Genome Publications
https://doi.org/10.61096/978-81-981372-2-7_22

Chapter 22

Future Horizons: Gene Editing, RNA Medicines, and Cell-Based Therapies

Dr. V. Balasubramaniam
Professor & Principal, Faculty of Pharmacy, Sri Venkateswaraa University, Ettayapuram, Thoothukudi District, Tamilnadu
Dr. KS. Jaganathan
Additional Director, Serum Institute of India, Pune, Maharashtra, India

Abstract: The evolution of therapeutics has moved beyond small molecules into a transformative era defined by genetic manipulation, RNA-based drugs, and cell-derived therapies. Gene editing technologies such as CRISPR-Cas systems, TALENs, zinc-finger nucleases (ZFNs), and prime editing have enabled unprecedented precision in correcting disease-causing mutations. In parallel, RNA therapeutics, including small interfering RNA (siRNA), antisense oligonucleotides (ASOs), mRNA vaccines, and emerging microRNA modulators, have opened new avenues for treating previously intractable conditions ranging from rare genetic disorders to cancer and infectious diseases. Meanwhile, cell-based therapies, encompassing mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), hematopoietic stem cells, and neural progenitors, offer regenerative solutions that bridge molecular medicine and tissue engineering. This chapter provides an in-depth exploration of these advanced modalities, examining their mechanisms, clinical progress, regulatory hurdles, ethical considerations, and integration into precision medicine frameworks. Comparative analysis with conventional pharmacology highlights the paradigm shift from symptomatic treatment to curative, disease-modifying strategies. Furthermore, emerging trends such as allogeneic "off-the-shelf" CAR-T products, synthetic biology-based programmable therapeutics, and exosome-mediated delivery systems are evaluated. Collectively, these innovations point toward a future where multi-omics data, artificial intelligence, and robotics will synergize to establish a globally accessible, ethically grounded, and personalized therapeutic ecosystem.

Keywords: Gene editing, RNA therapeutics, Cell-based therapies, Precision medicine, Regenerative pharmacology.

Citation: V. Balasubramaniam, KS. Jaganathan. Future Horizons: Gene Editing, RNA Medicines, and Cell-Based Therapies. *Modern Therapeutic Pharmacology: Precision Therapeutics Across Organ Systems*. 2025; Pp234-246. https://doi.org/10.61096/978-81-981372-2-7_22

22.0 INTRODUCTION

From Small Molecules to Living Medicines and Regenerative Therapies

For decades, pharmacotherapy was dominated by small molecules designed to modulate biochemical pathways through receptor binding or enzyme inhibition. While these agents have profoundly impacted disease management, their limitations off-target effects, partial efficacy, and lack of curative potential have fueled the search for more precise and durable interventions [1]. The emergence of biologics, including monoclonal antibodies and recombinant proteins, marked the first step toward targeted pharmacology. However, even these protein-based therapeutics are constrained by their inability to fundamentally correct underlying genetic or cellular pathologies.

The 21st century has seen an unprecedented convergence of molecular biology, genomics, and bioengineering, leading to the advent of living medicines. These include genetic interventions that reprogram the blueprint of life, RNA therapeutics that regulate gene expression post-transcriptionally, and cell-based therapies that regenerate damaged tissues or reconstitute immune function [2]. Unlike traditional pharmacology, which often relies on chronic administration, these novel modalities aim to achieve durable or even permanent therapeutic effects.

A key milestone in this transition was the development of the CRISPR-Cas system in 2012, which democratized gene editing by simplifying the process of sequence-specific DNA cleavage [3]. Similarly, the rapid deployment of mRNA vaccines during the COVID-19 pandemic demonstrated the speed and scalability of RNA-based therapeutics [4]. Concurrently, stem cell therapies and chimeric antigen receptor (CAR)-modified immune cells have validated the feasibility of using living cells as drugs, fundamentally redefining clinical pharmacology.

Despite these advances, significant challenges remain. The high cost of development, regulatory complexity, manufacturing scalability, and long-term safety monitoring pose substantial barriers to widespread adoption [5]. Furthermore, ethical debates surrounding genome editing and equitable access to advanced therapies highlight the societal dimensions of these scientific breakthroughs. Nonetheless, the trajectory of biomedical innovation suggests that gene editing, RNA medicines, and cell-based therapies will become integral pillars of future precision medicine.

