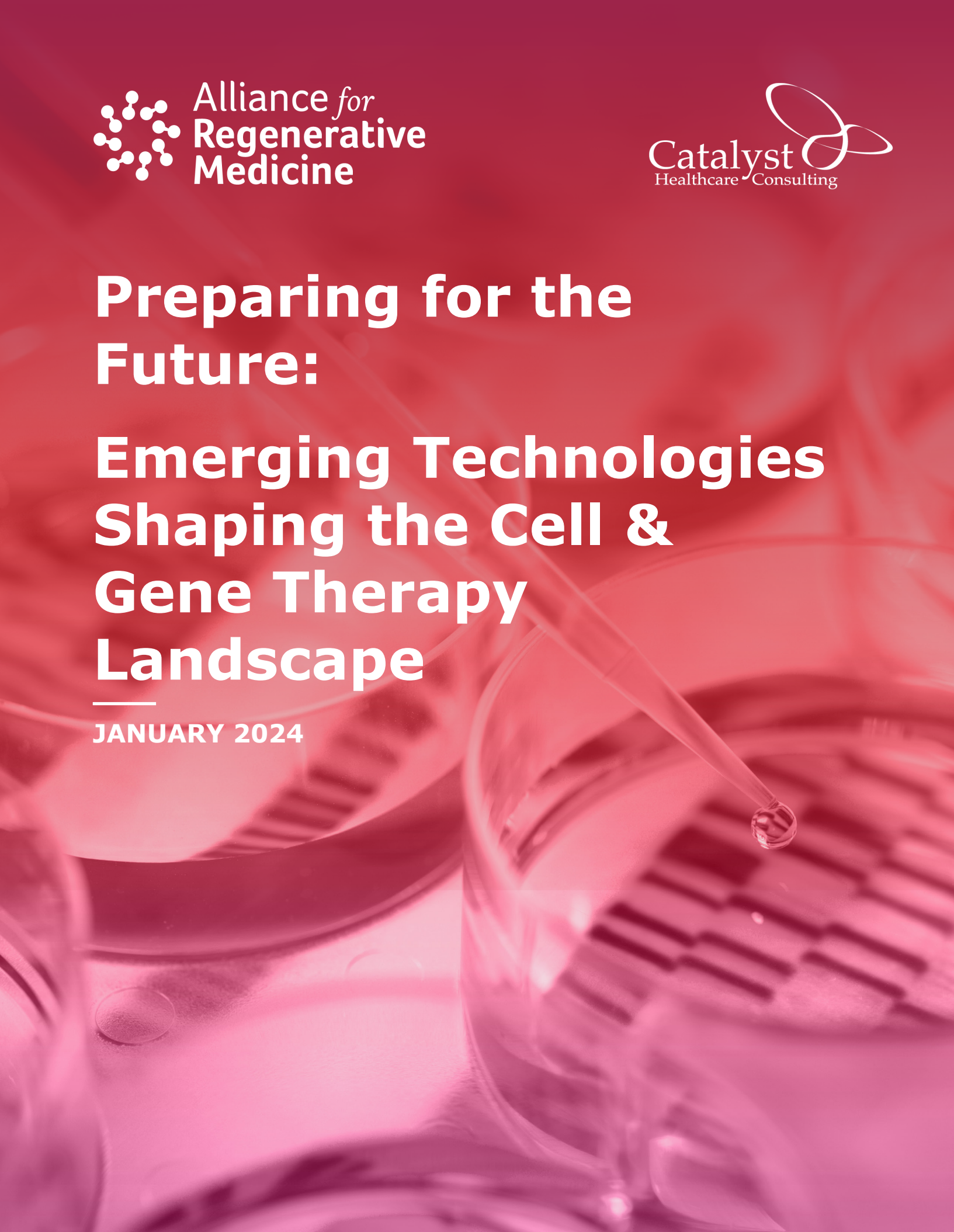
Alliance for Regenerative Medicine

Catalyst Healthcare Consulting

Preparing for the Future:

Emerging Technologies Shaping the Cell & Gene Therapy Landscape

JANUARY 2024



About The Authors

Taryn Serman: PhD, Senior Director of Communications and Research at Catalyst Healthcare Consulting

Nancy Bradish Myers: JD, CEO and Founder of Catalyst Healthcare Consulting

About The Alliance For Regenerative Medicine

The Alliance for Regenerative Medicine (ARM) is the leading international advocacy organization championing the benefits of engineered cell therapies and genetic medicines for patients, healthcare systems, and society. As a community, ARM builds the future of medicine by convening the sector, facilitating influential exchanges on policies and practices, and advancing the narrative with data and analysis. We actively engage key stakeholders to enable the development of advanced therapies and to modernize healthcare systems so that patients benefit from durable, potentially curative treatments. As the global voice of the sector, we represent more than 400 members across 25 countries, including emerging and established biotechnology companies, academic and medical research institutions, and patient organizations. Visit alliancerm.org to learn more.

About Catalyst Healthcare Consulting

Catalyst Healthcare Consulting is a dynamic, hands on regulatory affairs partner to global innovators, advocates, payors, providers, investors, and trade organizations helping to speed innovative healthcare solutions to market to improve the lives of patients. The Catalyst team is comprised of experts in the fields of regulatory strategy, public policy, FDA dynamics, patient engagement, reimbursement and government programs, and scientific communication who bring vision, expertise, energy, and creative solutions to the table. The team works side-by-side with clients to efficiently obtain critical regulatory and policy objectives and help clients build collaborations and propel their cutting-edge technologies forward. To learn more about Catalyst, visit catalysthcc.com.

EXECUTIVE SUMMARY

The field of Cell and Gene Therapy (C&GT) is growing by leaps and bounds as evidenced by industry pipelines and regulatory submissions. FDA Center for Biologics and Research (CBER) leadership has described their expectations for "exponential growth in C&GT submissions."[1] A major action taken by regulators to prepare for this future is the 2023 CBER reorganization to create Office of Therapeutic Products (OTP) Super Office to "address the substantial growth in the development of innovative, novel products and the ever-changing public health landscape."[2] The anticipated size and scope of C&GT emerging technologies will require regulators to engage in preemptive planning to ensure nimble reactions to submissions.

In the spirit of looking ahead, the Alliance for Regenerative Medicine (ARM) contracted with Catalyst Healthcare Consulting to conduct a horizon scan to identify emerging technologies in the for-profit C&GT sector that are most likely to require novel solutions for agency review and regulation over the next 3-10 year period.

The findings reported herein are intended to spur conversations around the state of the C&GT landscape and to increase awareness of technologies in development, especially those that are likely to challenge regulatory paradigms currently in place. The results of our efforts are presented in the Summary Table below, with detailed descriptions of the scientific background, current areas of therapeutic development, and future perspectives of each technology elucidated in the main text.

Summary Table: The top emerging technologies in C&GT likely to warrant a reassessment of existing regulatory frameworks.

Emerging Technology	Description	Unique Features that Challenge Existing Regulatory Frameworks
Engineered T cells for Autoimmunity	Cell therapies that target an imbalanced or overactive immune system.	Rather than creating a highly effective cytotoxic response (akin to CAR-T cell therapies), these therapies regulate immune cell signaling to restore balance, warranting the determination of the factors governing sufficient vs. excessive immunosuppression.
***in vivo* CAR-T cell Engineering**	Delivery of gene editing cargo to engineer (e.g. CAR-T) cells *in vivo* without the need for cell isolation from the patient or a donor.	Producing CAR-T cells directly in the patient rather than in an *ex vivo* setting will require new regulatory thinking for CMC and *in vivo* safety, efficacy, and toxicity studies.
Synthetic Biology	Cell therapies with at least two functional components that mimic a biological process with Boolean functionality (e.g., a "decision-making" switch). "	Programming cells to be decision-making sentinels rather than on-demand killers will require different ways of assessing their behavior in the patient and other novel regulatory considerations.

Non-double-stranded break-inducing Gene Therapies	Gene therapies that leverage machinery capable of modulating target genes without inducing double-stranded breaks.	New mechanisms for genetic manipulation will expand the potential for making tunable and/or multi-gene edits, requiring discussions and guidance around right-sized long-term safety and efficacy testing.
iPSC-derived Therapies	Cell therapies derived from induced pluripotent stem cells.	Starting with unique material from which to derive therapeutic cells may require regulatory development around novel ways of standardized identity and safety testing.
Engineered Immune Cells	Immune cells (non-αβ T cells) that have been genetically engineered to target specific disease-associated agents.	Harnessing functions of unique immune cells to unlock new therapeutic avenues may require new and potentially unique safety and efficacy testing guidance.
Nonviral Gene Therapy Delivery	Delivery of gene therapy machinery to cells without using a viral vector.	Characterizing a unique panel of vectors will require vector-specific safety and efficacy testing and scalable CMC that takes advantage of a least burdensome regulatory approaches.
Capsid Engineering	Engineering of viral capsids for enhanced targeting and tropism, through mechanisms such as enhanced cell-specificity, tissue penetration, immune evasion, intracellular trafficking, or other functional avenues.	Improving existing viral vectors to enhance the potency of gene therapies may require new regulatory considerations for approval timelines and building block type regulatory approaches.
Bioengineered and Xenotransplanted Organs	Engineering of cells, tissues, and matrices to replace or bolster organ missing a period after function.	Ensuring organ function and immune tolerance post-transplantation may require new thinking around appropriate safety and efficacy testing largely under compassionate-use settings.
Secretome and Exosome-based Therapeutics	Use of cell culture-derived media (secretome) or purified exosomes as therapeutics.	New mechanisms for genetic manipulation will expand the potential for making tunable and/or multi-gene edits, requiring discussions and guidance around right-sized long-term safety and efficacy testing.
Alternative RNA Therapeutics	RNA-based therapies that influence protein expression through mechanisms distinct from available RNA therapies.	Manipulating protein expression using unique RNA-targeted mechanisms may require new and potentially unique safety and efficacy testing guidance.

