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Genetic scissors: A new era in gene therapy

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ABSTRACT: The article gives a brief description of gene therapy, a new technology for treating genetic disorders, and the revolutionary effect of gene editing techniques which include CRISPR-Cas9. The introduction serves as a basis by covering the basics of gene therapy and the revolutionary aspect of genetic scissors as surgical instruments for genetic editing. In a brief overview, gene therapy is explained, which is meant to correct hereditary failing at their source, and subsequently ZFNs (zinc finger nucleases), CRISPR-Cas9 and TALENs are explored. The diverse applications are emphasized in the review, and the paper explores how genetic scissors are utilized in gene repair (correction of mutations which are responsible for diseases such as atherosclerosis, cancer, bone and cartilage repair etc.). It highlights the potential of genetic scissors in changing the therapeutic landscape. The future scope section provides a detailed illustration of the ever-changing capabilities and possibilities of the technology, thereby offering a glimpse of what may be around the next corner. To sum up, the review discusses the ground-breaking role of genetic scissors in gene therapy and stress the necessity of continuous research, ethical standards and collaboration to successfully apply these methods in personalized medicine and healthcare.

KEYWORDS: Gene therapy; genetic scissors; DNA modification; CRISPR-Cas9; disease treatment

1. INTRODUCTION

Gene therapy has become the game-changer in modern medicine, guaranteeing the possibility of tackling various genetic defects by addressing the causes of these disorders. The key point to the gene therapy effectiveness is the involvement in this process of genetic scissors, the cutting-edge molecular instruments designed to provide for the accurate alteration of the genome [1, 2]. Among the several ones, CRISPR-Cas9 has become very popular for its effectiveness and versatility in performing gene editing at target sites. While CRISPR-Cas9 is just one of several genetic scissors used for therapeutic purposes, it is one of the best and most effective genetic scissors to be explored for the same. We encompass here the wide genetic scissor spectrum, which includes zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and base editors, with their particular mechanisms and implementation in gene therapy in mind [3, 4]. We investigate the successes and difficulties of the utilization of genetic scissors among the different types of genetic disorders; occurring from monogenic disorders to multifactorial conditions [5, 6]. In addition, we consider emerging trends in delivery systems and the safety procedures that are predicting the future of gene therapy [7-9]. This review will bring together the current research and the latest developments in technology to give suggestions on how this technology can be used to come up with effective treatment of genetic diseases and opening the way for personalized precision medicine.

2. HUMAN GENE THERAPY

Human gene therapy (HGT), involves transferring DNA into a patient's cells to correct defects or enhance the production of beneficial proteins. This innovative approach, in biology and biotechnology has paved the way for therapeutic methods. By harnessing advancements in microbiology, virology, organic chemistry, and various other scientific fields human gene therapy serves as a cutting-edge method of drug delivery [10-13]. It encompasses more than gene transfer; it involves a process of discovery, production, testing, and refinement to develop effective gene therapy products for human use. Gene therapy offers advantages over treatments by addressing the root genetic causes of diseases targeting specific affected cells and tissues and providing long-lasting effects from a single treatment [14-16]. The inaugural clinical trial for gene therapy took place a decade ago when patients, with combined deficiencies underwent lymphocyte

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isolation followed by *ex vivo* gene transfer to introduce the missing adenosine deaminase (AoA) gene. The choice of the delivery vector among the three types of vectors i.e. viral, non-viral, and cell-based, must be injected directly into the tissue or given systemically in the bloodstream and is critical for gene therapy to begin [17, 18]. Being the vector it has to find the targeted cell, penetrate the cell barrier, and move toward the nucleus within the cell. Next, the therapeutic gene is encoded into a strand of mRNA which is also then translated to produce the therapeutic protein. The protein moves to the bloodstream and works on the cell or nearby cells whose receptor it can bind into as well as other locations. The receptor of the protein and proteins must interact, then a biological impact has to be derived that serves to bring therapeutic benefits [19, 20].