22.1 Gene Editing Technologies: CRISPR, TALEN, ZFN, and Prime Editing

Gene editing enables the precise alteration of genomic sequences to correct pathogenic mutations, modulate gene expression, or introduce novel traits. Among the various platforms, CRISPR-Cas has emerged as the most versatile, owing to its simplicity, cost-effectiveness, and adaptability [6]. The CRISPR-Cas9 system uses a single-guide RNA (sgRNA) to direct the Cas9 endonuclease to a specific genomic locus, where it introduces a double-strand break (DSB). Subsequent repair by non-homologous end joining (NHEJ) or homology-directed repair (HDR) allows for targeted gene disruption or correction [7].

Beyond CRISPR, other editing tools retain clinical relevance. Transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) preceded CRISPR and remain valuable for applications requiring high specificity with minimal off-target activity [8]. For instance, TALENs have been successfully employed in the ex vivo editing of T cells for therapeutic purposes, while ZFNs have advanced into clinical trials for treating HIV and lysosomal storage disorders [9].

Recent innovations such as base editing and prime editing offer even greater precision. Base editors can convert individual nucleotides without introducing DSBs, reducing the risk of insertion-deletion mutations, while prime editing employs a reverse transcriptase fused to Cas9 nickase to achieve template-directed sequence changes [10]. These tools significantly expand the therapeutic

landscape, enabling the correction of point mutations responsible for monogenic diseases, such as sickle cell disease and β-thalassemia, both of which are now in late-stage clinical trials [11].

Delivery remains a critical bottleneck. While viral vectors such as adeno-associated viruses (AAVs) are commonly used, their limited cargo capacity and immunogenicity pose challenges. Non-viral platforms, including lipid nanoparticles (LNPs) and engineered exosomes, are gaining attention for their improved safety and scalability [12].

Despite their promise, gene editing technologies face ethical scrutiny, particularly regarding germline editing and potential off-target effects. Regulatory agencies have thus mandated rigorous preclinical safety evaluation and long-term follow-up in clinical trials [13]. As these tools evolve, their integration with artificial intelligence-driven off-target prediction and high-fidelity Cas variants may help mitigate risks and accelerate clinical translation.

22.2 RNA-Based Therapies: siRNA, ASO, mRNA Vaccines, and microRNA Therapeutics

RNA-based therapeutics represent a paradigm shift in drug development by targeting disease pathways at the level of RNA rather than proteins. Small interfering RNAs (siRNAs) harness the RNA interference (RNAi) pathway to degrade complementary messenger RNAs (mRNAs), thereby silencing pathogenic genes [14]. The approval of patisiran, an siRNA drug for hereditary transthyretin-mediated amyloidosis, validated this approach clinically [15]. Similarly, givosiran for acute hepatic porphyria exemplifies the expanding scope of RNAi therapeutics.

Antisense oligonucleotides (ASOs) offer complementary functionality by binding target RNA sequences and modulating splicing or translation. Nusinersen, an ASO used to treat spinal muscular atrophy, demonstrates the therapeutic power of altering RNA splicing to restore functional protein production [16]. These modalities, however, often face challenges with delivery to extrahepatic tissues, necessitating chemical modifications such as phosphorothioate backbones and conjugation to targeting ligands like N-acetylgalactosamine (GalNAc) [17].

Messenger RNA (mRNA) therapeutics, propelled to prominence during the COVID-19 pandemic, offer transient expression of therapeutic proteins without the risk of genomic integration. mRNA vaccines, such as those developed for SARS-CoV-2, have showcased the scalability and speed of this platform [18]. Ongoing research extends mRNA applications to cancer immunotherapy, enzyme replacement, and regenerative medicine. Meanwhile, microRNA (miRNA)-based therapies are under development for diseases such as cancer and fibrosis, although clinical translation is limited by challenges in specificity and stability [19].

Despite these advances, the primary barriers to RNA therapeutics include delivery, immunogenicity, and manufacturing complexity. Lipid nanoparticles remain the leading delivery vehicle, but emerging systems like polymer-based carriers and extracellular vesicles promise to enhance tissue-specific targeting [20]. Collectively, RNA medicines exemplify how molecular pharmacology is transitioning from traditional receptor modulation to transcript-level intervention.

22.3 Cell-Based Therapies: MSCs, iPSCs, Hematopoietic and Neural Stem Cells

Cell-based therapies leverage living cells as functional therapeutic agents capable of repairing or replacing damaged tissues. Mesenchymal stem cells (MSCs) are among the most widely studied due to their multipotency and immunomodulatory properties. Clinical trials have demonstrated their utility in conditions such as graft-versus-host disease (GvHD), osteoarthritis, and inflammatory bowel disease, with several MSC-derived products receiving regulatory approval in Asia [21].

