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Review Article

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GENE THERAPY: CREDIBILITY OR CURSE?

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ABSTRACT

Background: Gene therapy is a promising therapeutic approach that involves introducing functional genetic material into defective or diseased cells to treat or prevent disease. Recent advances have led to significant success in managing inherited disorders such as cystic fibrosis, alpha-1 antitrypsin deficiency, hemophilia, beta-thalassemia, and sickle cell disease, and have also shown potential in treating cancers and infectious diseases, including HIV. Several gene therapy products have received regulatory approval for clinical use; however, concerns regarding their long-term efficacy and safety remain. **Methodology:** This review summarizes the current status of gene therapy by discussing major therapeutic approaches, including ex vivo and in vivo gene delivery, genome editing, somatic gene therapy, and germline gene therapy. It also examines the clinical outcomes of approved gene therapies and evaluates their associated safety challenges. **Results and Discussion:** Advances in gene therapy have demonstrated encouraging clinical outcomes, particularly for inherited genetic disorders. Somatic gene therapy has shown promising efficacy and an acceptable safety profile in approved clinical applications. In contrast, germline gene therapy still faces substantial technical, ethical, and safety concerns, limiting its clinical translation despite its potential to prevent hereditary diseases. **Conclusion:** Gene therapy represents a transformative strategy for treating genetic and acquired diseases. While somatic gene therapy has become a viable clinical option, germline gene therapy requires further technological refinement and rigorous safety evaluation before widespread clinical application. Continued research is expected to improve the safety, efficacy, and therapeutic potential of gene-based interventions.

1. INTRODUCTION

Gene therapy can be summarized as transferring genetic material to cure a disease or improve a patient's clinical condition. Reprogramming viruses into genetic shuttles that deliver a target gene into target cells is a core concept of gene therapy. Based on the kind of viral genome, these gene therapy vectors are categorized as RNA or DNA viral vectors. Many RNA viral vectors are derived from basic retroviruses such as murine

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leukemia virus. These vectors cannot transduce nondividing cells, a major limitation. This problem may be addressed by developing novel retroviral vectors derived from lentiviruses such as the human immunodeficiency virus (HIV). The most common DNA viral vectors are adenoviruses and adeno-associated viruses. Although existing vector systems can transfer genes into cells in vivo, the best delivery vehicle has not yet been identified [4]. As a result, existing viral vectors should be used with extreme caution in humans, and vector research needs further advancement.

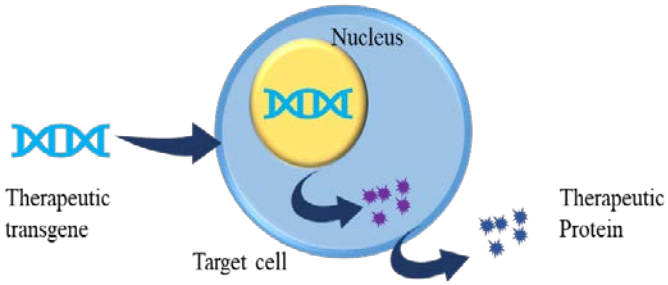


Figure 1: Basic concept of gene therapy

A gene therapy medicinal product is defined by the European Medicines Agency (EMA) as a *"Biological medicinal product containing an active substance that is a recombinant nucleic acid used in or administered to humans to regulate, repair, replace, add, or delete a genetic sequence, and the therapeutic, prophylactic, or diagnostic effect is directly related to the recombinant nucleic acid. In gene therapy medicinal products, vaccines against infectious diseases are not authorized."*

Nucleic acids are negatively charged, highly hydrophilic biomolecules. These polymers combine to form the most basic component of a living organism. The US Food and Drug Administration (FDA) defines gene therapy as products *"that are supplied as nucleic acids, viruses, or genetically modified microbes and exert their effects by transcription and/or translation of transferred genetic information and/or integration into the host genome. The products can be utilized to alter cells in vivo or to transfer cells ex vivo before administering them to the recipient.*" [5]

2. MATERIALS AND METHODS

The review searched databases including PubMed, Google Scholar, and ScienceDirect for research and review articles and books relevant to the terms or combinations of terms. We reviewed several articles and books to gain a solid understanding of gene therapy/nucleic acid-based drug delivery systems. Books on genetics helped inform the article. We collected articles on the history of gene therapy and its advantages and disadvantages.

