid advancements. Traditional CARs typically comprise four distinct structural regions: 1. A target binding domain, such as a single-chain variable fragment (scFv), responsible for conferring specificity in recognition; 2. A hinge region facilitating flexibility and proper orientation of the CAR construct; 3. A transmembrane domain enabling anchoring of the

CAR onto the cell membrane; and 4. Intracellular signaling domains encompassing costimulatory molecules and CD3 ζ signaling motifs. Currently, the fifth generation of CAR has emerged, primarily focusing on enhancing intracellular signaling regions to augment immune cell activation and cytotoxicity [187]. Furthermore, diverse iterations of CAR are also under development, including Bivalent CAR, LINK CAR, Stealth CAR, and SNIP CAR, among others [187]. The initial modification of immune cells focused on T cells; however, there has been a recent inclusion of other immune cell types such as NK cells and macrophages in the realm of cellular immunotherapy [188]. The treatment of hematologic malignancies has witnessed significant advancements with the remarkable progress achieved by CAR-T therapy. The currently approved CAR-T products mainly target CD19 and BCMA. According to the analysis of long-term therapeutic effect data, therapies targeting CD19 demonstrate objective remission rates ranging from 44 to 91% in patients with relapsed or refractory B-cell lymphoma or chronic lymphocytic leukemia, and complete remission rates ranging from 28 to 68% (follow-up time \geq 24 months, time range: 24–123 months). In the case of B-acute lymphoblastic leukemia patients, the initial remission rate ranges from 62 to 86% (follow-up time \geq 1 year, time range: 1–4.8 years). Limited available data exists for therapies targeting BCMA in patients with relapsed or refractory multiple myeloma; however, overall response rates are reported at levels between 73 and 100%, while complete remission or stringent complete remission rates range from 33% to 83% (follow-up time \geq 1 year, time range: 13–48 months) [189]. Currently, CAR-T therapies for solid tumors have not yet received market approval and are still in the clinical trial phase. The targets under development, based on the high expression of antigens in various tumor cell types, encompass HER2, IL-13R α 2, GD2, ROR1, EGFR, CEA, Claudin-18, and MUC1, among others. The identification of specific tumor antigens poses a significant challenge for CAR therapy in the context of solid tumors [190]. Furthermore, efficient delivery and infiltration of CAR into the tumor microenvironment remain formidable obstacles that need to be overcome. Additionally, preventing tumor cell escape and mitigating the immunosuppressive effects exerted by the tumor microenvironment present further challenges [191].

CRISPR technologies offer potential *ex vivo* therapies for the treatment of human cancers by engineering cellular immunotherapies. For instance, T cells can be modified using CRISPR-Cas9 gRNA-mediated knockout to specifically target PD1 or co-target the T cell receptor, inactivate immunosuppressive factors, and/or integrate CAR elements into the T cell receptor α -chain constant

locus for CAR T-cell engineering. These strategies have already progressed to clinical trials. However, *in vivo* CRISPR therapies are still in pre-clinical stages due to challenges such as efficient and selective delivery and editing [192].

Endocrine and metabolic diseases

Diabetes

Diabetes is traditionally classified into two types, namely Type 1 diabetes (T1D) and Type 2 diabetes (T2D). T1D is characterized by the autoimmune destruction of insulin-producing beta cells in the pancreatic islets, whereas T2D is characterized by cellular resistance to insulin, resulting in relative insulin insufficiency. In recent years, it has been recognized that both T1D and T2D share a common underlying mechanism involving functional impairment of beta cells; however, these mechanisms differ between the two types. The former primarily involves immune-mediated pathways leading to early-stage beta cell destruction, while the latter predominantly involves metabolic pathways with subsequent cell loss occurring at a later stage [193]. Over the past decade, more than 75 genetic signals associated with T1D have been unveiled through GWAS, including variations in HLA alleles [194]. Additionally, over 400 genes linked to T2D, such as AP3S2, GRB14, and TCF7L2, have been identified [195–198]. Currently, gene therapy for diabetes remains at its nascent stage.

The plasticity of pancreatic islet cells has been demonstrated by research findings. Studies conducted by Takaki Matsuoka and colleagues have revealed that Pdx1 can induce the differentiation of neurogenin 3 positive endocrine progenitor cells into insulin-positive cells, as well as transdifferentiate glucagon-positive α cell populations into β cells. Furthermore, MafA can enhance the functionality of Pdx1 in this process, offering promising prospects for diabetes treatment [199]. Subsequently, Xiangwei Xiao and collaborators utilized AAV to deliver Pdx1/MafA, successfully reprogramming α cells into β cells in mice and effectively normalizing blood sugar levels in diabetic model mice [200, 201]. Building upon these advancements, Genprex is currently investigating the therapeutic potential of GPX-002 (AAV-Pdx1/MafA) for treating T1D. As of November 14th, 2023, according to an official website announcement, this trial remains at the preclinical stage [202].