Induced pluripotent stem cells (iPSCs) represent another revolutionary platform. By reprogramming adult somatic cells into a pluripotent state, iPSCs bypass the ethical concerns associated with embryonic stem cells while retaining the ability to differentiate into any cell type [22]. This technology underpins emerging regenerative strategies for neurodegenerative diseases, myocardial infarction, and diabetes, where functional tissue replacement is required [23].

Hematopoietic stem cell transplantation (HSCT) remains the gold standard for hematologic malignancies and certain genetic disorders. Recent advances in conditioning regimens, HLA matching, and post-transplant immune modulation have significantly improved outcomes [24]. Similarly, neural stem cell therapies are being investigated for spinal cord injury and Parkinson's disease, where early-phase trials have demonstrated encouraging safety and feasibility profiles [25].

Key limitations of cell therapies include manufacturing complexity, immunological rejection, and variability in potency. The development of universal donor cell lines, gene-edited hypoimmunogenic cells, and automated bioreactor systems may help overcome these barriers [26]. Furthermore, integration with biomaterials and 3D bioprinting is anticipated to enhance engraftment and functional recovery in tissue regeneration.

As the field matures, regulatory agencies are establishing rigorous frameworks for potency assays, long-term monitoring, and product characterization to ensure safety and reproducibility [27]. With these advances, cell-based therapies are poised to become a cornerstone of regenerative pharmacology and precision medicine.

22.4 CAR-T and Beyond: Solid Tumor CAR-T and Allogeneic Products

Chimeric antigen receptor (CAR) T-cell therapy has emerged as a landmark innovation in immuno-oncology, particularly for hematologic malignancies such as B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma. CAR-T therapy involves the genetic engineering of autologous T cells to express synthetic receptors targeting tumor-associated antigens, leading to potent cytotoxic responses [28]. However, translating this success to solid tumors has proven challenging due to tumor heterogeneity, an immunosuppressive microenvironment, and limited T-cell infiltration [29].

To address these barriers, novel CAR designs incorporate additional costimulatory domains, such as 4-1BB and CD28, or utilize armored CAR-T cells that secrete cytokines like IL-12 to enhance activity in hostile tumor microenvironments [30]. Dual-targeting CARs and bispecific CAR constructs aim to reduce antigen escape, while regional delivery strategies, including intratumoral or intraperitoneal administration, are under investigation to improve efficacy in solid tumors [31].

Another key development is the emergence of allogeneic "off-the-shelf" CAR-T products derived from healthy donor T cells. These therapies promise to reduce manufacturing time and cost, which are major limitations of autologous CAR-T therapy. Companies are using gene editing platforms such as TALENs or CRISPR to eliminate T-cell receptors and major histocompatibility complex (MHC) molecules to prevent graft-versus-host disease and rejection [32]. Early-phase clinical trials for allogeneic CAR-T therapies in leukemia and lymphoma have demonstrated encouraging safety and efficacy, signaling a potential paradigm shift in scalable cellular immunotherapy [33].

Nevertheless, CAR-T therapy faces challenges including cytokine release syndrome (CRS), neurotoxicity, and durability of response. Advances in safety switches, synthetic regulatory circuits, and controlled CAR expression systems are being explored to mitigate these risks [34]. As manufacturing becomes more automated and integrated with synthetic biology, CAR-T and next-

generation engineered immune cells will likely expand to broader indications, including autoimmune disorders and infectious diseases [35].

22.5 Exosomes and Nanovesicles: Natural Delivery Vehicles for Next-Generation Therapeutics

Exosomes and extracellular nanovesicles are nanoscale lipid bilayer particles secreted by cells that play critical roles in intercellular communication. Their ability to transport nucleic acids, proteins, and lipids in a biocompatible manner has spurred interest in their therapeutic use as natural drug delivery systems [36]. Unlike synthetic nanoparticles, exosomes possess inherent tissue tropism and lower immunogenicity, making them particularly attractive for targeted delivery.

In oncology, tumor-derived exosomes are being investigated as both biomarkers and therapeutic targets, while engineered exosomes are under development for delivering small RNAs, CRISPR components, and chemotherapeutics [37]. Notably, exosomes have demonstrated the ability to cross the blood-brain barrier (BBB), positioning them as promising carriers for central nervous system (CNS) disorders such as glioblastoma and neurodegenerative diseases [38].