Just before gene therapy is applied in general medicine, its products have to be therapeutically and economically outputting. This reveals that such benefits should be way higher than the effects of the ordinary treatment, which is mostly cheap. Over the past years, significant progress has been made in our understanding of molecular biology and the pathophysiology of diseases, as a result, gene therapy has attracted significant interest (Table 1) [19, 21, 22]

Table 1. (Wiley clinical trials database) lists the gene delivery mechanisms (vectors) utilized in gene therapy clinical trials from 1992 to the present

Delivery system	Number of trials
Retrovirus	67
Liposome	59
Adenovirus	54
Cell-based	20
Poxvirus	26
Other methods[a]	16
Naked DN	8

[a] Electroporation, gene gun, HSV, etc.

3. GENETIC SCISSORS

Genetic Scissors include molecular instruments that utilize the strategic cutting and modifying of genetic sequences within DNA. Scissors are the tools used to make changes to the genetic code on a targeted basis, i.e. correcting mutations, insertions, or disruptions of the harmful genes. As a result of the insertion of these molecular scissors into cells, scientists can overcome genetic defects by repairing modifying, deleting, or changing a disease-causing gene or genes. The premise of the genetic scissors lies in the peculiar ability to edit DNA down to the base-pair level which makes it a potential candidate for the development of newer and more personalized therapies for many genetic disorders [23]. Some examples of genetic scissors are as follows:

CRISPR-CAS9

The word CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat), is used to refer to the organization of the repeated and short DNA elements in the genome of the prokaryotes, and this organization is unique. Genome engineering is a synonym for a general process of the intentional manipulation of a genome and/or its contexts (e.g., epigenetic marks) or products (e.g., transcripts) [13]. Eukaryotic cell culture technology has a broad range of applications, the most important of which is the ability to achieve fundamental science, biotechnology, and medicine transformation simply and effectively [19]. CRISPR-Cas-9 is a defense mechanism that helps prokaryotes to autoimmunity to drive off viruses and bacteriophages. Streptococcus pyogenes was the first source of Cas protein used which led to genome editing. It is a big protein that contains 1368 amino acids, acting as a "DNA endonuclease" and breaking the DNA's double strand by forming a double-strand break. Three basic steps make up the CRISPR/Cas-9 genome editing mechanism: identification, differentiation, and problem-solving [21, 24]. The C5rRNA-matching property of the sgRNA operationalized by the engineered Cas-9 is exploited to direct the cleavage of the target sequence in the gene of interest. Only when sgRNA is provided can CAS-9 protein begin its work. The Cas-9 nucleases are positioned upstream of the PAM and later result in the formation of DSBs through DNA DSBs. The number of base pairs (bp) in the PAM sequence is 14 in the short range (2–5 bp) which can vary for a specific bacteria. The open pit is located upstream from the stream. Afterward, the Cas-9 is bound to the target PAM allowing the DNA helix strand to melt, subsequently, the RNA-DNA hybrid is removed. The Cas-9 protein that performs the function of DNA cleavage is the protein that initiates this process. The base-pairing functionality of the 5'cRNA is used as the

backbone of a synthetic sgRNA which when coupled with Cas-9 guides the cleavage of the target gene sequence. The Cas-9 protein that causes the intended DNA break cannot operate efficiently unless sgRNA is present. The Cas9 nuclease can recognize a PAM sequence that is paired with three bases upstream and causes the DSB creation through DNA double-strand breaks (DSBs). The PAM sequence length is 14bpa (2–5bp) for the short range, and it depends on different bacteria. It is located downstream from an uncovered area. Then the Cas-9 complex is guided by a special PAM to a DNA-target and here the local DNA melting initiates, which is followed by the excision of the RNA-DNA hybrid. The Cas-9 protein which is the cutting-molecule, initiates the process. The HNH domain performs the cleavage of the complementary strand, whereas the RuvC domain does the rest of the strand. As a result, the blunt-ended DSBs are usually formed which is an intermediate of the repair process. In the end, the cellular machinery will repair the DNA ends that are broken. One of the two pathways of correcting the DSBs (double-strand breaks) which are formed by Cas-9 protein in CRISPR/Cas-9 mechanism is homologous recombination (HDR) and the other is non-homologous end joining (NHEJ). In contrast to other repair pathways that work in some cell cycle phases, NHEJ is active 24 hours a day. This exercise involves an enzymatic process called nonhomologous end joining (NHEJ), which excludes homologous DNA. Genes like HDR can be used as a homologous DNA template and may also be very precise at the same time. Here both H and S-phases are important for the replication of DNA, but this is mostly what the RNA plays a leading role. HDR requires that the donor DNA specify the sequence of interest, which is used for CRISPR-gene editing by gene therapists [22, 25, 26]. Gene modification can be achieved in 1 to 2 weeks after the target design, and clonal cells with modified genes can be derived within 2 to 3 weeks as well [27]. From CRISPR-Cas9 applications in fungal and plant science to the re-examination of the path of agricultural research, this technology has the potential to dramatically change the way we think about and conduct agricultural research. The technology will be in the future improved by designing or finding smaller Cas9 variations with more specific, even better to deliver in human cells [28].