Stem cell therapy has demonstrated advancements in the field of diabetes treatment, exemplified by VX-880. However, the efficacy of stem cell therapy may be hindered by transplant rejection in recipients. VCTX210 integrates stem cell therapy with CRISPR-Cas9 gene editing technology to modify stem cells, thereby disrupting or introducing genes associated with immune rejection

(B2M, PD-L1, HLA-E) to achieve immune evasion. Currently, VCTX210 is undergoing phase 1 clinical trials and administration to the first patient has been completed [203]. The other investigational drug GARV-AAV2-A20, currently under research, targets distinct genes [204]. The TNFAIP3 gene encodes the ubiquitin-editing enzyme A20, which modulates immune cell activation by elevating the threshold of NF- κ B signals. Overexpression of A20 effectively mitigates inflammatory mediators, thereby safeguarding islet transplants against inflammatory assaults [205]. Currently, these drugs are still in the nascent stages; however, over the course of the next decade, they hold immense potential to revolutionize diabetes treatment.

Obesity

Obesity can be classified into polygenic obesity, monogenic obesity, and obesity syndrome based on genetic characteristics, with polygenic obesity being the most prevalent form. The development of polygenic obesity is influenced by a combination of polygenic factors and environmental factors. Rare monogenic obesity primarily involves gene defects related to the leptin-melanocortin pathway, such as the LEP gene encoding leptin, the LEPR gene encoding leptin receptors, and SH2B1. SH2B1 encodes the important SH2B adapter protein 1 that plays a crucial role in mediating leptin-induced signal transduction [206]. Gene therapy for treating obesity is still at an early stage.

In mammals, two distinct types of adipose tissue exist: white adipose tissue (WAT) and brown adipose tissue (BAT). While WAT primarily stores excess fuel, BAT expresses the unique uncoupling protein 1 (UCP1) and possesses the characteristic of energy dissipation. Therefore, activating BAT can increase energy consumption and become a potential target strategy for treating obesity. Chih-Hao Wang and his colleagues utilized the CRISPR-SAM system to activate UCP1 expression in white fat cells, promoting the transformation of WAT into BAT, resulting in significant therapeutic effects in a mouse model. The CRISPR-SAM system has demonstrated its ability to activate endogenous gene expression [207]. Ruhang Tang et al. utilized adeno-associated virus serotype 9 (AAV9) as a vector for delivering the FST gene, and observed its preventive efficacy against high-fat diet-induced obesity [208]. In addition, a recent large-scale exome genome sequencing study involving 645,626 individuals has identified GPR75 as being significantly associated with a lower body mass index. Subsequent investigations using GPR75 gene knockout mice have demonstrated resistance to obesity [209]. Therefore,

targeting the downregulation of GPR75 expression may hold promise as a potential therapeutic strategy for combating obesity.

Other diseases

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by symmetrical, polyarticular inflammation of small joints. The pathogenesis of RA remains unclear, and it can affect multiple organ systems [210]. Autoimmune reactions and inflammation are the primary mechanisms involved, encompassing various signaling pathways such as JAK-STAT, MAPK, PI3K-AKT, Wnt, Notch, and NF- κ B signaling pathways. Additionally, epigenetic modifications also play a crucial role in the onset and progression of RA. Understanding these molecular mechanisms offers a plethora of targets for drug development, encompassing the development of various small molecule inhibitors such as Ruxolitinib, a JAK1/2 inhibitor, VX-702, a p38 MAPK inhibitor, and SHR0302, an exceptionally selective JAK1 inhibitor [211]. In contrast to these small molecule drugs, gene therapy for RA is currently in its nascent preclinical stage. Haobo Han et al. have developed a fluorinated polyamidoamine dendrimer carrier that can be targeted to inflamed joints via intravenous injection for the delivery of miR-23b, a non-coding oligonucleotide that regulates gene expression at the post-transcriptional level. By modulating the NF- κ B pathway in proliferating synovium, miR-23b promotes anti-inflammatory and anti-proliferative responses [212]. Additionally, Fang Wang et al. have employed an AAV carrier to co-deliver TNFR-Fc/CTLA4-FasL (AAV-TFCF), which represents another promising gene therapy strategy [213]. The single nucleotide polymorphisms (SNPs) of protein tyrosine phosphatase non-receptor types 2 and 22 (PTPN2/22), specifically PTPN2:rs478582 and PTPN22:rs2476601, may be associated with hyperimmune response and exacerbation of inflammation in RA patients. These SNPs may serve as potential therapeutic targets for managing RA [214]. Moreover, MYC and FOXO1 genes, SNP rs6927172, TNFAIP3, OLIG3 gene, and miR-155 were identified as promising candidate genes for CRISPR-Cas9 therapy [215].