Beyond oncology, exosomes have shown potential in fibrosis, cardiovascular repair, and inflammatory diseases. For example, mesenchymal stem cell (MSC)-derived exosomes exhibit immunomodulatory and regenerative properties that are being evaluated in clinical trials for acute respiratory distress syndrome (ARDS) and chronic kidney disease [39]. Nanovesicles derived from platelets or red blood cells are also being explored as bioinspired drug carriers with enhanced circulation time and reduced clearance [40].

Despite their promise, large-scale manufacturing, cargo loading efficiency, and regulatory standardization remain hurdles. Emerging technologies, including microfluidic-based exosome isolation and synthetic nanovesicle engineering, may address these limitations [41]. By combining exosome biology with RNA therapeutics and gene editing, future exosome-based delivery systems could enable precision medicine at an unprecedented level.

22.6 Synthetic Biology: Programmable Biosensors and Logic-Gated Therapeutics

Synthetic biology applies engineering principles to biological systems, allowing the design of programmable therapeutic agents capable of performing complex tasks. In pharmacology, this field is enabling the development of “smart” therapeutics that sense disease-specific signals and respond dynamically [42].

Logic-gated CAR-T cells exemplify this concept by requiring the simultaneous recognition of multiple antigens to activate cytotoxic function, thereby reducing off-target toxicity [43]. Similarly, synthetic gene circuits have been engineered to control drug release, trigger apoptosis selectively in cancer cells, or modulate immune activity in response to inflammatory cues [44].

Programmable biosensors embedded within engineered cells can detect pathological biomarkers and initiate therapeutic responses. For instance, synthetic biology-based bacterial systems have been designed to colonize tumors and secrete anti-cancer agents locally, minimizing systemic exposure [45]. Moreover, synthetic toggle switches using CRISPR interference (CRISPRi) enable reversible control of gene expression, paving the way for more precise and safer interventions [46].

This emerging discipline also integrates with RNA medicines and exosome-based delivery systems. Researchers are developing modular RNA-based logic circuits that execute Boolean operations in cells, enabling highly selective targeting of diseased tissues [47]. While these

innovations remain largely preclinical, they hold the potential to redefine pharmacology by merging biological computation with therapeutic intervention.

The translation of synthetic biology to the clinic faces significant regulatory challenges, particularly in standardizing safety assessments and addressing ethical concerns surrounding self-replicating or autonomous systems [48]. Nevertheless, the convergence of synthetic biology, gene editing, and artificial intelligence offers a transformative path toward adaptive, precision-guided therapeutics.

Table 22.1: Key Advancements in Gene Editing, RNA, and Cell-Based Therapies

Technology	Recent Breakthroughs	Clinical Applications	Key Limitations
CRISPR/Prime Editing	High-fidelity Cas variants	Sickle cell disease, β-thalassemia	Off-target effects, delivery
RNA Therapeutics	siRNA and mRNA vaccines	Rare genetic diseases, cancer	Immunogenicity, tissue targeting
CAR-T Therapy	Dual-antigen and armored CARs	Hematologic malignancies, solid tumors (investigational)	CRS, neurotoxicity, cost
Exosome-Based Delivery	Engineered exosomes for RNA drugs	CNS disorders, fibrosis	Manufacturing scalability
Synthetic Biology	Logic-gated gene circuits	Cancer, autoimmune disorders	Regulatory complexity