3. GENE TRANSFER SYSTEMS AND DELIVERY STRATEGIES

Genetic materials are introduced into a patient's cells through several processes. These processes involve taking blood or tissue samples from patients and grafting them back into the patient's body [7]. The processes are discussed below, along with the diseases each currently treats.

3.1 Ex-vivo gene therapy

Ex vivo gene therapy is most commonly used with hematopoietic stem cells (HSCs), which are important in blood and immunological disorders, cancer, and inheritable conditions affecting organs and tissues readily accessible via blood cells.

Early gene therapy research used murine viral vectors and was mostly inefficient because genetically transformed HSCs were poorly retained. The first reports of successful gene therapy trials involving ex vivo transfer of c cDNA encapsulated in Moloney murine leukemia virus into autologous HSCs of patients with X-SCIDs were published in 2000. The euphoria aroused in the medical and scientific communities was rapidly quenched by the development of leukemia in multiple individuals in two distinct clinical locations in Europe. Clinical trials were canceled, and concerns about gene therapy safety were reaffirmed. The study is still ongoing to determine the success rate of gene therapy in treating leukemia.

3.2 In vivo gene therapy

In vivo gene transfer techniques may introduce the gene therapy vector directly to the desired organ or distribute it via the circulatory system into vessels supplying that organ. In vivo gene transfer offers an advantage over ex vivo techniques because it eliminates the time-consuming (and costly) process of removing cells from the patient, modifying them in vitro, and returning the genetically modified cells to the patient.

In vivo gene therapy is favorable because it is straightforward and requires only a small amount of material delivered to the brain. These characteristics make it an excellent tool for targeting a single, tiny portion of the brain. Viral entry and protein production may activate dangerous endogenous viruses,

a drawback of in vivo gene transfer. Random mutagenesis may also produce replication-competent recombinant viruses [11]. Retroviruses, for example, can incorporate themselves into the host genome and trigger inadvertent malignant modification. Even in an 'immune-privileged' organ like the brain, inflammation and immune responses are of special concern.

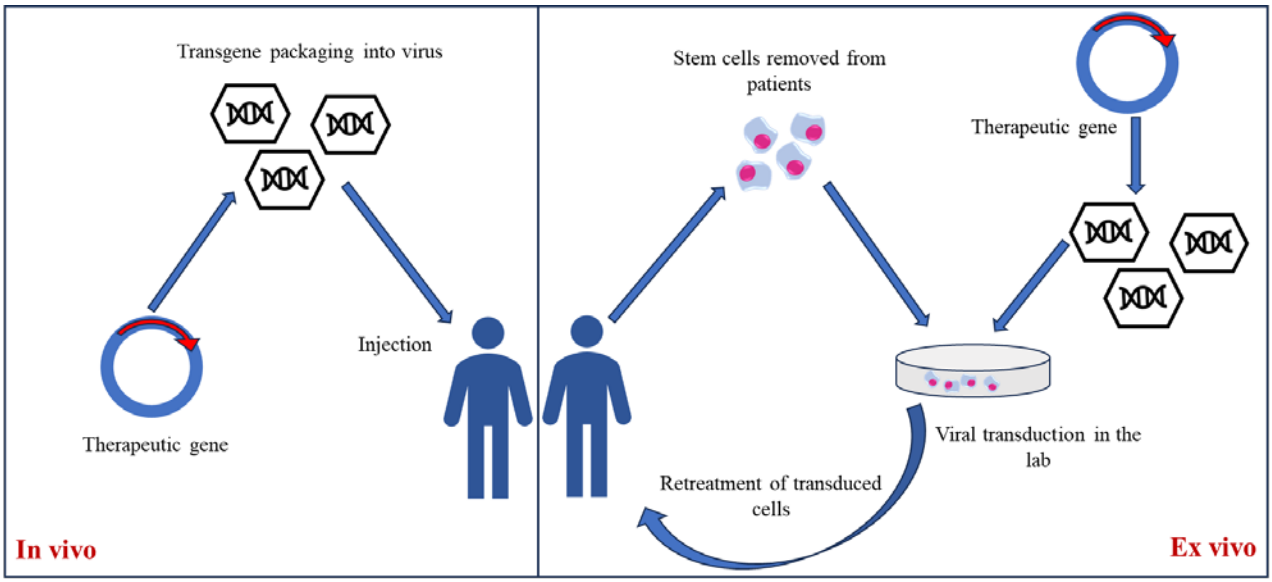


Figure 2: In vivo vs. Ex vivo

The introduction of highly adaptable genome-editing tools in recent years has allowed researchers to incorporate sequence-specific alterations into the genomes of a diverse range of cell types and species quickly and affordably. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases or mega nucleases are the fundamental technologies currently most commonly used for facilitating the editing of genomes.