This report summarizes the narrative and methodology used by Catalyst Healthcare Consulting to complete the horizon scan effort. We carefully considered many approaches and chose the one described in the report. Some could argue that the use of a different methodology could modify these rankings, so we encourage readers to view these identified emerging technologies not as a complete list of promising technology, but rather, as an example of what is to come.

We hope this report will illuminate the path ahead and generate ideas on how the industry, regulators, and stakeholders can prepare the way for bringing promising science to patients.

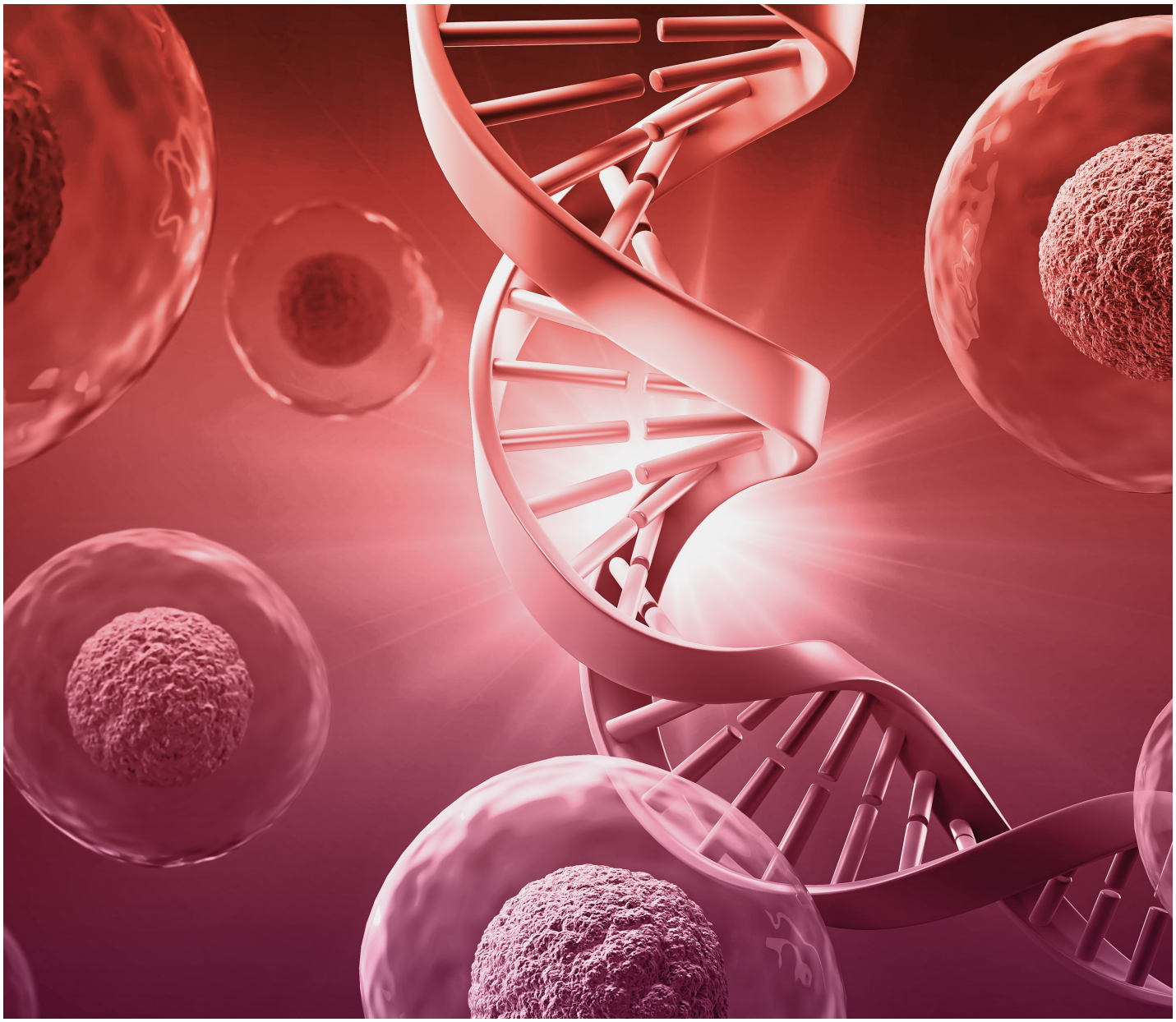
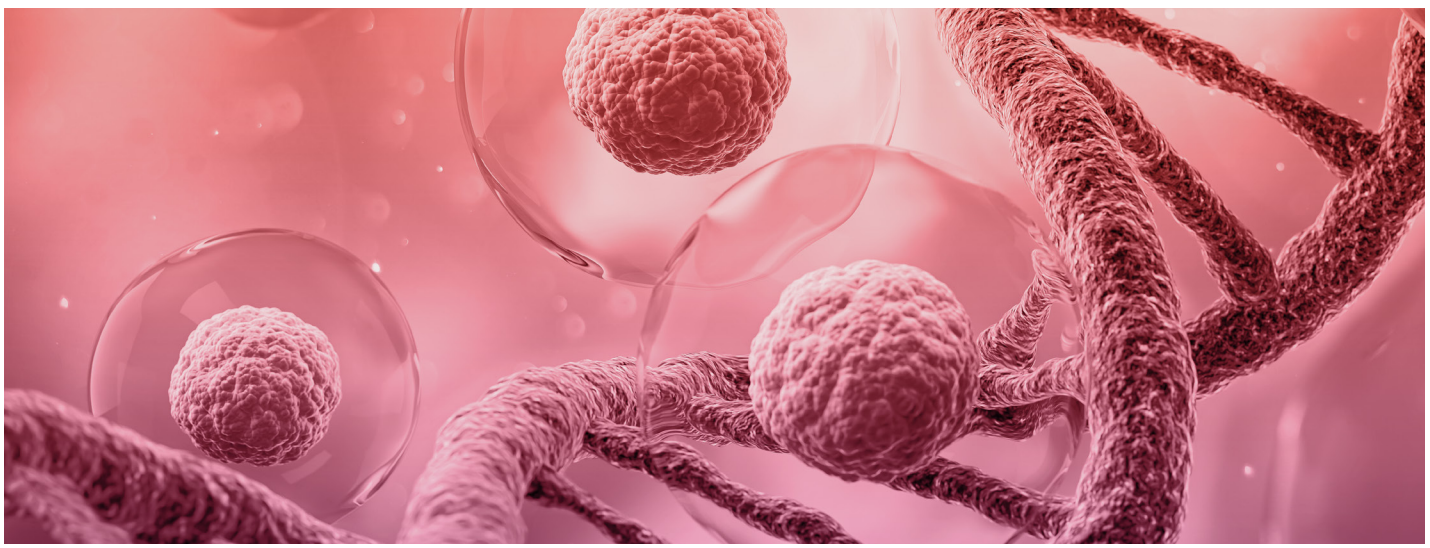


TABLE OF CONTENTS

Section 1: Introduction

Section 2: The Top Emerging Technologies in the C&GT space

Section 3: Conclusions



INTRODUCTION

The Cell and Gene Therapy Revolution

Cell and Gene Therapies (C&GTs) have transformed the way we approach previously incurable and difficult-to-treat diseases by repurposing cells as living medicines or altering the genetic code to restore function. Innovations in fundamental research, translational medicine, and manufacturing are rapidly advancing the C&GT space, as evidenced by the increasing number of companies, clinical trials, and approved therapies emerging in this space.[3] This booming growth is expected to continue, putting pressure on regulatory bodies to address the growing need for new policies, review strategies, and expedited paths to approval.

The Regulatory Landscape for C&GTs

In the United States, the sustained increase in the number of C&GT products entering the clinical phase has corresponded with an increase in applications and meeting requests to The Food & Drug Administration (FDA). To ensure the timely evaluation and approval of novel C&GTs, the Center for Biologics Evaluation and Research (CBER) at FDA has converted the former Office of Tissue and Advanced Therapies (OTAT) into a new super office called the Office of Therapeutic Products (OTP) dedicated primarily to the review and approval of C&GTs.[4,5] Despite the exponential growth in workload, the Director of CBER, Dr. Peter Marks, has indicated that, "the FDA is prepared to evaluate any IND application associated with a proposed clinical study or use of an investigational C&GT treatment and determine whether the risks of the proposed study or treatment are reasonable in light of the potential unproven benefits anticipated."[6]

Methodology for Horizon Scan of Emerging Technologies

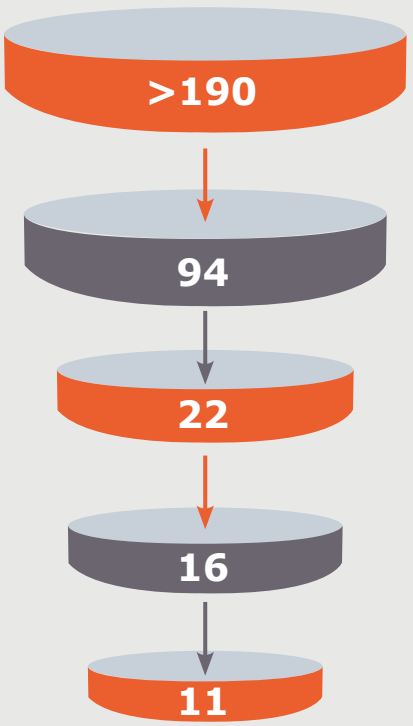
There are many ongoing efforts seeking to enhance the efficacy and safety of available C&GT strategies.[7] However, this whitepaper, based on a recently completed horizon scan, seeks to identify emerging technologies in C&GT.