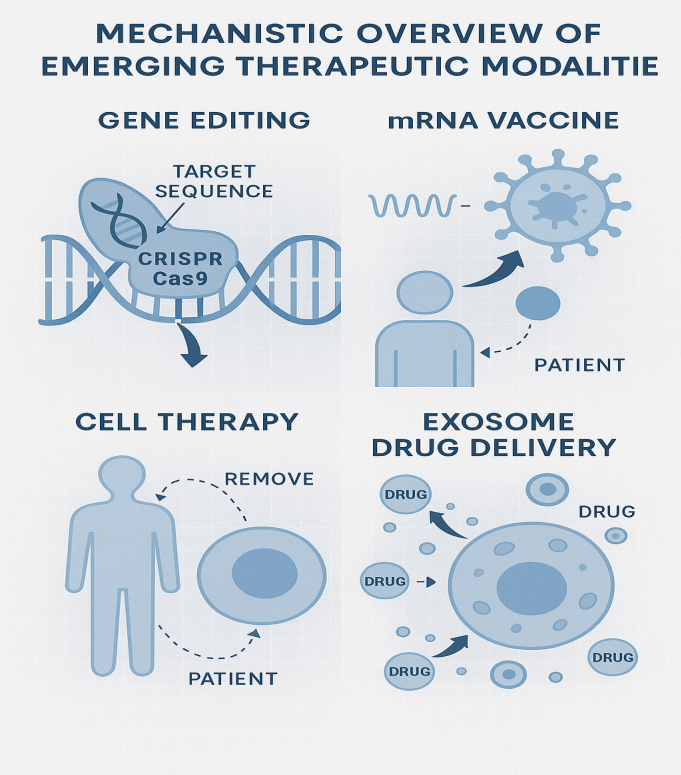

Figure 22.1: Mechanistic Overview of Emerging Therapeutic Modalities

22.7 Regulatory and Translational Barriers

The rapid evolution of gene editing, RNA medicines, and cell-based therapies has created unprecedented regulatory challenges. Unlike conventional small molecules, these therapies involve highly complex biological products with unique manufacturing, quality control, and safety considerations [49]. Regulatory agencies such as the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have implemented specific guidelines for Investigational New Drug (IND) submissions that include detailed Chemistry, Manufacturing, and Controls (CMC) data, preclinical safety assessments, and clinical trial protocols tailored to advanced therapy medicinal products (ATMPs) [50].

Manufacturing represents a critical hurdle, particularly for personalized therapies such as autologous CAR-T cells, which require stringent control of cell sourcing, genetic modification, and scalability [51]. Moreover, gene editing therapies demand rigorous evaluation of off-target effects, long-term genotoxicity, and the potential for germline transmission, even when therapies are somatic [52].

Long-term monitoring is essential for RNA and cell-based therapies, as delayed adverse effects may not manifest during initial clinical trials. Regulatory bodies increasingly mandate post-marketing surveillance programs, patient registries, and real-world evidence collection to assess durability, safety, and immunogenicity [53]. For instance, the FDA's requirement for 15-year follow-up in gene therapy trials underscores the complexity of monitoring these treatments [54].

Standardizing potency assays and quality metrics remains challenging, especially for exosome-based therapeutics, where heterogeneity in particle size, cargo composition, and isolation techniques complicates regulatory evaluation [55]. Similarly, synthetic biology-based therapies pose novel regulatory dilemmas because they involve dynamic, programmable biologics that do not fit into traditional drug frameworks [56]. Collaborative initiatives between regulators, industry stakeholders, and academic experts are thus critical to harmonizing standards and accelerating safe clinical translation.

22.8 Access and Ethical Concerns

While gene editing, RNA therapeutics, and cell-based therapies promise transformative benefits, their high cost and ethical implications raise concerns about equitable access. The pricing of therapies such as CAR-T, which can exceed hundreds of thousands of dollars per treatment, has highlighted the financial strain these innovations place on healthcare systems [57]. Efforts to implement alternative payment models, such as outcomes-based reimbursement, are being explored to improve affordability [58].

Beyond cost, the risk of genetic discrimination and stigmatization represents an important ethical consideration. Although legislation such as the Genetic Information Nondiscrimination Act (GINA) in the United States offers some protection, gaps remain in global regulatory frameworks [59]. Additionally, the prospect of germline editing, particularly following the controversial case of CRISPR-modified embryos, has sparked international debate regarding the moral limits of human genetic intervention [60].

The use of allogeneic cell therapies also raises questions about donor consent, ownership of biological materials, and equitable distribution of treatment across different socioeconomic groups [61]. Moreover, ethical issues surrounding synthetic biology include the potential creation of self-replicating systems or misuse for non-therapeutic purposes, requiring the development of robust biosecurity measures [62].

Patient engagement and transparent communication about the risks, limitations, and realistic expectations of these therapies are essential to maintain public trust. Ethical oversight committees and global governance frameworks will be necessary to ensure that innovation aligns with societal values and avoids exacerbating health disparities [63].

22.9 Future Roadmap: Integrating Omics, AI, and Global Precision Medicine

The future of gene editing, RNA therapeutics, and cell-based therapies lies in the integration of multi-omics technologies, artificial intelligence (AI), and advanced manufacturing platforms. Genomic, transcriptomic, proteomic, and metabolomic profiling will allow unprecedented personalization of treatment strategies, enabling therapies to be tailored not just to specific diseases but to individual patients [64].

AI and machine learning are already being employed to predict CRISPR off-target effects, optimize RNA secondary structures for stability and translation, and streamline CAR-T cell design [65]. Similarly, robotic automation and digital biomanufacturing are poised to revolutionize the production of living medicines, reducing costs and improving scalability [66].