Osteoarthritis

Osteoarthritis (OA) is a chronic and progressive disease characterized by the degeneration of joint cartilage, clinically presenting as joint pain and stiffness. Despite its prevalence among more than 500 million individuals, effective treatments for OA are currently lacking [216, 217]. Gene therapy for OA is being actively investigated. Follistatin (FST) functions as a potent inhibitor of muscle growth and an activin binding protein [218]. In their study, Tang et al. employed AAV9-mediated delivery of the FST gene to mitigate inflammatory factors in joint

synovial fluid, suggesting that this pathway may hold therapeutic potential for injury-induced OA [208]. The clinical trial NCT05835895 is a phase 1b study designed to investigate the safety, tolerability, and efficacy of GNSC-001 Gene Therapy. GNSC-001 is an AAV-based gene therapy product that carries a gene encoding interleukin-1 receptor antagonist (IL-1Ra). Through intra-articular injection, it transduces cells to produce IL-1Ra, exerting anti-inflammatory and joint protective effects for the treatment of OA. Currently, in the recruitment phase since June 12, 2023, this trial is anticipated to conclude in 2028. In this study [219], Zhao Lan et al. demonstrated that the CRISPR-mediated deletion of nerve growth factor (NGF), matrix metalloproteinase 13 (MMP13), and IL-1 beta genes in a mouse model of post-traumatic OA preserved joint pain management and joint structure. The utilization of CRISPR/Cas9-based gene editing presents a promising therapeutic approach for the treatment of OA.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is characterized by autoimmune response-mediated inflammation and organ damage, predominantly affecting young women. Given the pivotal role of B cells in self-antigen recognition during its pathogenesis, gene-engineered immune cell therapy has recently emerged as a promising approach for SLE treatment [220]. In the study conducted by Andreas Mackensen et al., a cohort of 5 SLE patients, who were unresponsive to several immunosuppressive agents, was included. Lentiviral vectors were employed for transducing patient-derived T cells with anti-CD19 CARs. Following lymphocyte depletion pretreatment, the modified T cells were reinfused into the patients at a dose of 1×10^6 CAR T cells per kilogram of body weight. Over a median follow-up period of 8 months, sustained remission without medication was achieved [120]. These findings highlight the potential application of genetically engineered cellular immunotherapy in autoimmune diseases alongside its well-established therapeutic efficacy in cancer treatment. Currently, multiple ongoing clinical trials are investigating potential treatments for recurrent refractory SLE patients. These trials include CAR-NK therapy targeting CD19(NCT06010472), CAR-T therapies targeting both CD19 and BCMA (NCT05858684, NCT05474885), as well as CAR-T therapy specifically targeting CD19(NCT06056921).

Future prospects

In the past decade, significant advancements have been achieved in the field of gene therapy, particularly in monogenic diseases and hematological malignancies, with the approval of numerous therapeutic products. Simultaneously, promising prospects have emerged for

non-monogenic or complex diseases. By employing comprehensive whole-genome association studies, mendelian randomization analysis, single-cell sequencing of the genome, genetic association research, artificial intelligence machine learning techniques, and other methodologies, we are progressively unraveling the underlying pathogenic mechanisms of complex diseases [221–224]. This approach enables us to effectively identify pathogenic genes implicated in multi-gene disorders and offers novel therapeutic targets for complex disease management. Simultaneously, the progress in genetic engineering technology enables us to intervene in diseases at the DNA or RNA level, supplementing targets that are beyond the reach of conventional drugs. Furthermore, the development of gene therapy products relies on carriers exhibiting high efficiency, robust specificity, low immunogenicity, potent loading capacity, and prolonged duration. The advancement of these carriers is imperative, both viral and nonviral. For instance, in vivo gene therapy extensively employs AAV carriers that can be optimized and modified to acquire exceptional therapeutic properties through approaches like natural isolates, directed evolution, and artificial intelligence-assisted design [225, 226]. For monogenic diseases, the ultimate objective is to achieve long-term or even lifelong cures, as these diseases typically manifest symptoms from birth. Conversely, polygenic and complex diseases tend to emerge later in life. Hence, our focus should not solely be on treating clinical manifestations but rather on early identification of susceptible populations for the purpose of gene prevention and therapy.

Conclusions

Gene therapy holds great promise as a treatment modality, particularly in the realm of monogenic disorders and tumors. However, with the advancement of our understanding regarding disease pathogenesis, gene therapy is now being applied to address polygenic diseases. Furthermore, given that most polygenic disorders manifest later in life (with symptoms not present at birth), it becomes imperative to identify susceptible individuals for genetic prevention prior to symptom onset. The era of gene therapy is upon us.

Public summary

1. Significant advancements have been made in the field of gene therapy, leading to the approval of various gene therapy drugs, particularly in monogenic conditions.
2. Building upon the progress achieved in gene therapy for monogenic diseases and cancers, extending its application to polygenic or complex diseases would enable targeting a broader range of patient populations.

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Authors' contributions

W.T. drafted the manuscript. T.V.L. and H. Y. revised and approved the final manuscript.

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Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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