Prior to initiating the horizon scan, a team from Catalyst Healthcare Consulting and Alliance for Regenerative Medicine worked to create an unbiased approach to scan the C&GT industry. This approach began with defining "emerging technologies" as **innovative technologies with substantial untapped clinical potential, capable of shaping the future of the biotech industry over the next 10 years.** The horizon scan included any technology that will be **regulated by CBER OTP** and **can be directly translated into or enable the translation of a marketed product.**

For example, engineered cell therapies and genetic medicines with the potential for a durable, transformative effect on patient outcomes were considered, while existing non-persistent RNA therapeutics, including mRNA vaccines, antisense oligonucleotides (ASOs), RNA interference (RNAi), and RNA aptamers were not within this scope of work. Medical devices were also not in scope, except those that might be part of a combination product that meets the aforementioned criteria.

Once "emerging technologies" was defined, the team assembled a list of C&GT companies that procured IPOs or start-up funding between 2021-2023.[8,9] From there, each company's C&GT products were assessed individually, and any products that did not leverage technologies that fit our definition of "emerging" were excluded. These exclusion criteria included: (1) any products classified as traditional adoptive cell therapies (i.e., chimeric antigen receptor (CAR)-T cell therapies, tumor-infiltrating lymphocytes (TILs), and (TCR)-T cell therapies), (2) AAV-based gene replacement therapies, (3) manufacturing systems, or (4) broadly applicable, optimization-focused platforms such as artificial intelligence (AI). Further, any products that are already in late-stage clinical development (i.e., in phase 3 clinical studies or beyond) were also excluded from the candidate list. Finally, products developed by companies still in stealth mode were not considered, due to a lack of detailed technology descriptions. This left a refined candidate list of novel C&GT products with IND/IDE/BLA potential in early development stages from companies with active websites that publicly describe product pipelines (**Figure 1**).

Figure 1: Process for scanning the C&GT industry for emerging technologies



C&GT companies that procured start-up funding or announced IPOs in 2021-2023

C&GT companies developing "emerging" products

Categories of emerging C&GT technologies identified in scan

Categories of emerging C&GT technologies eligible ranking and objective scoring

Top scoring emerging C&GT technologies described in this report

Figure developed by Catalyst Healthcare Consulting

From there, individual products in development were grouped into broad technology categories. A cross-functional internal team of PhDs and C&GT experts then discussed the refined emerging technology categories, researched additional companies developing technologies in these categories (that were not already described in the IPO tracker or start-up funding summary reports), and re-assessed the categories using the aforementioned exclusion criteria. Categories that (1) lack disruptive innovation (i.e., technologies that do not challenge the current regulatory standards or scientific paradigms), (2) are unique to a single company, or (3) have already been developed into marketed products were also excluded. The agreed-upon technology categories were then ranked by five members of the internal team who each provided a list of the top 5 technologies likely to influence the industry and patient lives in the next 3-10 years. A point was given to each technology that ranked among each team member's Top 5 list as one half of our "C&GT expert scoring system". To round out the C&GT expert scoring system, the list of refined emerging technology categories was sent in the form of a survey to a variety of industry, academic, and investment experts in the C&GT space for their subjective ranking and input. A point was given each time a technology ranked in the eleven survey responders' Top 6 list (**Figure 1**).

The emerging technology categories were then filtered into an objective scoring system that added numerical values to qualities based on an objective set of criteria (**Table 1**). For each of the four objective scoring criteria, the highest scores were given to technologies with (1) 4-7 companies developing the technologies, (2) more than $100M averaged funding, (3) academic papers (or press releases in lieu of academic papers) first describing the technology published in 2020 or more recently, and (4) in preclinical or IND-enabling stages of development.

The rationale governing the number of points for each criteria are as follows:

01 Technologies being developed by 4-7 companies suggests high likelihood that at least one company will take a product to market, while regulators may still be unaware of the technology.

02 Publications from 2020 onward likely describe technologies with a scientific basis that challenges current technological and regulatory paradigms.

03 Technologies being developed by companies with more than $100M in funding likely have the financial support to take a product to market.

04 Key candidates in IND-enabling stages of development have clinical promise but may still be unknown to regulators.

To calculate objective scores, the "year of first publication" and "funding" results were averaged and the resulting value was used to assign the individual scores for these two criteria. For the "development stage", the point value assigned to the development stage of each product in the category was determined, then the values were averaged to assign the final numerical score (**Table 1**).

Table 1: Objective Scoring Criteria

Quality Measured	Measurement	Criteria	Score
Need for updated regulation	Number of companies developing the technology	1-3	2
		4-7	4
		7+	3
Novelty of innovation	Year of first publication by company describing technology	before 2015	0
		2015-2020	2
		2020-2023	4
Resources to develop technology	Average developing company funding ($USD)	<$50M	2
		$50-$100M	3
		$100M+	4
Predicted time to market	Development stage of key candidates	Discovery	1
		Preclinical	3
		IND-enabling	4
		Phase 1	2
		Phase 2	1
		Phase 3+	-2

The point values from this objective scoring step were summed with the point values from the expert C&GT scoring values. Through this filter, a list of the top emerging technologies in the C&GT space came to light (**Figure 1**).

Summary of findings

From this scanning approach, a top ten list was developed (**Table 2**), though a three-way tie for the 10th spot resulted in a top 11 list. Below, the scientific basis of these technologies is described as well as future perspectives based on the current state of the industry. The technologies are presented in the text in a manner that allows for the accumulation of scientific background information for each therapy, rather than presented in the ranked order of final scores.

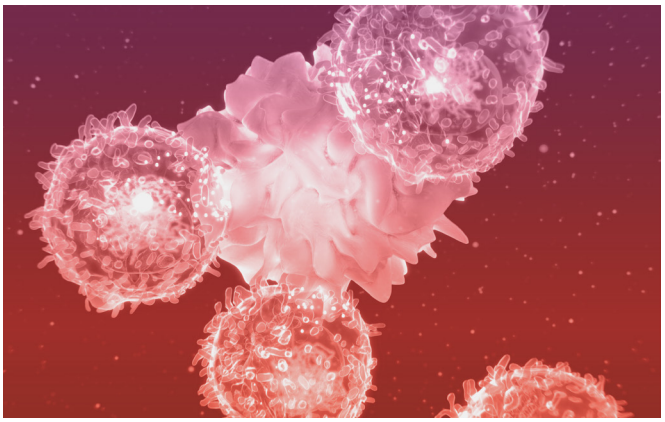
Table 2: The Top Emerging Technologies in C&GT identified by the described methodology

Rank	Technology
1	Engineered T cells for Autoimmunity
2 (tie)	*in vivo* CAR-T cell Engineering
3 (tie)	Synthetic Biology
4	Non-double-stranded break-inducing Gene Therapies
5	iPSC-derived Therapies
6	Engineered Immune Cells
7	Nonviral Gene Therapy Delivery
8	Capsid Engineering
9 (tie)	Bioengineered and Xenotransplanted Organs
10 (tie)	Secretome and Exosome-based Therapeutics
11 (tie)	Alternative RNA Therapeutics

The Top Emerging Technologies in the C&GT space

Engineered T cells for Autoimmunity

01 Background



Systemic autoimmune diseases are primarily caused by the production of autoantibodies or autoreactive cytotoxic T lymphocytes (CTLs), both of which recognize self-antigens as foreign and result in an excessive, damaging immune response. Autoantibodies, which are produced by self-reactive B cells, bind to self-cells in the body and mediate their destruction. Similarly, autoreactive CTLs use their T cell receptors (TCRs) to interact with major histocompatibility complex class I (MHC-I) molecules on the surface of self-cells, resulting in apoptotic death. Cell death mediated by these self-reactive immune cells results in widespread inflammation that can ultimately damage tissue, among other severe health complications.[10] Current treatments for autoimmune diseases, such as lupus, multiple sclerosis, inflammatory bowel disease (IBD), and more, rely largely on systemic immunosuppressant medications. These non-specific treatment regimens are associated with nausea and vomiting, fatigue, and increased risk of infection, facilitating the need for alternative treatment strategies, such as T cell-based therapies.[11]

There are three major subsets of T cell-based therapies currently in development to target an imbalanced or hyperactive immune system associated with autoimmunity: CAR-T cell therapies for autoimmunity, engineered regulatory T cell (Treg) therapies, and Treg transplants.[12]