International collaboration will be central to building a global precision medicine ecosystem. Shared clinical data platforms, harmonized regulatory pathways, and cooperative research initiatives could accelerate therapeutic development and ensure equitable access worldwide [67]. The convergence of gene editing, RNA medicines, cell therapies, and synthetic biology with next-generation computational and manufacturing technologies has the potential to redefine medicine from reactive treatment to proactive disease interception [68].

Ultimately, future breakthroughs will likely involve combination modalities, such as exosome-delivered RNA-guided gene editing or synthetic biology-enhanced CAR-T therapies, creating intelligent, adaptive therapeutic systems capable of responding dynamically to disease evolution. This integrated approach could mark the beginning of a new era where precision medicine becomes globally accessible, ethically grounded, and technologically seamless [69].

Table 22.2: Regulatory and Ethical Challenges in Advanced Therapeutics

Challenge	Example	Mitigation Strategies
CMC complexity	CAR-T manufacturing variability	Automated closed-system manufacturing
Long-term safety monitoring	Gene therapy follow-up (15 years)	Registries, real-world evidence
High cost	CAR-T >$400,000	Outcomes-based pricing models
Genetic discrimination	Misuse of genetic data	Strengthening legal protections
Ethical concerns in germline editing	CRISPR embryo controversy	International moratorium, ethics panels

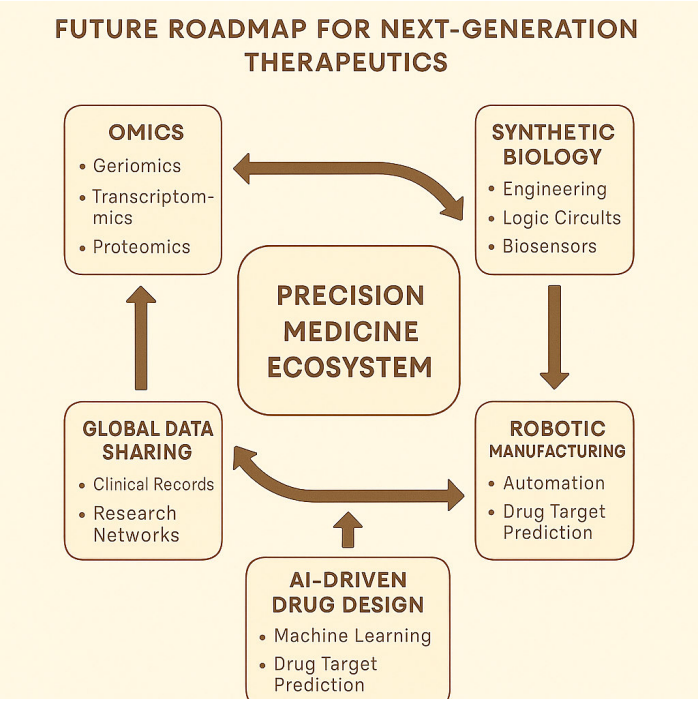

Figure 22.2: Future Roadmap for Next-Generation Therapeutics

22.10 CONCLUSION

The convergence of gene editing, RNA-based medicines, and cell-based therapies marks a transformative era in modern pharmacology, transitioning from conventional small-molecule drugs to precision-engineered, living therapeutics. Gene-editing technologies such as CRISPR-Cas systems, TALENs, zinc finger nucleases, and prime editing have expanded the boundaries of molecular medicine, enabling the precise correction of pathogenic mutations and the development of novel therapeutic modalities for previously intractable diseases. Similarly, RNA-based interventions, including siRNA, antisense oligonucleotides, mRNA vaccines, and microRNA therapeutics, have demonstrated their ability to modulate gene expression and drive targeted biological responses with unprecedented specificity and adaptability.

Cell-based therapies, ranging from mesenchymal stem cells and induced pluripotent stem cells to hematopoietic and neural stem cell platforms, have shifted the therapeutic paradigm toward tissue regeneration and functional restoration. Meanwhile, innovations in CAR-T cell therapy, allogeneic off-the-shelf products, and engineered immune cells have redefined cancer immunotherapy, while exosomes and nanovesicles have emerged as natural and versatile delivery vehicles for complex biological payloads. These advancements are further reinforced by synthetic biology approaches, where programmable biosensors and logic-gated therapeutic circuits provide new opportunities for dynamic and responsive interventions.