4. ZINC FINGER NUCLEASES (ZFN)

The position of zinc finger nucleases (ZFNs), which are the most advanced molecular tools and are most commonly used in gene therapy, is on the very top of the list. A recent study shows that ZFNs may be used for the frequency of targeting up to 20% of a human species-causing gene. Future studies may as well exhibit whether zinc finger nucleases are involved in unwanted genomic instability and apply these *in vitro* findings to *in vivo* models [29]. In this way, ZFNs act as a team of two that bind specifically to the genome based on a unique DNA sequence alone. These artificial nuclease domains are constituted by a zinc finger domain DNA binding site that has been genetically engineered. FokI domains are known to dimerize and further form a site-specific DSB that is DNA-specific. It is the transition of these homology-directed repair (HDR) and non-homologous end joining (NHEJ) events, which mainly comprise the cellular DNA level repair mechanism. The frameshift mutations most often arise when a gap is created as a result of an insertion or deletion (indel) at the cleavage site during the nonhomologous end joining (NHEJ) repair process. This is the fact that one strand of DNA is cut at the site where the indels are introduced. In addition to insertions and deletions, misalignments at the cleavage site can be also caused by frameshift mutations affecting substantially the function of the gene. Most of such events are driven by NHEJ, which is the primary process of HR. While HDR is also utilized to create DSBs, a sophisticated method of homologous recombination is employed during the process, allowing for high-precision editing and the success of the cell therapy will be dependent on the ability to identify and select successfully those cells which are the ones carrying the desired genetic changes, and this step will be the key in this process. ZFN-directed gene engineering, which is a novel technology, has a great chance to offer new therapies due to its booster revolution. This kind of treatment may involve gene editing for the restoration of genes that are responsible for debilitating genetic disorders, killing genes that are associated with the disease and the creation of custom designed immune cells that are unique for each patient and could lead to more effective immunotherapeutic strategies. The advent of this latest technology heralds a new era in the history of precision medicine with immense chances of specialised treatment designs that not only help patients suffering from various genetic illnesses and disorders to overcome the diseases but also improve their quality of life quality [30-34].

An array of studies have shown that ZFN may be cytotoxic, which is a crucial drawback. It is likely that apoptotic and lethal events unrelated to target sites are the consequence of either excessive cleavage or cytotoxicity. Hence, the ZF DNA-binding domains could be viewed as a point where the target site is not perfectly recognized [35]. It is the case that highly specific ZFNs must be developed for ZFN technology to be applied therapeutically. Three clinical trials using the ZFN-based approach have been completed and are in

current progress. In one of these strategies, T cells are removed from the body of an HIV-positive individual and subjected to zinc finger nucleases that disrupt the chemokine receptor type 5 (CCR5) gene function, thus making the cells resistant to viral infection [36-37].