Given their efficacy in recognizing cancer-related antigens and destroying tumor cells, CAR-T cell therapies are an established platform that is currently being leveraged for autoimmunity applications. All approved CAR-T cell therapies are designed to target antigens associated with proliferative B cells (CD19 or BCMA), achieving success in targeting liquid cancers caused by B cell permutations (e.g., leukemias, lymphomas, and myelomas).[13] Since some autoimmune diseases are characterized by an increase in autoantibodies produced by dysfunctional B cells, CD19- and BCMA-targeting CAR-T cells may be repurposed to reduce autoantibody production by mediating B cell killing (**Figure 2A**).[14,15]

Tregs are a heterogenous population of immune regulatory cells that can help reduce the killing activity of CTLs to prevent a hyperactive immune response. Tregs are a subset of CD4+ T cells that are characterized by their co-expression of CD25 and FOXP3, the latter of which is an essential transcription factor for mediating their immunosuppressive activity.[16] Polyclonal Treg therapy – in which Tregs are isolated from a patient, expanded in culture, and reinfused into the patient– has had mixed results in terms of therapeutic efficacy for autoimmune disease patients.[17] Therefore, cell therapy developers have begun using Tregs as a candidate cell type for gene-modified cell therapy.[18]

02 Areas of active therapeutic development

CAR-T cell therapies for autoimmunity currently in development seek to target CTLs to hyperactive, autoantibody-producing B cells. To do so, CARs are engineered to express the autoantigen itself, facilitating the binding of CAR-T cells to the autoantibodies on B cells to mediate their destruction (**Figure 2B**).[19,20,21]

Outside of traditional CAR-T cell approaches, developers have also begun engineering Tregs to express CARs or TCRs to direct their immunosuppressive activity to sites of autoimmune disease. For example, Tregs have been engineered to induce immunosuppression at sites of self-reactive lymphocyte-mediated cellular destruction through the expression of TCRs or CARs that recognize antigens on the cells that are being destroyed (**Figure 2C, D**). For example, TCR-Treg cells engineered to recognize pancreatic islet cells are being developed to suppress their destruction by self-reactive CTLs for Type I diabetes indications.[22,23] Moreover, TCR-Tregs have been designed to hone to and mediate immunosuppression at sites of myelin accumulation, the underlying cause of progressive multiple sclerosis, thus reducing disease severity (**Figure 2C**).[23]

As an alternative mechanism to directly recognizing antigens on cells likely to be destroyed by self-reactive T cells, CAR-Tregs have also been engineered to recognize inflammatory cells that contribute to autoimmune severity (**Figure 2D**). For example, Tregs engineered with CARs recognizing IL-23, a receptor that is overexpressed by inflammatory cells in the GI tract of Crohn's disease patients, have been shown to reduce local inflammation associated with disease.[24] Similarly, CAR-Tregs that recognize certain classes of HLA molecules present on newly transplanted organs are being developed to suppress the immune reactivity to these foreign antigens to reduce the risk of graft-vs-host disease (GvHD).[25]

Finally, cell therapy developers are also optimizing enrichment protocols to select and expand for allogeneic, non-engineered Tregs from healthy cord blood that can naturally hone to sites of inflammation and mediate immunosuppression for a variety of disease indications.[23]

Figure 2: Engineered T-cell therapies for autoimmunity in development

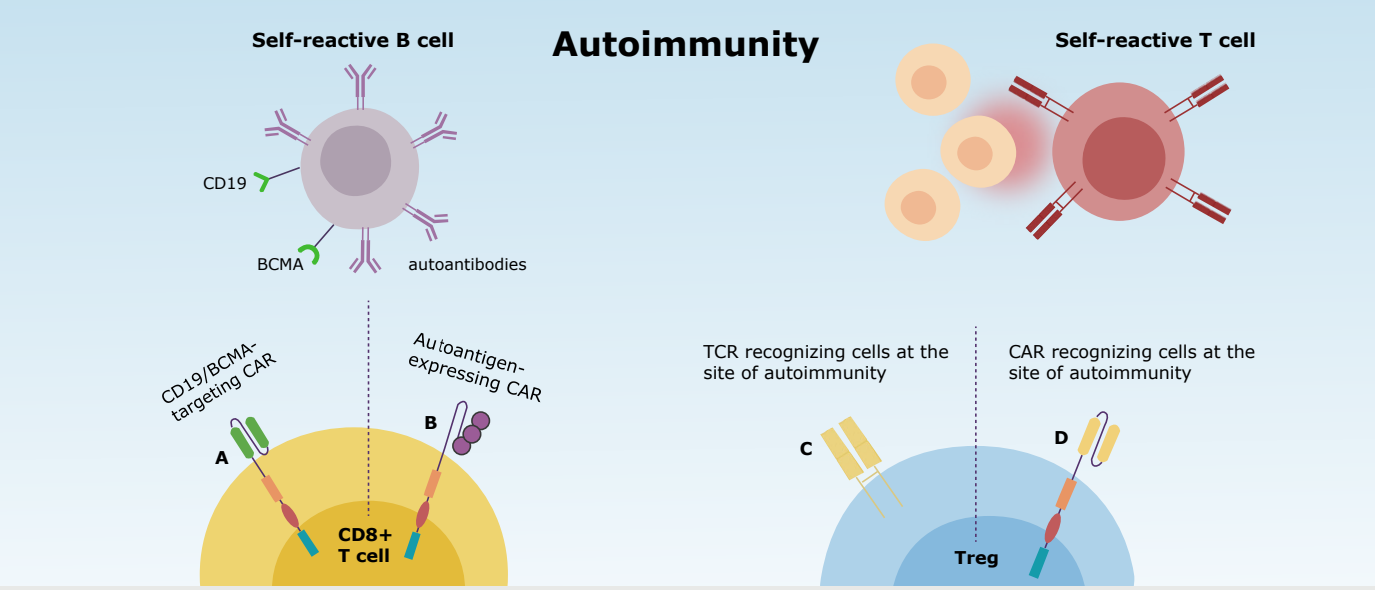


Figure created by Catalyst Healthcare Consulting with biorender.com

03 Looking ahead

As T cell therapies for autoimmune diseases– especially Treg therapies– continue to advance, several key considerations must be made. A main concern is the potential for Treg therapies to promote broad, "off-target" immunosuppression. Treg cells are known to promote "infectious tolerance," whereby the recognized epitopes are spread via Treg signaling to dampen other immune cell responses. This concern is particularly important for Treg therapies that target general, non-self antigens (like certain HLA classes) presented on transplanted organs. Therefore, dosing regimens must be carefully considered and assessed to ensure the appropriate degree of immunosuppression. Additionally, the stability and long-term effects of Tregs must also be assessed to ensure the chance of efficacy outweighs the potential risks. These studies will require clear assays and markers to assess the specificity and degree of immunosuppression mediated by Treg therapies. Moreover, guidance is needed surrounding clinical study design, including the selection of endpoints and the potential to apply these therapies to multiple autoimmune disease indications in a single study. To maximize the therapeutic potential of Treg therapies, more focused clinical research will be needed to understand how to precisely balance their immunosuppressive function, prolong their stability and longevity in patients, and prevent CAR-Treg exhaustion.[17]

Engineered Immune Cells

01 Background

Cytotoxic T cells have long been the focus of developing cell therapies, especially given the clinical success of CAR-T cell therapies in revolutionizing immuno-oncology. However, many other immune cells in the body target cancer and other diseases, therefore motivating the development of novel therapies that harness the activity of other immune cells, including natural killer (NK) cells, macrophages, γδ -T cells, and B cells.

NK cells are innate immune lymphocytes with remarkable cytotoxic capabilities that do not rely on antigen presentation cells (APCs) or recombination-dependent polymorphic antigen receptors to recognize and target abnormal cells in the body. Instead, NK cells have both killer activating receptors (KARs) and killer inhibitory receptors (KIRs) that allow them to recognize cells that lack self-antigens (e.g., cancer cells) (Figure 3A) as well as cells that express non-self-antigens (e.g., non-commensal bacterial cells during an infection). Once activated, NK cells release cytotoxic granules containing enzymes that induce apoptosis in target cells.[26]

Macrophages are innate immune cells differentiated from monocyte precursors that engulf invading pathogens or damaged cells via phagocytosis. Once engulfed, macrophages release cytokines to recruit adaptive immune cells (T cells and B cells) and present antigens to these cells via MHC-I molecules to activate the adaptive immune response (**Figure 3B**).[27]

γδ-T cells are a subset of innate-like T cells that express a TCR comprised of γ and δ chains. Unlike conventional αβ-T cells, like cytotoxic and helper T cells, γδ-T cells are involved in immune surveillance at barrier tissues like the skin and mucous membranes, where they respond to invading pathogens and tumor cells. These cells act as a bridge between the innate and adaptive immune response since they function both to secrete pro-inflammatory cytokines and to induce potent anti-tumor cytotoxicity (**Figure 3C**).[28]

Finally, B cells are specialized adaptive immune cells that, when activated by antigen-presenting cells, differentiate into plasma cells that secrete highly specific antibodies. These antibodies recognize and bind to target cells expressing the cognate antigen, marking them for destruction. Importantly, B cells can develop memory, allowing the immune system to mount a rapid response upon subsequent encounters with the same antigen, ultimately providing long-lasting immunity (**Figure 3D**).[29]