Despite this progress, challenges remain in regulatory oversight, manufacturing scalability, long-term safety monitoring, cost management, and ethical concerns, particularly regarding genetic equity and the potential misuse of these powerful technologies. However, with the integration of multi-omics, artificial intelligence, robotics, and global precision medicine initiatives, the future holds the promise of more accessible, effective, and personalized therapeutic strategies. Ultimately, the successful translation of these innovations will depend on collaborative efforts among scientists,

clinicians, regulators, and policymakers to ensure that these breakthroughs lead to safe, equitable, and transformative healthcare solutions.

REFERENCES

1. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096.
2. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149–157.
3. Kim Y, Kweon J, Kim A, Chon JK, Yoo JY, Kim HJ, et al. A library of TAL effector nucleases spanning the human genome. Nat Biotechnol. 2013;31(3):251–258.
4. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013;14(1):49–55.
5. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2010;11(9):636–646.
6. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med. 2017;377(18):1713–1722.
7. Karikó K, Weissman D. mRNA vaccines: time to deliver. Nature. 2021;591(7848):336–340.
8. Kulkarni JA, Witzigmann D, Thomson SB, Chen S, Leavitt BR, Cullis PR, et al. The current landscape of nucleic acid therapeutics. Nat Nanotechnol. 2021;16(6):630–643.
9. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. Science. 2018;359(6382):1355–1360.
10. Setten RL, Rossi JJ, Han SP. The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov. 2019;18(6):421–446.
11. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med. 2018;379(1):11–21.
12. Crooke ST, Witztum JL, Bennett CF, Baker BF. RNA-targeted therapeutics. Cell Metab. 2018;27(4):714–739.
13. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science. 2007;318(5858):1920–1923.
14. Liu S, Trapnell C. Single-cell transcriptome sequencing: recent advances and remaining challenges. F1000Res. 2016;5:F1000 Faculty Rev–182.
15. Rezvani K, Rouce RH. The future of cellular therapy. Br J Haematol. 2020;191(2):209–221.
16. Gauthier J, Turtle CJ. Insights into cytokine release syndrome and neurotoxicity after CD19-specific CAR-T cell therapy. Curr Res Transl Med. 2018;66(2):50–52.
17. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 2021;11(4):69.
18. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. Off-the-shelf allogeneic CAR-T cells: development and challenges. Nat Rev Drug Discov. 2020;19(3):185–199.
19. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439–448.

20. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977.
21. Lener T, Gimona M, Aigner L, Börger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials an ISEV position paper. J Extracell Vesicles. 2015;4:30087.
22. Zhao Z, Ukidve A, Kim J, Mitragotri S. Targeting strategies for tissue-specific drug delivery. Cell. 2020;181(1):151–167.
23. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature. 2017;546(7659):498–503.
24. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci. 2019;9:19.
25. Chan HF, Zhang Y, Ho YP, Chiu YL, Jung Y, Leong KW. Rapid formation of multicellular spheroids in double-emulsion droplets with controllable microenvironment. Sci Rep. 2013;3:3462.
26. Xie M, Chen Q, He S, Feng Y, Chen Y, Yu C, et al. Advances in exosome-based drug delivery and tumor therapy. Front Oncol. 2021;11:733465.
27. Way JC, Collins JJ, Keasling JD, Silver PA. Integrating biological redesign: where synthetic biology came from and where it needs to go. Cell. 2014;157(1):151–161.
28. Nissim L, Bar-Ziv R, Milo R, Barenholz Y, Alon U. Synthetic biology-based therapeutic cancer circuits: integrating logic gates and sensors. Nat Rev Cancer. 2017;17(12):707–718.
29. Ruder WC, Lu T, Collins JJ. Synthetic biology moving into the clinic. Science. 2011;333(6047):1248–1252.
30. Xie M, Fussenegger M. Designing cell function: assembly of synthetic gene circuits for cell biology applications. Nat Rev Mol Cell Biol. 2018;19(8):507–525.
31. Morrison C. Fresh from the biotech pipeline: 2020. Nat Biotechnol. 2021;39(2):123–128.
32. FDA. Long Term Follow-Up After Administration of Human Gene Therapy Products. Guidance for Industry. 2020.
33. EMA. Guideline on quality, non-clinical and clinical aspects of gene therapy medicinal products. 2018.
34. Horgan D, Romao M, Morré SA, Kalra D. Artificial intelligence: power for civil society and the citizen in digital health. Public Health Genomics. 2019;22(5–6):145–161.
35. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. Nat Med. 2019;25(1):44–56.
36. Xu X, Tay Y. New frontiers in CRISPR-based therapeutic approaches. Trends Biotechnol. 2021;39(9):898–913.
37. Cavazza A, Moiani A, Mavilio F. Mechanisms of retroviral integration and mutagenesis. Hum Gene Ther. 2013;24(2):119–131.
38. Cummings J, Lee G, Zhong K, Fonseca J, Taghva K. Alzheimer's disease drug development pipeline: 2021. Alzheimers Dement. 2021;7(1):e12179.
39. Yang L, Zhang X, Wang Q, Wei X, Ding Y, Luo Y. Nanotechnology platforms for cancer immunotherapy. Biomaterials. 2020;255:120153.
40. Haeussler M, Schönig K, Eckert H, Eschstruth A, Mianne J, Renaud JB, et al. Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR. Genome Biol. 2016;17:148.