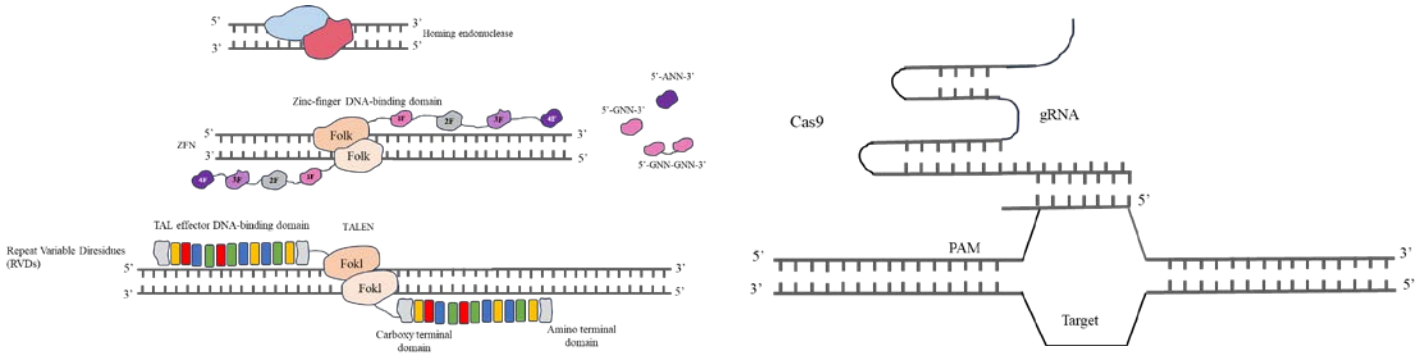


Figure 3: Genome-editing tools

3.3 Germ-line gene therapy

Germline gene therapy alters genes, so the offspring develops a new trait. Germline gene therapy is contentious. It would insert 'normal' human DNA into the parents' eggs or sperm, or into the fertilized egg or early embryo. The purpose is to alter the offspring's inherited genetic makeup.

This might be done to prevent a hereditary condition or to introduce a 'enhancing' genetic variation. No human germline gene therapy studies have been conducted, and the scientific community informally bans such research. Both its practicality and value are questionable. However, it is used in plants and animals to produce more productive and disease-free offspring.

3.4 Somatic gene therapy

In somatic gene therapy, genes are introduced into somatic cells. The ethical implications of somatic gene therapy have been intensively debated, and a consensus has formed authorizing genetic manipulation of a patient's somatic cells to treat serious illnesses.

Somatic tissue gene therapy treatments include direct complementation of nDNA, mitochondrial gene modifications, and other methods to complement pathogenic mtDNA alterations [28]. In a mouse model of Ant1 deficiency, an nDNA null mutation was successfully generated in muscles by infusion of an adeno-associated virus (AAV)-borne Ant1 cDNA. In future decades, this method will most likely be employed to treat mitochondrial myopathies and cardiomyopathies.