Figure 3: Immune cell functions harnessed by emerging engineered immune cell therapies

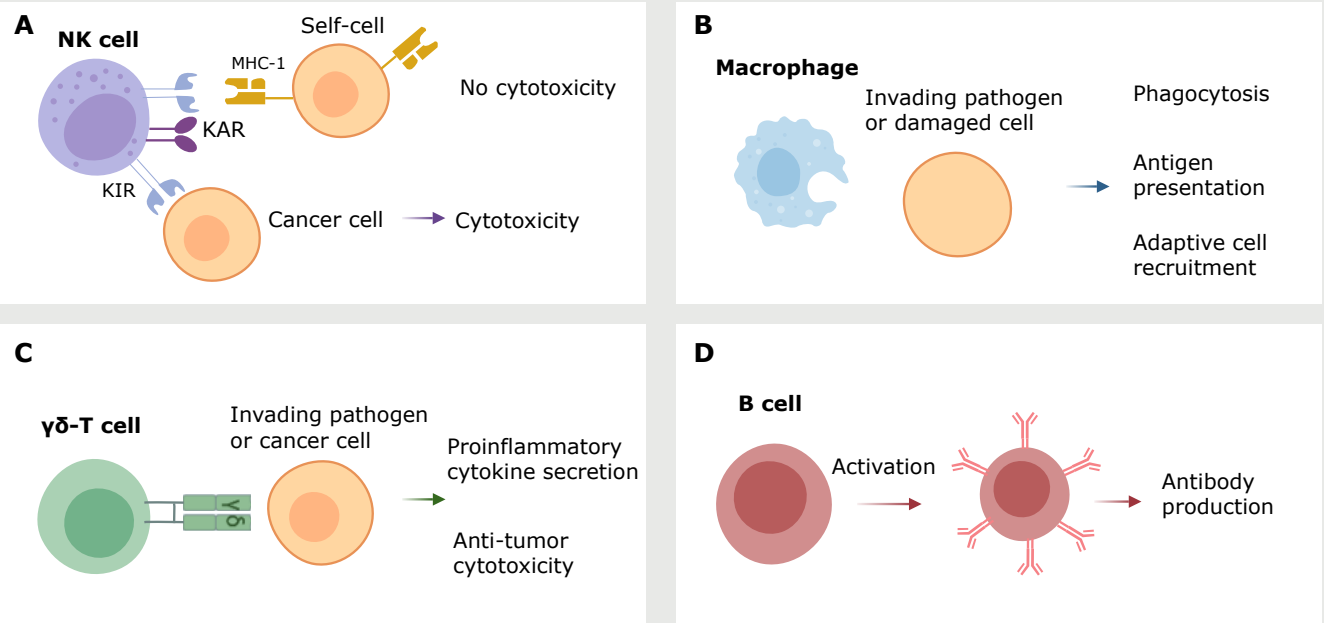


Figure created by Catalyst Healthcare Consulting with biorender.com

Leveraging the activities of these diverse immune cells through the design of novel therapies can treat diseases in ways distinct from currently available therapies.

02 Areas of active therapeutic development

Many engineered immune cells rely on the expression of CARs for a variety of downstream applications.

For example, CAR-macrophage– or "CAR-M"– therapies are generated by first isolating monocytes from patients and engineering them with CARs to generate CAR-Monocytes. These engineered CARs possess not only the extracellular antigen-targeting domain but also several intracellular domains to stimulate phagocytic and cytokine secretion activities that contribute to tumor cell destruction. Studies have shown that CAR-Monocytes alone possess potent anti-tumor activity, and can therefore be used directly as therapeutics, or can be further differentiated into CAR-M cells.[30] CAR-M cell therapies seek to address certain limitations of CAR-T cell therapies in treating cancer. For example, CAR-T cells possess a limited ability to penetrate solid tumors due to the highly immunosuppressive microenvironment surrounding the tumor.

However, since native macrophages are actively recruited to solid tumors, CAR-M therapies may overcome this limitation. Additionally, macrophages are inherent stimulators of the adaptive immune response, so once they penetrate a tumor, they can recruit adaptive immune cells to the solid tumor and stimulate a robust adaptive response. Finally, the significant cell to cell heterogeneity within a solid tumor mass has prevented CAR-T cell infiltration as CAR-T cells traditionally recognize only the single antigen encoded by the CAR-T. In contrast, macrophages natively present many antigens and can therefore activate the adaptive immune response against multiple tumor-specific antigens present within the solid tumor. CAR-M therapies are currently being developed to treat indications such as HER-2 solid tumors, mesothelin-positive solid tumors, prostate cancer, liver fibrosis, autoimmune disease, and more.[31]

The generation of CAR-M cells is similar to the process for CAR-T cell generation, whereby cells are first isolated from a patient. From the cellular population, CD14+ monocytes are selected for and expanded. These monocytes can then be directly engineered with a CAR to produce CAR-Monocyte therapies, directly engineered with the CAR then subsequently differentiated into CAR-M cells, or differentiated into macrophages via treatment with stimulating factor GM-CSF and subsequently engineered to express the CAR. The resulting CAR-Monocyte or CAR-M cells are then re-infused into the patient.[32]

CAR-NK cells in development also seek to address limitations faced by available CAR-T cell therapies. The development of allogeneic CAR-T cell therapies has been limited by their associated risk of inducing GvHD, an inflammatory disorder mediated primarily by T cells. Since NK cells generally do not induce GvHD (or other CAR-T-associated side effects like neurotoxicity and cytokine release syndrome), these therapies may cause less potential harm to patients.[33] Additionally, NK cells can kill tumors without prior sensitization by APCs, bypassing the need for expansion in response to tumor antigens, which CAR-T cells require.

Allogeneic CAR-NK cells in development are engineered to express CARs that recognize standard T cell antigens, like CD1934, or solid tumor-associated antigens.[35] Other developing CAR-NK cells overexpress a fusion protein containing CAR domains and domains of the native NK cell KAR, NKG2D. NKG2D recognizes a variety of antigens associated with tumor cells to induce their cytotoxic destruction and subsequent T-cell recruitment. These developing CAR-NK cell therapies also express membrane-bound IL15, a costimulatory molecule supporting CAR-NK cell expansion and proliferation.[34]

Like CAR-NK cells, CAR-γδ T cells can also perform tumor-killing functions without co-stimulation by MHC molecules, representing a higher potential for allogeneic success compared to CAR-T cell therapies. Their additional immune surveillance function also equips these cells with better tissue homing and infiltration, providing an advantage for penetrating solid tumors over traditional CAR-T cell therapies. Some developing CAR-γδ T cell therapies also co-express TCRs or T Cell Receptor-Like monoclonal antibodies (TCRLs), which enable recognition of both extracellular tumor antigens and intracellular tumor antigens, respectively.[36]

Beyond engineering these diverse immune cell types to express CARs, cell therapy developers are also leveraging the native features and benefits of B cells, particularly the antibody-secreting activity of mature B cells (also called plasma cells), to create novel therapies called B cell "factories". These therapies use the antibody-secreting function of plasma cells to instead express enzymes, antibodies, structural proteins, or signaling proteins that are lacking or dysfunctional in certain disease indications. This strategy offers an alternative to enzyme replacement therapies, which require weekly, time-consuming infusions of proteins into patients. These therapies also offer an advantage over traditional gene therapy strategies because they can be re-dosed to patients, bypassing the need to optimize a single dose to ensure stable expression.[37]