5. TRANSCRIPTION ACTIVATOR-LIKE EFFECTOR NUCLEASES (TALENs)

TALEs, a new kind of molecular tool, are used as a genome editing tool for gene therapy to cause a desired genome change. These engineered proteins can be DNA-binding because they encompass the nuclease catalytic domain and the transcription activator-like effector (TALE) DNA-binding domain, which can bind and recognize specific DNA sequences in the genome with a pair of TALENs. TALENs can be used in the context of scientific research and medicine to quickly and effectively edit genes. The potential of this technology is evident in the possibility of developing new therapies for hereditary diseases [38]. TALENs accomplish this because the nuclease function of their dimers enables targeted DNA recognition and double-strand break (DSB) creation, which is repaired by the cellular repair mechanisms, primarily the homology-directed repair (HDR) and the non-homologous end joining (NHEJ). NHEJ is the mediator of the repair process, which can give rise to two main mutations: frameshift mutations and gene function disruption, through small inserts or deletions introduced into the DNA. In contrast to the traditional HDR-mediated repairing which requires site replacement or insertions, the more precise HDR-mediated repairing approach utilizes the external DNA template to make targeted replacements or insertions. The downstream applications could be realized in a better way by a correct and specific selection of the cells having the genetic modifications that are needed. Molecular medicine has been brought to a revolution by a major positive event called TALEN-mediated gene engineering which can offer novel therapeutic interventions such as the cure of inherited diseases, genome editing mostly to remove disease-causing genes, and personalized engineering of immune cells for tailored immunotherapeutic approaches. These novel breakthroughs thus herald a new era of precision medicine by making it possible to create patient-specific and effective medications to treat several genetic illnesses and diseases that have plagued humanity for so long [39-40]. The evaluation protocols and procedures for the created TALENs and their derivatives are additional hurdles that make TALENs be a technology which is not standardized in the application to diverse model organisms. At present, the study of tailor-made TA-LENs is mostly being tested in yeast based assays and in vitro cleavage assays [41]. Genetic technologies with advantages and their limitations are mentioned in Table 2.

6. DIFFERENT MEDICAL CONDITIONS TREATED BY GENE THERAPY

All the different medical conditions treated by gene therapy are mentioned in Table 3.

6.1. Bone and cartilage repair

Working long hours on a computer or standing for extended periods puts a strain on the body and may lead to musculoskeletal disorders, which is an important matter for public health [42]. In the US more than 66 million people visit doctors for musculoskeletal diseases and the medical expenditure is expected to be $873 billion annually [43]. The trend toward the management of difficult musculoskeletal injuries and pathologic conditions in compromised biological environments continues, so there is still a need for accurate clinical treatment methods. This is despite the progress in medicines, implant materials, and surgical techniques. The predisposing factors are, for instance, biomechanics changes, inflammation, and traumas. Patients with cartilage defects may require surgical treatment as a conservative treatment that is often applied does not give the expected outcomes [44]. In orthopedics, the local problems of articular cartilage remain unsolved. These lesions do not heal by themselves, but no complete cartilage regeneration occurs (there is no lasting regeneration) when treatment is not given [45]. The genetic knife is of great significance in the process of bone and cartilage repair through the correction of gene mutations that cause skeletal disorders. Scientists are aiming to fix the genetic errors that are responsible for diseases like osteogenesis imperfecta, achondroplasia, and other skeletal malformations by going after the particular genes that cause the disease. This method provides an opportunity to prevent aggravating or even to reverse the

Table 2. Genetic technologies with advantages and their limitations

Sr. No	Technology	Advantages	Limitations	Ref
1	Human Gene therapy	• It involves transferring DNA into a patient's cells to correct defects.	• Unwanted immune system reaction.	14-16

2	Genetic scissors	• It addresses the root genetic causes of diseases targeting specific affected cells and tissues and providing long-lasting effects from a single treatment. • Used for correcting mutations, insertions, or disruptions of the harmful genes • Overcome genetic defects by repairing modifying, deleting, or changing a disease-causing gene or genes.	• Genetic testing can provide only limited information about an inherited condition • CRISPR/Cas9 raises ethical concerns • Some cells, like neurons and muscles, can't be removed from the body, making them unsuitable for gene editing	23
3	Zinc finger nucleases (ZFN)	• ZFN-based approach e.g. used where T cells are removed from the body of an HIV-positive individual and subjected to zinc finger nucleases that disrupt the chemokine receptor type 5 (CCR5) gene function, thus making the cells resistant to viral infection	• It may be cytotoxic, which is a crucial drawback	35-37
4	Transcription activator-like effector nucleases	• TALENs are highly specific. • TALENs can be used to study cell mutations and investigate gene function.	• TALENs require a thymine to be present before the 5' end of the target sequence • TALENs have a risk of producing false-negative and false-positive results	39-40