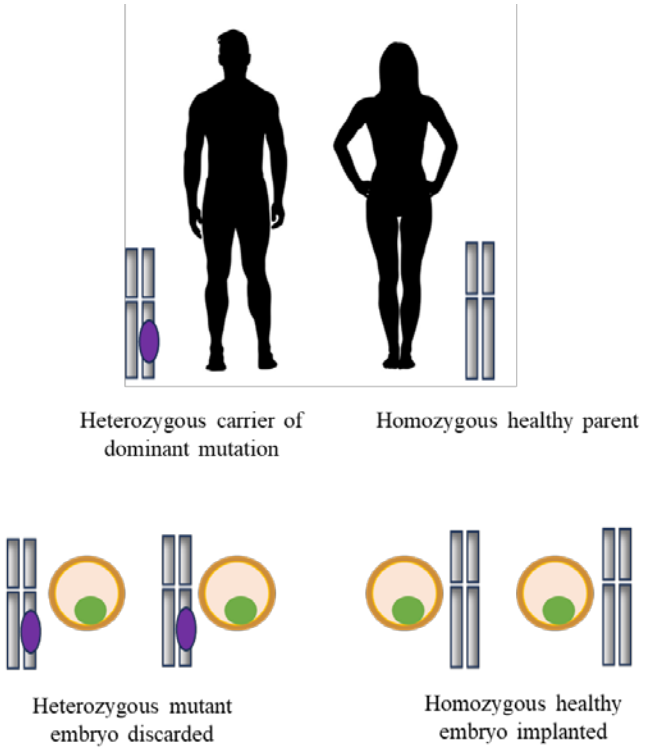


Figure 4: Germline gene therapy

3.5 Choosing the Vector

Choosing DNA or RNA viral vectors for gene therapy depends on several criteria, including treatment objectives, target-cell properties, and safety concerns. Viral vectors such as lentiviral vectors can transduce specific cell types, including dividing and non-dividing cells, and the target cell type is crucial for gene therapy. While DNA vectors, when incorporated into the host genome, provide stable, long-term expression, RNA vectors, such as mRNA, allow only temporary transgene expression. In gene therapy, safety is paramount. RNA vectors are considered safer because they do not integrate with the genome, which lowers the possibility of insertional mutagenesis. RNA and DNA vectors elicit immune responses, and mRNA vaccines provide potent but brief responses. Vector immunogenicity affects gene therapy goals and treatment success. Another crucial factor is the vector delivery technique. Certain delivery strategies may suit specific vectors better. Adeno-associated viruses (AAVs), for instance, are frequently used in in vivo gene therapy because they can infect many tissues.

3.6 Genome editing tools

The effectiveness, precision, and safety of genome editing methods such as CRISPR-Cas9, TALENs (Transcription Activator-Like Effector Nucleases), and ZFNs (Zinc Finger Nucleases) vary. Every technology offers a unique set of benefits and drawbacks. For precise genome alterations, CRISPR-Cas9 is highly effective, while TALENs are also effective but may require more design effort. ZFNs are also efficient, although they can be more difficult to design and use than other nucleases. Technological developments in CRISPR attempt to reduce off-target effects; careful design and optimization of TALENs and ZFNs can enhance specificity. Because of the potential for off-target effects, genome editing tools like CRISPR-Cas9, TALENs, and ZFNs raise safety questions. ZFNs and TALENs use comparable delivery mechanisms, while CRISPR-Cas9 uses a range of delivery methods, such as viral vectors and lipid nanoparticles. Gene therapy and functional genomics research are appealing applications for these technologies, but safety concerns must be carefully considered. TALENs and ZFNs may suit certain design parameters or delivery preferences; nevertheless, careful design and validation are necessary [46]. In clinical contexts, the choice of genome-editing techniques depends on safety, target-cell properties, and therapeutic use. CRISPR-Cas9 is widely used because of its efficacy, although other techniques are also useful.

3.7 Principle behind gene transformation

Griffith's experiment explains how a non-virulent pneumococcal strain became virulent. In that study, he infected mice with an amalgam of live bacteria from the non-virulent R form of Type I pneumococcus and heat-inactivated bacteria from the virulent S form of Type II pneumococcus [19]. The results surprised him because they revealed that the mice developed pneumonia infections and died. Griffith also extracted colonies of Type II pneumococcus S from the blood of these mice. Because the original virulent S form of Type II pneumococcus was heat-inactivated, he deduced that not only the R form but also the pneumococcal type had to change from Type I to Type II. This was a once-in-a-lifetime incident [32]. A year later, Dawson and Sia verified Griffith's findings and even developed an in vitro transformation process. Afterward, new investigations

supplemented previous efforts 21. Only in 1941 did Avery and McCarty dedicate themselves to purifying the transforming substance to identify the component responsible for transformation. McCarty and Avery finally discovered that deoxyribonucleic acid (DNA) caused the transformation. This occurred in 1944, and most geneticists, including Avery, believed that genes had to be protein-based. Since then, scientific understanding of the molecular foundation of life has changed significantly, and DNA has become an emotive topic. In 1990, the first therapeutic gene transfer was performed on an ADA patient, and China was the very first nation to approve a gene therapy-based medicinal product in 2003 [22].

Gene therapy has cured illnesses that were previously incurable or unresponsive to conventional treatments. SMA (spinal muscular atrophy), for example, is a dreadful childhood disorder. Only 8% of individuals with monogenic motor neuron illness caused by an SMN1 mutation survived 20 months without constant ventilatory assistance [34]. In contrast, in a clinical study, all children who received the gene therapy Zolgensma lived to be 20 months old with improved motor function [25].