41. Wang H, La Russa M, Qi LS. CRISPR/Cas9 in genome editing and beyond. Annu Rev Biochem. 2016;85:227–264.
42. Mout R, Ray M, Lee YW, Scaletti F, Rotello VM. In vivo delivery of CRISPR/Cas9 for therapeutic gene editing: progress and challenges. Bioconjug Chem. 2017;28(4):880–884.
43. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339(6121):823–826.
44. Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S, et al. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat Biotechnol. 2015;33(9):985–989.
45. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther. 2020;5(1):1.
46. George LA, Sullivan SK, Giermasz A, Rasko JEJ, Samelson-Jones BJ, Ducore J, et al. Hemophilia B gene therapy with a high-specific-activity factor IX variant. N Engl J Med. 2017;377(23):2215–2227.
47. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262–1278.
48. Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science. 2020;367(6481):eaba7365.
49. High KA, Roncarolo MG. Gene therapy. N Engl J Med. 2019;381(5):455–464.
50. Marks P, Witten CM, Califf RM. Clarifying FDA regulation of regenerative medicine products. *N Engl J Med*. 2017;376(6):500–502.
51. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med*. 2017;9(9):1183–1197.
52. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol*. 2018;36(8):765–771.
53. EMA. Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products. *EMA/CAT/701802/2014 Rev.1*. 2018.
54. FDA. Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products. *FDA Guidance for Industry*. 2015.
55. ISEV. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). *J Extracell Vesicles*. 2018;7(1):1535750.
56. Greely HT. The ethical challenges of the expanded frontier of synthetic biology. *Nat Rev Genet*. 2021;22(3):185–195.
57. Whittington MD, Pearson SD. A framework for assessing the value of cell and gene therapy. *J Manag Care Spec Pharm*. 2020;26(3):674–681.
58. Garrison LP, Towse A, Briggs A, de Pouvourville G, Grueger J, Mohr P, et al. Performance-based risk-sharing arrangements—good practices for design, implementation, and evaluation: report of the ISPOR Good Practices for Performance-Based Risk-Sharing Arrangements Task Force. *Value Health*. 2013;16(5):703–719.
59. Clayton EW. Ethical, legal, and social implications of genomic medicine. *N Engl J Med*. 2003;349(6):562–569.
60. Cyranoski D. What CRISPR-baby prison sentences mean for research. *Nature*. 2020;577(7791):154–155.

61. Hogle LF. Ethical challenges in the development of cell-based interventions for children. *Theor Med Bioeth*. 2016;37(4):283–300.
62. National Academies of Sciences, Engineering, and Medicine. *Biodefense in the Age of Synthetic Biology*. Washington, DC: National Academies Press; 2018.
63. Jasanoff S, Hurlbut JB, Saha K. CRISPR democracy: Gene editing and the need for inclusive deliberation. *Issues Sci Technol*. 2015;32(1):25–32.
64. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. *Genome Biol*. 2017;18(1):83.
65. Zhu J, Chen X, Liao Z, He C, Hu X, Chen Y, et al. Machine learning-based prediction of CRISPR-Cas9 cleavage efficiency. *Bioinformatics*. 2019;35(3):459–466.
66. Paull D, Sevilla A, Zhou H, Hahn AK, Kim H, Napolitano C, et al. Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells. *Nat Methods*. 2015;12(9):885–892.
67. Feero WG, Guttmacher AE. Genomics, personalized medicine, and pediatrics. *Acad Pediatr*. 2014;14(1):14–22.
68. Khoury MJ, Galea S. Will precision medicine improve population health? *JAMA*. 2016;316(13):1357–1358.
69. Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med*. 2015;372(9):793–795.