development of bone deformities. Not only does it enable the targeting of the crucial signaling pathways and regulators responsible for the growth, homeostasis, and repair of cartilage and bone, but it also facilitates manipulation of the same. Concerning the genes involved in osteogenesis, chondrogenesis, and matrix remodeling, researchers could modify these genes and the processes involving differentiation, proliferation, and synthesis of extracellular matrix. This particular regulation of gene expression offers great opportunities to develop pharmacological strategies for treating many diseases. The possibility to manage gene expression with great precision could be a game changer in the field of orthopedics, since it could be used to prompt tissue regeneration in bone fractures, osteoarthritis etc [39, 40, 46]. A tissue looking like hyaline cartilage of the joint can be successfully used to restore cartilage when a growth factor cDNA is transferred to perichondrium-derived mesenchymal cells in a model of partial-thickness defects [47].

6.2. Cancer

Tumor suppressor genes and oncogenes are the two major gene groups that are related to the process of carcinogenesis. The former stem from cell progression mechanism while the latter uses an apoptosis process to destroy the malignant cells [48, 49]. CRISPR-Cas9, gene editing technology that is being assessed for the purpose of cancer research and therapy, is the most relevant for colon cancer. This tool, as a pair of molecular scissors, performs the task of snipping out DNA sequences of the genome allowing it to introduce desired traits. One of the major ways scientists can inhibit cancer-propagating pathways is by gene-targeted editing, which can be targeted to oncogenes and reactivate tumor suppressor genes [50]. This technology has an extraordinary promise to dive into the molecular basis of cancer using the single genes approach of those found to be involved in cancer development. Thanks to the use of patient-specific models of cancer and CRISPR-Cas9 screens, it is possible to discover new drug targets and the technology to design more efficient personalized treatment schemes that are unique to each individual. On the other hand, through the use of CRISPR-Cas9 technology, it is possible to alter immune cells for enhanced recognition of and removal of cancer cells. While there are promising chances of these technologies, more research must be carried out with safety in mind and clinical application for colon tumours, as well as other malignancies [51, 52]. Over the last two decades, cancer gene therapy has become a fast-moving area. These times are characterized by having some drugs on the market, but others are still in clinical trials [53, 54].

Table 3. Diseases with treatment remedy

Sr. No	Diseases	Treatment	Ref
1	Musculoskeletal disorders	Genetic scissors	[42]
2	Skeletal disorders / Articular cartilage lesions	Bone and cartilage repair through the correction of gene mutations with the help of genetic knife	[45]
3	Colon cancer	CRISPR-Cas9, gene editing technology, make use of molecular scissors, that performs the task of snipping out DNA sequences of the genome allowing it to introduce desired traits.	[50]
4	Atherosclerosis cardiovascular disease	CRISPR/Cas9 therapy	[58, 59]
5	Paroxysmal autoimmune disease type 1 diabetes	Gene editing techniques presented by CRISPR-Cas9 RNP	[66]
6	Hearing loss	Gene therapy with genetic editing techniques	[71, 72]