Meanwhile, concerns about gene therapy's safety and ethics have been raised since its inception. Several experimental studies, particularly in the early days of gene therapy, failed to deliver therapeutic benefits or had unforeseen severe outcomes, including patient deaths. Fortunately, most safety issues have been resolved or will be in the near future because of major improvements in research [30]. Nonetheless, discussions on gene therapy continue, fueled in part by the development of new technologies such as germline gene editing. In this article, we will examine the history of gene therapy, the techniques used, and its benefits and drawbacks [30].

Initial gene therapy experiments for SCID-X1, an immunodeficiency disorder characterized by a deficiency of T cells, impaired B-cell function, the absence of natural killer (NK) cell proliferation, and a shortage of -chain (c) dependent cytokines, verified the concept that gene-corrected cells had an edge in selection and could thus effectively reconstitute immune competency in treated patients. However, using a murine retroviral vector for ex vivo gene transfer led to leukemia in five of the twenty people treated, raising safety concerns about retroviral vectors. In contemporary gene therapy approaches, self-inactivating (SIN) viral vectors have reduced the risk of insertion-related mutagenesis and clonal dominance [38]. Germline editing raises safety and ethical concerns, including unforeseen repercussions and societal injustices. To fully understand these risks and potential biases, more research is needed [40]. The complex concept of consent in germline editing raises concerns about future generations and the impact on social norms. The potential for designer babies and regulatory challenges requires international cooperation to ensure appropriate guidelines and governance for future generations. Major obstacles include safety management, unresolved ethical questions, equity and consent principles, avoiding social repercussions, and coordinating regulatory standards across nations [41].

3.8 Ethical dilemmas for human trials

Prioritizing safety is crucial, since preclinical findings suggest realistic safety expectations, particularly regarding unintentional DNA modifications. Researchers can manage risks by increasing them progressively and starting with smaller doses. When safety data are limited, guidelines call for scientific justification, ethical trial design, and avoiding germline cells to prevent heritable genetic changes. To prevent inheritable genetic changes without adequate safety information, most guidelines recommend restricting gene therapy to somatic cells exclusively.

Until we better understand potential risks, guidelines prefer gene therapy techniques that influence gene expression rather than make permanent DNA changes. To ensure ethical and scientific rigor, experts from a variety of fields should oversee gene therapy trials in a multidisciplinary manner and avoid enhancement uses such as intelligence or athletic ability [49].

4. RESULT AND DISCUSSION

Gene therapy has brought new hope to science. Treatment of hereditary diseases, cancer, as well as several other previously untreatable non-genetic diseases are now possible with gene therapy. Scientists have studied gene transfer methods since the early days, but technological advances have now made it possible to implement these theories.

From the studies we reviewed while writing this article, we found that although gene therapy has brought a new ray of hope in science, it also has drawbacks. There are some ethical restrictions in the practice of gene therapy. The biggest issue comes in practicing germline gene therapy in humans. In some cases, the body may not respond to the therapy, and the immune

system may attack the protein produced by the newly added gene as foreign material. While treating complex non-genetic diseases like diabetes, heart disease, and neurodegenerative disorders with gene therapy presents ethical and scientific challenges, it also presents enormous potential for advancement.

Targets in complex illnesses are ambiguous because they involve numerous genes, epigenetics, and environmental interactions. Editing more than one gene creates ethical ambiguities, challenges delivery, and increases safety hazards. Because gene editing is harder to access than treatments for hereditary disorders, it raises ethical questions in human trials. By altering disease-related pathways, reducing the need for ongoing care, slowing neurodegeneration, targeting multiple genetic factors in complex diseases, and enabling regenerative capacity to address underlying biology such as heart damage, gene therapy can treat incurable conditions. So, while major challenges remain, the potential rewards of moving gene therapy into complex disease areas could be revolutionary advances in treatment. Careful translation is warranted.

5. CONCLUSION

Gene therapy treats genetic disorders and some non-genetic disorders in a personalized manner. It also treats acquired and previously incurable diseases. It is an advanced drug delivery system that can improve quality of life by reducing a patient's dependence on traditional treatments. While gene therapy promises to treat several diseases, it also has some challenges. It is highly expensive, less accessible, and carries the risk of rejection by the body’s immune system. Gene therapy is particularly effective for hereditary illnesses caused by single gene mutations. Developing successful gene therapies for complicated, non-genetic disorders is more difficult. Ethical issues of gene therapy are also a big concern. Another challenge is insufficient gene transfer and targeting the correct site. The safety of transferring genes with a viral vector is also questionable.