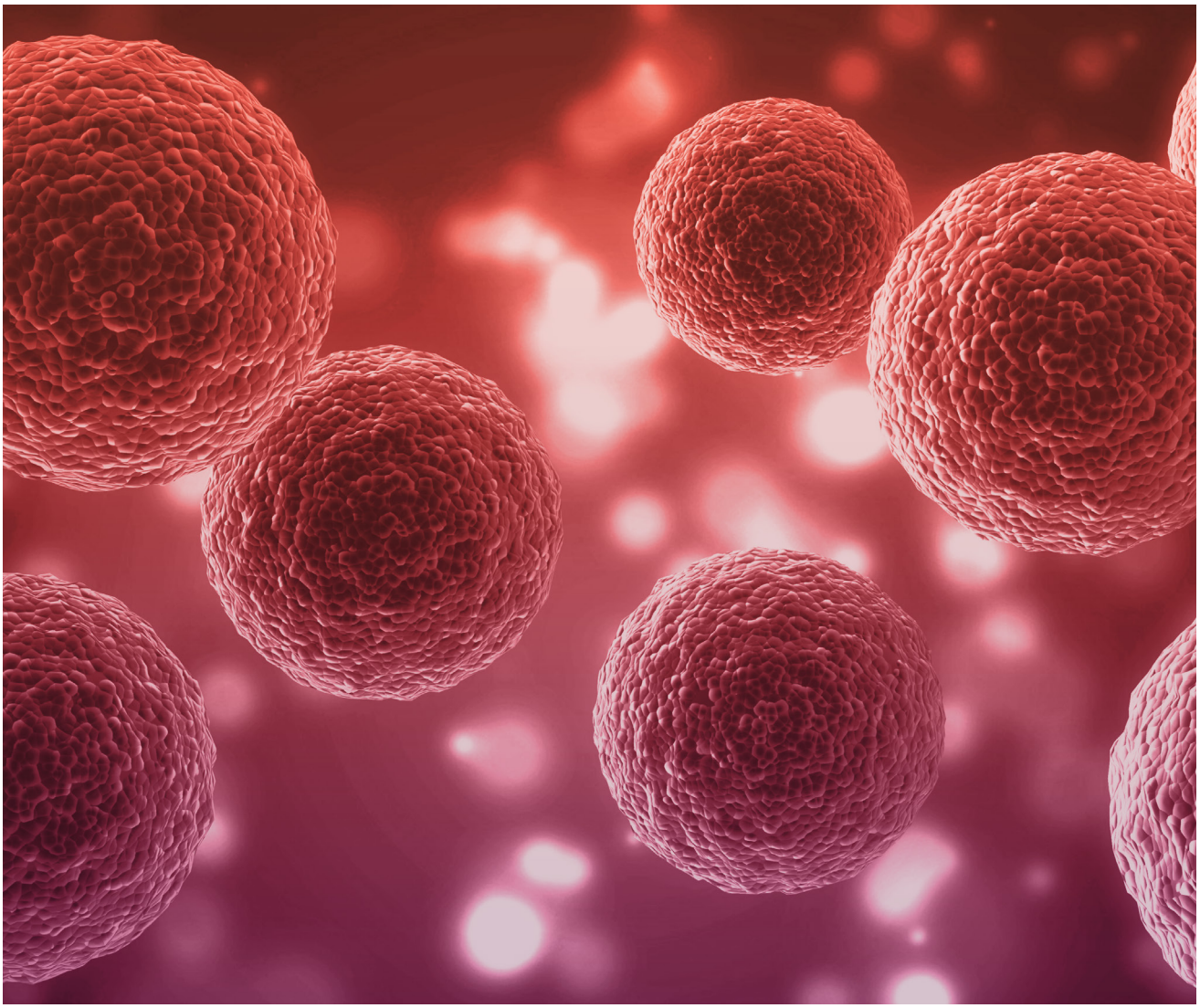
Similar to CAR-T cell therapies, B cell therapies also start with collection of blood from patients, followed by the purification of B cells, genetic engineering using homology-directed repair to replace native antibody secretion with therapeutic protein secretion, and differentiation into plasma cells. These "biofactories" are then optimized to express the therapeutic protein of interest prior to injection or re-infusion back into the patient.[38,39]

03 Looking ahead

A variety of challenges may arise as engineered immune cells continue to transition from the preclinical space toward large-scale production. A major area to optimize is the enrichment of low-abundant immune cells prior to genetic engineering and infusion into the patient. For example, macrophages cannot proliferate by themselves *in vitro* or *in vivo*, limiting their cell number to those directly isolated from the patient.[40] Similarly, γδ-T cells comprise only 1-5% of total T cells, requiring highly effective purification and expansion strategies to ensure sufficient cell numbers for efficacy.[36] Likewise, NK cells are notoriously difficult to culture and require feeder cells to provide them with extracellular secretions needed for proliferation.[41] The co-culture of immune and feeder cells will require an extra layer of optimization, standardization, and regulation as these therapies progress into the clinical space.[39]

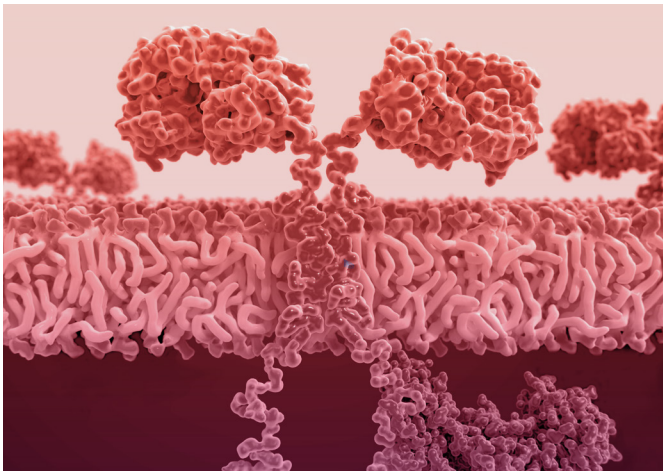
Beyond ensuring sufficient cells for efficacy, the delivery of these therapies to the correct biological sites must be optimized. For example, exogenous macrophages tend to cross the lung after injection and accumulate in the liver, lowering their therapeutic potential.[40] More preclinical and clinical studies will be needed to address these potential limitations of engineered immune cells.

For B cell factories, the manufacturing process for large-scale production will need to be proven. Moreover, as B cells are notoriously hard to genetically manipulate due to inherent factors, most B cell factories in development use transposon-mediated gene editing (discussed in a later section), a non-traditional method for gene-editing that has not yet been used in any approved cell therapy product. The insertion of therapeutic protein transgenes in B cells must be very specific to prevent the risk of integration near cancer-promoting genes, which carries a potential risk of inducing B cell cancers in treated patients.[42]



Synthetic Biology

01 Background



Synthetic biology is a broad term and its use extends to areas beyond cell and gene therapies. For the purposes of this paper, synthetic biology refers to **systems with at least two functional components that mimic a biological process with Boolean functionality.** For cell therapy applications, synthetic biology describes the engineering of cells with "decision-making" capabilities that allow them to carry out multiple desired functions. that allow them to carry out multiple desired functions. Synthetic biology for cell therapy seeks to address challenges encountered by currently available CAR-T cell therapies, such as off-tumor toxicity, antigen heterogeneity, poor persistence, and functional suppression due to the tumor microenvironment (TME).

The promise of more efficacious adoptive cell therapies can mean huge therapeutic payoffs for patients with a variety of cancers, including acute myeloid leukemia (AML), colorectal cancer, hepatocellular carcinoma, epithelial ovarian cancer, clear cell renal cell carcinoma, and other solid cancers.[43]

02 Areas of active therapeutic development

Example technologies in development that utilize synthetic biology for cellular decision-making include allogeneic, logic-gated CAR-NK cells. These cells are designed with activating CARs targeting specific antigens on multiple target cell types, as well as inhibitory CARs that prevent the clearance of healthy cells. Additional features, such as calibrated release cytokines, can also be programmed into these cells, enabling autocrine and paracrine stimulation and expansion of the CAR-NK cells *in vivo*.[44]

Similar to logic-gated NK cells, integrated circuit T (ICT) cell products are in development to treat a variety of cancers. These cells express two CARs recognizing two distinct target antigens, both of which must be present to initiate killing, thereby limiting off-tumor toxicity. Further engineering can enhance the *in vivo* expansion of ICTs in the body by encoding silencing RNAs to reduce expression of ligands recognized by host apoptotic pathways and/or by host immunosuppressive pathways.[45]

03 Looking ahead

The engineering of additional inhibitory receptors into CAR-NK cells may greatly enhance their specificity, providing "safeguards" that may make allogeneic therapies a reality. The development of allogeneic therapies greatly reduces timelines to providing patients with effective therapies. Beyond providing an avenue for more effective allogeneic cell therapies, these multi-armed immune cells may also limit tumor escape as multiple antigens will be required to mutate to achieve escape, providing a lower chance for relapse than offered by traditional CAR-T cell therapies.

However, the more complex engineering steps required to generate multi-receptor cell therapies may limit the starting population of cells, a factor that has historically limited the efficacy of engineered cell therapy products. More generally, logic-gated cells will need to be evaluated in additional proof-of-principle studies for their ability to overcome challenges traditionally associated with CAR-T cell therapies, such as their ability to infiltrate the immunosuppressive microenvironment of solid tumors and to persist in the body long-term.[46]

Induced pluripotent stem cell-derived cell therapies

01 Background

Pluripotent stem cells, which exist in nature only as embryonic stem cells (ESCs), can differentiate into every cell type in the body.[47] In 2007, scientists discovered how to reprogram any adult cell type into an induced pluripotent stem cell (iPSC) by the ectopic co-expression of four transcription factors, called Yamanaka factors.[48,49] These immune-naïve cells can propagate in culture indefinitely and can be differentiated into any of the three germ-layer cell lineages when exposed to certain small molecules under certain culture conditions.[50] Therefore, iPSCs provide a wealth of cells for many downstream therapeutic applications.

02 Areas of active therapeutic development

Primary areas for ongoing iPSC-based therapeutic development include (1) autologous cell-based therapies for regenerative applications or (2) "off-the-shelf" allogeneic cell banks.[51]

Stem cell therapies seek to replace, repair, or rejuvenate damaged cells, tissues, or organs in the body. Once isolated, stem cells are typically injected into the damaged patient site, where they differentiate into the surrounding cell types to replace functional activity that has been lost due to damage or degeneration.[52] Many cell therapy developers are focusing on regenerating damaged cells using iPSC technology, since these cells boast the pluripotency of ESCs without the associated ethical obstacles. For example, iPSCs are currently being leveraged as starting cells for differentiation into adult cells that can replace (1) pancreatic beta cells to restore insulin secretion for use in the treatment of Type I diabetes[53], (2) native or gene-corrected neurons to restore dopaminergic signaling in sporadic and genetic Parkinson's disease patients[54,53,] (3) cardiomyocyte grafts to promote immunomodulatory repair and stimulate contractions in heart failure patients55, and (4) photoreceptors to restore light-sensing in Inherited Retinal Disease patients.[54]

Since immune rejection and GvHD are major complications limiting "off-the-shelf" allogeneic cell therapies, several developers have turned to hypoimmunogenic iPSC-derived cell lines as starting material.