6.3. Atherosclerosis

Among the whole world, atherosclerotic cardiovascular diseases remain the leading cause of death even though the therapy has gone a long way and there are many developments [55]. Considering that for years we have been researching to understand the fundamental steps of atherogenesis, reperfusion-induced cardiac damage, and ischemic heart failure, many target genes have been identified as the ones that can be taken as important in the initiation and progression of atherosclerotic vascular disease [56, 57]. One of the preventive features of CRISPR/Cas9 therapy for atherosclerosis is gene editing, which helps a specialist to focus on the direct cause of this condition – which is the underlying genetic factors. Atherosclerosis is a disease which is characterized by the same plaque that is deposited in the arteries and this later leads to the arteries narrowing down, heart attacks, and stroke. Researchers are now able to perform the CRISPR/Cas9 gene editing technique using which they can target mainly those genes which are responsible for the lipid metabolism, inflammation, and plaque formation. This approach creates an opportunity for the introduction of a high-accuracy tool that adjusts the genetic characteristics correlated to atherosclerosis which can, in turn, stop the disease evolution and thus, significantly lower the risk of cardiovascular events. On the other hand, the researchers are developing methods that eliminate the use of viruses as a delivery system. This might be done through nanoparticles or lipid-based carriers to deliver the CRISPR Cas9 system components to the targeted cells that are bordered by the arterial wall. Current research has it as experimental but an interesting strategy to mitigate atherosclerosis and its downstream diseases. For animal trials to be successful, more studies have to be made to give an answer to which mode of delivery is the best, how to make sure the treatment is effective and also the safety concerns that might come up before clinical translation [58, 59].

6.4. Diabetes

Paroxysmal autoimmune disease type 1 diabetes (T1D) is based on the destruction and death of insulin-producing β-cells in pancreatic isles by autoreactive cytotoxic T-lymphocytes, which results in insulin deficiency and increased blood sugar levels [60]. However, thanks to the Human Genome Project and advanced molecular biology, gene therapy. The main target of gene therapy for diabetes mellitus (DM) remains to keep blood sugar within normal range with normal food consumption [61]. Despite a lot of progress already being made in each of these areas, the strategy is always met with a malicious immune response that often leads to the islets disappearing [62]. Exogenous insulin administration can no doubt ameliorate but not eliminate these conditions that ultimately lead to a high mortality rate among such individuals [63]. Gene editing is a powerful tool and the key to understanding diabetes at the level of genetic regulatory mechanisms that control beta cell function and the risk of diabetes. The most recent findings in the gene therapy field concerning the transcriptional cascade responsible for β-cell growth and developmental biology provide us with the tools to apply both *ex vivo* and *in vivo* gene therapy techniques together with cell therapy in animal models of diabetes [64, 65]. An experiment, through which a CRISPR-based intervention strategy was successfully implemented to primary human islet cells. By injecting Cas9 ribonucleoproteins (Cas9 RNP) complexes and guiding RNAs into the cell systems, scientists could target both coding and non-coding regions related to diabetes susceptibility. Using this approach, we found genetic targets interacting with elements containing diabetes-risk single nucleotide polymorphisms (SNPs), and this was the first step in the study of their functional significance in the context of diabetes pathogenesis. This investigation singled out the sMPHOSPH9 gene enhancer, establishing the correlation between diabetes susceptibility and this gene. In

addition, directing CRISPR-Cas9 RNP to cis-regulatory elements within the PCSK1 gene proved to be an effective method for editing important regulatory regions that are relevant to insulin processing. The combined insight of this multi-level approach showed that beta cells' control of PCSK1 and insulin secretion was compromised, pinpointing the key role of genetic regulation in the development of diabetes. Consequently, gene editing techniques presented by CRISPR-Cas9 RNP are strong instruments in finding out the genetic basis of diabetes which will lead to the development of new treatment approaches to help in diabetes management as well as treatment outcomes [66]. The most recent Diabetes Control and Complications Trial established a new paradigm for the treatment of this disease and provided strong scientific evidence of the benefits of strict control of blood glucose levels. Now, there are only two methods for strict control available: the first is using insulin injections multiple times in a day and the second is graded hormone infusion through programmable pumps, both of which require close communication between the patient and the physician and high self-discipline [67].