Further research is needed to optimize liposomal chemotherapy delivery, improve biomarker-based response prediction, assess safety effects on healthy cells, and explore liposomes' potential to augment other therapies, such as immunotherapy. Future directions include using anti-cancer liposomes as a neoadjuvant to shrink tumors before surgery; imaging-enabled therapies to visualize drug delivery and release within tumors; and targeted liposome binding, stimuli-triggered release, and imaging-enabled therapies. Liposomal delivery technology could be more effective against breast cancer with enhanced targeting, controlled release, imaging integration, and innovative combinations, while addressing knowledge gaps will advance the field.

Further studies on the immunogenicity, durability, and off-target effects of gene therapies are needed to improve specificity, understand potential unintended editing effects, determine whether higher doses are needed, and prevent harmful immune responses. Wider illness objectives, such as HIV, cancer, heart disease, and complicated neurological disorders, are part of the future scope. Delivery barriers may be overcome by the synergistic potential of combination medicines and in vivo gene editing.

Individualized manufacturing, delivery barriers, and high costs of custom gene therapies pose significant challenges to wider availability, particularly in less wealthy nations, necessitating adaptation of regulatory and payment models. In addition, ethical considerations surrounding gene editing and the potential for misuse also need to be addressed. Furthermore, collaboration among governments, pharmaceutical companies, and research institutions is crucial to accelerate the development and accessibility of gene therapies worldwide. Further innovation to address these gaps and limitations, as well as expansion into new disease areas, will continue advancing the scope of gene therapy in the future. But challenges remain.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Ameena Ahmed contributed to conceptualization, literature review, methodology, data curation, manuscript writing (original draft), and final manuscript preparation. Mrinmoy Pratim Chetia conducted the literature review, data analysis, critical revision of the manuscript, and validation of scientific content. Satyabrat Sarma contributed to conceptualization, supervision, critical review and editing of the manuscript, project administration, and approval of the final version for publication.

REFERENCES

[1] Tang R, Xu Z. Gene therapy: a double-edged sword with great powers. *Molecular and Cellular Biochemistry*, **474(1–2)**, 73–81 (2020) https://doi.org/10.1007/s11010-020-03834-3

[2] Zu H, Gao D. Non-viral vectors in gene therapy: recent development, challenges, and prospects. *The AAPS Journal*, **23(4)**, 78 (2021) https://doi.org/10.1208/s12248-021-00608-7

[3] Cavazzana M, Bushman FD, Miccio A, André-Schmutz I, Six E. Gene therapy targeting haematopoietic stem cells for inherited diseases: progress and challenges. *Nature Reviews Drug Discovery*, **18(6)**, 447–462 (2019) https://doi.org/10.1038/s41573-019-0020-9

[4] Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. *Gene*, **525(2)**, 162–169 (2013) https://doi.org/10.1016/j.gene.2013.03.137

[5] Aied A, Greiser U, Pandit A, Wang W. Polymer gene delivery: overcoming the obstacles. *Drug Discovery Today*, **18(21–22)**, 1090–1098 (2013) https://doi.org/10.1016/j.drudis.2013.06.014

[6] Greely HT. The revolution in human genetics: implications for human societies. *South Carolina Law Review*, **52(2)**, 377–381 (2001)

[7] Gaj T, Sirk SJ, Shui SL, Liu J. Genome-editing technologies: principles and applications. *Cold Spring Harbor Perspectives in Biology*, **8(12)**, a023754 (2016) https://doi.org/10.1101/cshperspect.a023754

[8] Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA, editors. *SEER Cancer Statistics Review, 1975–2012*. Bethesda, MD: National Cancer Institute (2015)

[9] Bose P, Vachhani P, Cortes JE. Treatment of relapsed/refractory acute myeloid leukemia. *Current Treatment Options in Oncology*, **18(3)**, 17 (2017) https://doi.org/10.1007/s11864-017-0456-2

[10] Rowe JM, Tallman MS. How I treat acute myeloid leukemia. *Blood*, **116(17)**, 3147–3156 (2010) https://doi.org/10.1182/blood-2010-05-260117

[11] Ljungman P, Urbano-Ispizua A, Cavazzana-Calvo M, Demirer T, Dini G, Einsele H, et al. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: definitions and current practice in Europe. *Bone Marrow Transplantation*, **37(5)**, 439–449 (2006) https://doi.org/10.1038/sj.bmt.1705265