Some of these cell lines use gene editing to knock out certain genes, such as HLA-II and HLA-A, to generate "cloaked" cells that evade detection by the adaptive immune system. In the absence of these receptors, patient-matched HLA-B and HLA-C classes can then be expressed to prevent recognition by NK cells.[56] Expression of CD47 receptors can also prevent recognition by NK cells.[57]

Natively hypoimmunogenic or genetically engineered hypoimmunogenic iPSC cell banks can then be used to generate multiple cell types for cell replacement therapies or to generate adoptive cell therapies.

The use of iPSCs can address both major obstacles limiting the development of engineered immune cells: low abundant starting populations and genetic manipulation-resistant cell types. Since iPSCs can theoretically replicate indefinitely in culture and can be readily manipulated, many cell therapy developers are using iPSCs as a starting material for immune cell engineering.[47,58] Since iPSCs grow more robustly in culture than do many immune cell types, they are amenable to additional engineering steps beyond the addition of cell-targeting CARs and TCRs.[35,59–61] For example, some iPSC-induced CAR-NK (iCAR-NK) cells in development are enhanced by the deletion of negative regulator genes, thus enhancing iNK cell persistence in the body.[59] Other iCAR-NK cells in development harbor three additional functional modifications in addition to the CAR that function to limit off-target cytotoxicity, to enhance iNK cell activity and proliferation, and to prevent self-cytotoxicity.[62] Additionally, iPSC-derived CAR-T cell therapies are being developed with similar added benefits, some with up to seven modifications to promote trafficking to solid tumors and prevent clearance by the immune system.[60]

03 Looking ahead

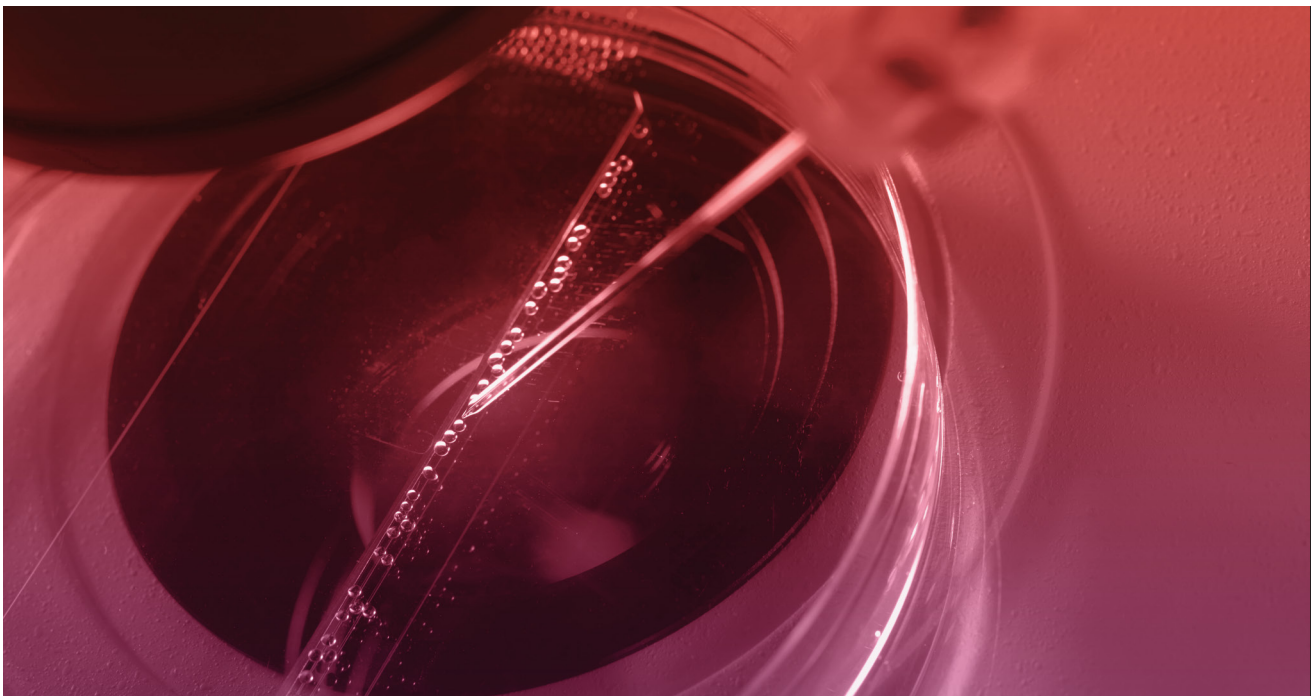
The future of iPSC-derived cells as an enabling technology for a variety of therapeutic products will require many new manufacturing innovations, safety standards, and regulatory guidance to ensure their timely commercialization.

The manufacturing for iPSC-derived therapies adds the additional step of cell reprogramming to the already long and complicated process of generating autologous cell therapies. The sourced patient cells must be sufficiently expanded prior to reprogramming, which can take anywhere from 7-28 days. Moreover, the process of reprogramming the cells by genetically introducing Yamanaka factors can take up to 4 months. After reprogramming, the cells must be assessed for loss of reprogramming factors (plasmids used to express Yamanaka factors) and the population must be cleared of cells that may have spontaneously differentiated during expansion. After reprogramming, the cells must be differentiated into the therapeutic cell type, which requires additional identity testing and characterization.

Currently, these steps lack clear regulatory guidance for quality control testing, which will likely be addressed in the future. Moreover, advances in the manufacturing of iPSCs, including using scalable technology, automation, and closed systems are needed to ensure reproducible and scalable manufacturing.[61]

Moreover, clarification from regulators will be needed regarding donor cell testing requirements and cell harvest requirements, including the qualifications necessary to be considered high-grade starting material for the derivation of an initial iPSC cell bank. Finally, regulatory clarification on required potency assays for determining the differentiation capacity of iPSCs will be important.[63,47]

Ensuring the safety of iPSCs will also be extremely important as these therapies enter the clinic. Certain safety risks relevant to iPSCs, such as genomic instability and teratoma formation, will need to be assessed by standardized QC assays clearly described in regulatory guidelines.



Bioengineered and Xenotransplanted Organs

01 Background

Organ transplantation can save lives and dramatically improve the quality of life for recipients, effectively reversing end-stage organ failure. A failing organ can be the result of multiple genetic and environmental factors, and organ transplantation is often the only available way to rescue function.[64] However, in the United States, over 100,000 people are on the waitlist for an organ transplant.[65] Several factors contribute to organ transplant shortages, including a limited number of donors, the short window of organ viability for transplantation, and geographic distribution disparities in organ availability. Moreover, even when organs become available, the recipient of an organ transplantation faces the risk of rejection mediated by the recipient's immune system. The rejection of an organ is primarily caused by cell-mediated or antibody-mediated rejection, in which either T cells or antibodies, respectively, recognize foreign antigens on the surface of the transplanted organ and initiate a widespread immune response. Both types of underlying immune responses lead to widespread inflammation and tissue damage that can ultimately lead to loss of organ function, thus facilitating the need for a new transplantation.[64] To prevent immune rejection, transplant recipients must adhere to long-term immunosuppressant regimens, which are associated with serious complications as a result of both reduced immune cell functioning, such as increased susceptibility to infections and cancer, and unintended drug-related side effects, such as renal failure and cardiovascular disease.[66]

Researchers have been exploring the use of bioengineered organs and tissues as a potential alternative to relying on organs from deceased or living donors for many years, with the shared goal of combatting organ shortages, minimizing organ transplant waiting periods, and reducing disparities in access to care. Two emerging strategies that rely on bioengineering have emerged in the organ transplant space to address both organ shortages and organ rejection: (1) decellularization and recellularization of organ matrices and (2) xenotransplantation.

Decellularization of organs refers to the process in which all cellular components of a donor organ are removed via chemical, physical, or biological (enzymatic) means, while the extracellular matrix (ECM) of the organ is maintained.The ECM maintains the organ's anatomical structure and supports its physiological function.

The resulting decellularized organ can thus serve as a scaffold for the *in vitro* recellularization of the organ with the recipient's cells or cells from a different donor.[67] While there has been some success in the recellularization of simple biological structures, such as the trachea, this process has not seen success for more complex organs, like kidneys, which are the most frequently needed organ for transplant.[68]

The second technology emerging in this space is the complete xenotransplantation of organs from other species into humans. Pigs are the species of choice for organ harvest since they can be easily genetically engineered and have similar anatomical structures to human organs. Moreover, pigs are highly bred and a common food source, reducing ethical concerns about their use in xenotransplantation. While pigs have high homology to humans, xenotransplantation of organs has been limited by immune-mediated incompatibilities between species that result in organ rejection. To address this, researchers have worked to identify porcine genes that encode proteins associated with immune rejection by the human immune system. Results of these studies found that three porcine carbohydrate molecules induce 95% of human antibodies formed against porcine organs.[69] Moreover, increased antibody-mediated complement system activation results in coagulation dysfunction, further mediating immune rejection. To address this, researchers have overexpressed human coagulation regulatory proteins in animal donors.[69,67]

By generating transgenic pigs with these genetic modifications, xenotransplantation has had some success in human recipients. In 2021, a kidney isolated from a transgenic donor pig lacking immune-stimulating carbohydrates was transplanted into two brain-dead human recipients in a ground-breaking proof-of-concept study. Importantly, the pig's thymus gland, which is responsible for removing self-reactive lymphocytes from the immune system, was also transplanted along with the kidney. The recipients were monitored for 54 hours on a ventilator while the transplanted kidney was analyzed for biological function and immune rejection. Analysis at the 54-hour endpoint revealed active kidney function with no signs of hyperacute or antibody-mediated rejection in either of the two recipients.[70] Additionally, FDA approved a pig-to-human heart transfer under a compassionate use exemption in 2022, resulting in short-term success within a living donor, who ultimately died two months after the transplant. The donor pig harbored 10 genetic modifications – four knocked-out porcine genes and six overexpressed human genes. The cause of death was potentially attributed to a pig virus detected in the transplanted heart.[71]

02 Areas of active therapeutic development

Preclinical research on organ transplantation has generally been slow due to the lack of conserved immune responses governing organ rejection between humans and existing animal models. Moreover, the use of animal models with high similarity to humans, such as non-human primates, is associated with increased regulatory requirements, specialized personnel/facilities, and ethical concerns.