6.5. Hearing loss

The most commonly identified sensory deficiency among humans is hearing loss, the most prevalent chronic illness in the world. By the year 2050, unfortunately, one out of every ten people will have a hearing problem that is debilitating [68]. To date, more than 25% of adult-onset or progressive hearing loss and the majority of congenital hearing impairment are due to inherited hearing impairment. Uncertainty remains as there is no medication yet discovered that can cure inherited deafness, although more than 130 genes have been linked with the disease [69]. Gene therapy by which a defective gene is replaced with a functional one was shown in mice with key features of the human disease deafness, which was the focus of several recent preclinical studies [70]. The use of gene editing techniques represents a unique medical development that has significantly changed the chances of such an approach being applied to humans, however, many issues need to be resolved. These challenges range from finding the most critical therapeutic time windows to validating the longevity and safety of the treatment and improving its efficiency [71, 72]. As anatomical isolation of the cochlea and the blood-labyrinth barrier that separates it from the systemic bloodstream are the reasons for these therapeutic modalities to be tested in the inner ear. Thanks to the peculiar property, it is a good gene therapy option for it reduces the probability of any possible off-target systemic dispersion. Thus, therapeutic vectors can be directly injected into the cochlea's fluid space, which in turn will diffuse along its entire length to the target cell type [73]. Currently, the most used gene-based strategy for treating monogenic hearing disorders is the replacement of a faulty gene with a normal one [74]. Although the latest developments in AAV-based gene therapy have been promising, more investigations are necessary before the FDA and EMA approve the procedure and make it a therapeutic option for hearing loss patients [75-78]. This is generally related to the peculiarities and limitations that characterize the novel technique, which is currently being applied in the field [79]. Gaps in our understanding remain in terms of the potential side effects, immune response to CRISPR/Cas9, and appropriate duration of treatment, which are still largely unknown, especially in humans. The upcoming clinical trials and the ongoing research will play a main role in uncovering part of these remaining questions [80-84].

7. CONCLUSION

To sum up, the review has shown that through knowledge of genetics, humans are able to use gene therapy like precision "genetic scissors" to overturn various medical conditions. Through a consideration of a wide range of disease areas in which gene therapy could be potentially applied, including inherited genetic disorders or acquired diseases, gene therapy turns out to be a promising future in medicine. Through the use of molecular tools, for example, CRISPR-Cas9 and other gene-editing technologies, researchers crack the complicated genetic component and create novel diagnostic and therapeutic measures for various diseases. Uncovering the mechanisms of how disease happens at the molecular level has led to the direct treatment where not only the symptoms of the disease can be improved, but also the cause of the disease. The range of conditions covered in this review suggest that gene therapy can be highly personalized and precise, with individual patients receiving treatments designed for their specific problems. While some concern areas have been addressed, technical problems together with the ethical considerations remain to be solved. Nevertheless, the collaborative works of scientists, clinicians, and policy makers have ensured that the field keeps moving forward, causing the rate of innovation in gene therapy to keep on rising, making a future for genetic therapy as the cornerstone of twenty-first century medicine a certainty.

8. FUTURE CHALLENGES

Gene delivery methods, both viral and nonviral, are used in gene therapy to try and reverse diseased phenotypes [85-86]. Considerable progress has been made in enabling long-term transgene expression and improving the efficiency, tolerability, and non-immunogenicity of gene transfer vehicles [87]. The gene editing technology is confronted with significant problems, mainly those of precision, specificity, and effectiveness. The key strategy in delivery is to enable the cells to select the intended cells and to treat the disease. Ex vivo techniques, performed outside the body, have natural advantages such as high controllability and specific quality checks of transplanted cells before they are engrafted. This is a technique that aims to repair the DNA with utmost precision, to prevent any unintentional damage using genomics as a guide. Ex vivo protein transduction facilitates shorter exposure to nuclease rather than employing DNA in vivo routes that last long. Yet, difficulties come with ex vivo manipulation processes namely the successful engraftment and immune escape of the cells. Then again, in vivo applications are associated with the risk of off-target consequences. This clearly calls for the choice of appropriate delivery vehicles and gene-targeting mechanisms in order to curtail the undesired effects. AAV delivery, which sustains CAS9 expression may cause off-target risks that continue raising for a long time, since therapy is involved [88, 89]. However, these obstacles do not rule out the possibility of further developments and improvements within these systems that would make them more suitable for clinical use. Still, this must be noted that on the other hand, ensuring long term effects and the possible balance between pros and cons calls for a complete and reflective preclinical evaluation before the actual applying of the treatment in humans [90, 91]. Gene therapy is still primarily used in research labs today, and its applications are still experimental. The majority of trials take place in Australia, Europe, and the United States [92-101].

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