[12] Lichtman MA. Battling the hematological malignancies: the 200 years' war. *The Oncologist*, **13(2)**, 126–138 (2008) https://doi.org/10.1634/theoncologist.2007-0228

[13] Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *New England Journal of Medicine*, **257(11)**, 491–496 (1957) https://doi.org/10.1056/NEJM195709122571102

[14] Little MT, Storb R. History of haematopoietic stem-cell transplantation. *Nature Reviews Cancer*, **2(3)**, 231–238 (2002) https://doi.org/10.1038/nrc748

[15] Miller AD, Law MF, Verma IM. Generation of helper-free amphotropic retroviruses that transduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene. *Molecular and Cellular Biology*, **5(3)**, 431–437 (1985) https://doi.org/10.1128/mcb.5.3.431-437.1985

[16] Shimotohno K, Temin HM. Formation of infectious progeny virus after insertion of herpes simplex thymidine kinase gene into DNA of an avian retrovirus. *Cell*, **26(1, Part 1)**, 67–77 (1981) https://doi.org/10.1016/0092-8674(81)90034-9

[17] Cepko CL, Roberts BE, Mulligan RC. Construction and applications of a highly transmissible murine retrovirus shuttle vector. *Cell*, **37(3)**, 1053–1062 (1984) https://doi.org/10.1016/0092-8674(84)90440-9

[18] Kantoff PW, Kohn DB, Mitsuya H, Armentano D, Sieberg M, Zwiebel JA, et al. Correction of adenosine deaminase deficiency in cultured human T and B cells by retrovirus-mediated gene transfer. *Proceedings of the National Academy of Sciences of the United States of America*, **83(17)**, 6563–6567 (1986) https://doi.org/10.1073/pnas.83.17.6563

[19] Friedmann T. A brief history of gene therapy. *Nature Genetics*, **2(2)**, 93–98 (1992) https://doi.org/10.1038/ng1092-93

[20] Wallen M, Aqil F, Spencer W, Gupta RC. Exosomes as an emerging plasmid delivery vehicle for gene therapy. *Pharmaceutics*, **15(7)**, 1832 (2023) https://doi.org/10.3390/pharmaceutics15071832

[21] Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, et al. T lymphocyte-directed gene therapy for ADA− SCID: initial trial results after 4 years. *Science*, **270(5235)**, 475–480 (1995) https://doi.org/10.1126/science.270.5235.475

[22] Rein LAM, Yang H, Chao NJ. Applications of gene editing technologies to cellular therapies. *Biology of Blood and Marrow Transplantation*, **24(8)**, 1537–1545 (2018) https://doi.org/10.1016/j.bbmt.2018.03.021

[23] Branca MA. Gene therapy: cursed or inching towards credibility? *Nature Biotechnology*, **23(5)**, 519–521 (2005) https://doi.org/10.1038/nbt0505-519

[24] Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *The Journal of Clinical Investigation*, **118(9)**, 3132–3142 (2008) https://doi.org/10.1172/JCI35700

[25] Baum C. Gene therapy for SCID-X1: focus on clinical data. *Molecular Therapy*, **19(12)**, 2103–2104 (2011) https://doi.org/10.1038/mt.2011.252

[26] Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. Repurposing CRISPR as an RNA-guided platform

for sequence-specific control of gene expression. *Cell*, **152(5)**, 1173–1183 (2013) https://doi.org/10.1016/j.cell.2013.02.022

[27] Barrangou R. Cas9 targeting and the CRISPR revolution. *Science*, **344(6185)**, 707–708 (2014) https://doi.org/10.1126/science.1252964

[28] Jiao Y, Xia ZL, Ze LJ, Jing H, Xin B, Fu S. Research progress of nucleic acid delivery vectors for gene therapy. *Biomedical Microdevices*, **22(1)**, 16 (2020) https://doi.org/10.1007/s10544-020-0469-7

[29] Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, **163(3)**, 759–771 (2015) https://doi.org/10.1016/j.cell.2015.09.038

[30] Fagerlund RD, Staals RHJ, Fineran PC. The Cpf1 CRISPR-Cas protein expands genome-editing tools. *Genome Biology*, **16**, 251 (2015) https://doi.org/10.1186/s13059-015-0824-9

[31] Davis AJ, Chen DJ. DNA double strand break repair via non-homologous end-joining. *Translational Cancer Research*, **2(3)**, 130–143 (2013) https://doi.org/10.3978/j.issn.2218-676X.2013.04.02

[32] Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annual Review of Biochemistry*, **79**, 181–211 (2010) https://doi.org/10.1146/annurev.biochem.052308.093131

[33] Cornu TI, Mussolino C, Cathomen T. Refining strategies to translate genome editing to the clinic. *Nature Medicine*, **23(4)**, 415–423 (2017) https://doi.org/10.1038/nm.4313

[34] Mao Z, Bozzella M, Seluanov A, Gorbunova V. Comparison of nonhomologous end joining and homologous recombination in human cells. *DNA Repair*, **7(10)**, 1765–1771 (2008) https://doi.org/10.1016/j.dnarep.2008.06.018

[35] Ferrari G, Cavazzana M, Mavilio F. Gene therapy approaches to hemoglobinopathies. *Hematology/Oncology Clinics of North America*, **31(5)**, 835–852 (2017) https://doi.org/10.1016/j.hoc.2017.06.010

[36] Taylor J, Xiao W, Abdel-Wahab O. Diagnosis and classification of hematologic malignancies on the basis of genetics. *Blood*, **130(4)**, 410–423 (2017) https://doi.org/10.1182/blood-2017-02-734541

[37] Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *New England Journal of Medicine*, **368(22)**, 2059–2074 (2013) https://doi.org/10.1056/NEJMoa1301689

[38] Heckl D, Kowalczyk MS, Yudovich D, Belizaire R, Puram RV, McConkey ME, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. *Nature Biotechnology*, **32(9)**, 941–946 (2014) https://doi.org/10.1038/nbt.2951

[39] Brabetz O, Alla V, Angenendt L, Schliemann C, Berdel WE, Arteaga MF, Mikesch JH. RNA-guided CRISPR-Cas9 system-mediated engineering of acute myeloid leukemia mutations. *Molecular Therapy–Nucleic Acids*, **6**, 243–248 (2017) https://doi.org/10.1016/j.omtn.2016.12.012

[40] Howard J, Friedman L. Protecting the scientific integrity of a clinical trial: some ethical dilemmas. *Clinical Pharmacology & Therapeutics*, **29(5)**, 561–569 (1981) https://doi.org/10.1038/clpt.1981.78

[41] Daikos GK. Ethical dilemmas encountered during clinical drug trials. *International Journal of Antimicrobial Agents*, **24(1)**, 24–31 (2004) https://doi.org/10.1016/j.ijantimicag.2004.03.004

[42] Almeida M, Ranisch R. Beyond safety: mapping the ethical debate on heritable genome editing interventions. *Humanities and Social Sciences Communications*, **9**, 139 (2022) https://doi.org/10.1057/s41599-022-01147-y

[43] Gyngell C, Douglas T, Savulescu J. The ethics of germline gene editing. *Journal of Applied Philosophy*, **34(4)**, 498–513 (2017) https://doi.org/10.1111/japp.12249

[44] Koplin JJ, Gyngell C, Savulescu J. Germline gene editing and the precautionary principle. *Bioethics*, **34(1)**, 49–59 (2020) https://doi.org/10.1111/bioe.12609

[45] Adli M. The CRISPR tool kit for genome editing and beyond. *Nature Communications*, **9**, 1911 (2018) https://doi.org/10.1038/s41467-018-04252-2

[46] Kanchiswamy CN, Maffei M, Malnoy M, Velasco R, Kim JS. Fine-tuning next-generation genome editing tools. *Trends in Biotechnology*, **34(7)**, 562–574 (2016) https://doi.org/10.1016/j.tibtech.2016.03.007

[47] Guha TK, Wai A, Hausner G. Programmable genome editing tools and their regulation for efficient genome engineering. *Computational and Structural Biotechnology Journal*, **15**, 146–160 (2017) https://doi.org/10.1016/j.csbj.2016.12.006

[48] Moon SB, Kim DY, Ko JH, Kim YS. Recent advances in the CRISPR genome editing tool set. *Experimental & Molecular Medicine*, **51(11)**, 1–11 (2019) https://doi.org/10.1038/s12276-019-0339-7

[49] Khalil AM. The genome editing revolution: review. *Journal of Genetic Engineering and Biotechnology*, **18(1)**, 68 (2020) https://doi.org/10.1186/s43141-020-00078-y