Despite this, several developers are working towards the decellularization and recellularization of complex organs.For example, advanced bioengineering strategies are currently being applied to the decellularization of whole human hearts from donors and the repopulation of the heart with endothelial and parenchymal cells derived from human iPSCs isolated from the recipient (**Figure 4A**).[72]

Other developing decellularization and recellularization strategies don't rely on a human donor at all, but rather use organs from pigs as the starting material for the generation of a decellularized scaffold. After cells are removed using perfusion decellularization, in which the pig organ is soaked in a strong detergent to leave behind a non-cellular matrix, human patient-derived stem cells are introduced into the matrix (within a bioreactor) to bioengineer new organs, including kidneys and livers (**Figure 4A**).[73]

In the field of xenotransplantation, research into porcine genes responsible for immune rejection and human proteins that may mitigate this reaction is ongoing. These developing strategies also seek to reduce the potential risk of the transmission of viruses between species, which may have resulted in the lack of long-term success in the first pig heart-human transplantation.[71] Even in the absence of active viral infections, the genomes of all species harbor endogenous retroviruses, which are remnants of once infectious retroviruses that became stably integrated within the genome over evolutionary history. The potential for reactivation of porcine endogenous retroviruses is thus an additional concern for xenotransplantation.

The most advanced technological developments in the xenotransplantation space rely on the genetic inactivation of all endogenous retrovirus sequences within the transgenic pig donor's genome using CRISPR/Cas-9 technology. The genomes of these same animals are also engineered to knock out multiple porcine genes and knock in multiple human genes. The DNA-containing nuclei of genetically edited *ex vivo* pig fibroblast cells, which ultimately contain nearly 70 genetic edits, are then injected into pig egg cells that are implanted into the uterus of an adult pig. These donor pigs are then raised in a pathogen-free environment to reduce the risk of viral transmission to humans (**Figure 4B**).[74]

Figure 4: Ongoing advances in bioengineered and xenotransplanted organs

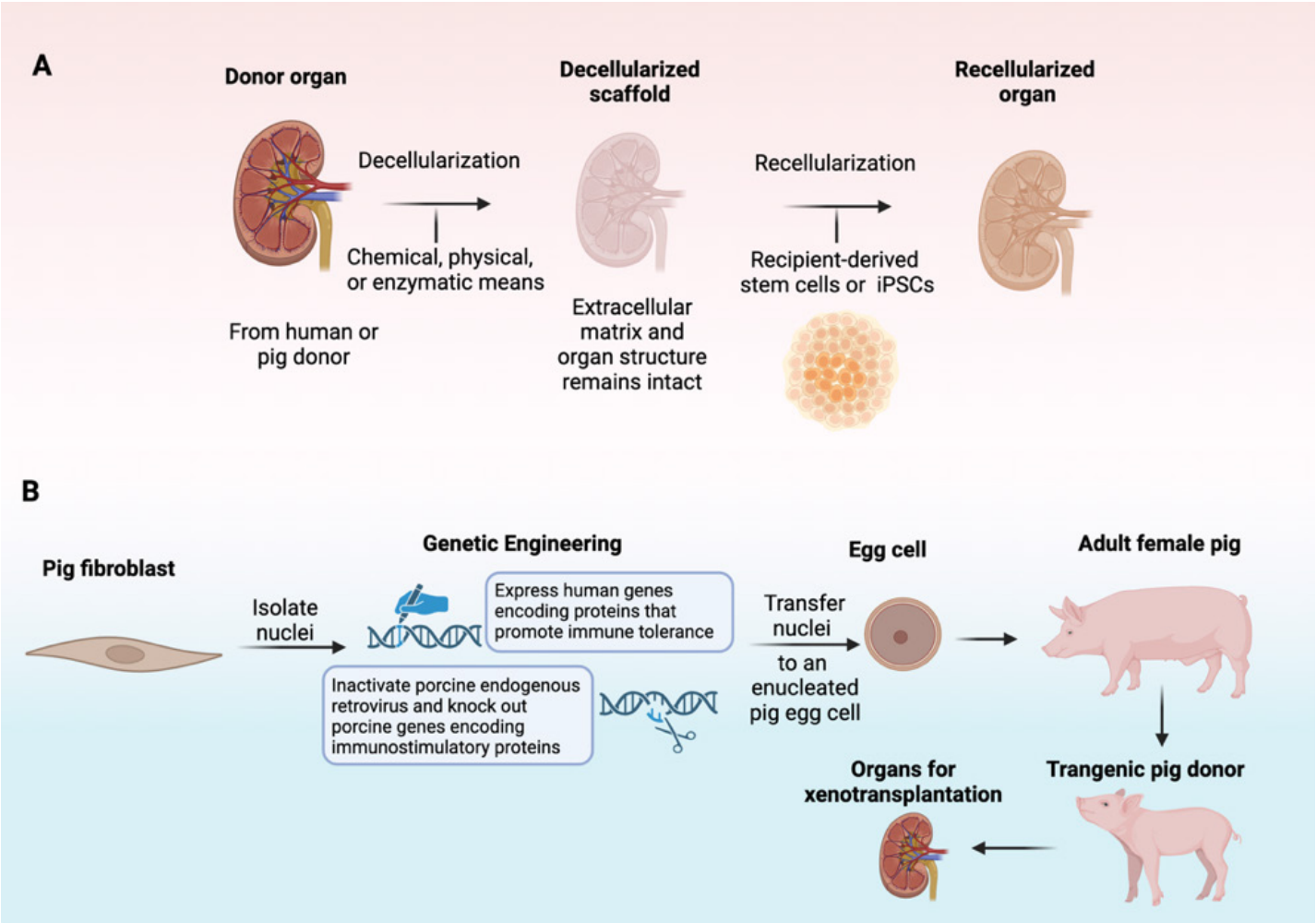


Figure created by Catalyst Healthcare Consulting with biorender.com

03 Looking ahead

The revolutionary field of bioengineered and xenotransplanted organs will greatly impact the lives of patients if successful. However, the process for developing these technologies still requires many standardized protocols. For example, the biomarkers that identify and predict the risk of immune rejection should be clearly established and standardized prior to the transition of bioengineered or xenotransplanted organs into clinical studies. These biomarkers will likely include T-cell markers, antibody markers, and markers of cellular injury.[75] Moreover, protocols for safety (including ensuring microbial sterility of organs), transplantation techniques, and post-transplantation monitoring must be standardized.

Secretome and Exosome-based Therapeutics

01 Background

A secretome is the collective term used to describe all paracrine soluble factors produced by a certain cell type. These soluble factors include peptides, membrane particles, small proteins, and extracellular vesicles called exosomes. Stem cell secretomes are of particular interest as research has shown that some stem cell-based therapeutics mediate their regenerative and immunomodulatory effects through their secretomes, which are studied in the form of stem cell-conditioned media.[76] Therefore, the secretome itself may have therapeutic applications in the regenerative medicine space. Additionally, the use of secretomes has both biological and manufacturing advantages over using stem cells themselves. Biologically, secretomes aren't associated with the stem cell engraftment–associated risks of teratoma formation or immune rejection.[77] From a manufacturing standpoint, secretomes can be readily scaled, stored, and used as off-the-shelf, ready-to-go products while maintaining the therapeutic benefits of stem cells.[78]

Exosomes are a major component of secretomes and may be responsible for a large portion of their underlying therapeutic advantages. Exosomes are single-membrane organelles that can be secreted by almost any cell type, including cancer cells and immune cells.[79] These cell-derived nanovesicles are often bestowed with native characteristics of the cell of origin and can carry a wide range of cargo including proteins, lipids, nucleic acids, and signaling molecules.[80] Researchers seek to harness the small size, high stability, and intercellular shuttling of exosomes for a variety of therapeutic applications, including the management of neurodegenerative disorders, cancer, musculoskeletal disorders, and cardiovascular disorders.[81,79]

02 Areas of active therapeutic development

Both whole secretomes and isolated exosomes are currently being investigated as novel therapeutic agents. Categories of developing secretome or exosome-based therapies include: regenerative secretomes or exosomes, immunomodulatory secretomes or exosomes (which include immuno-oncology applications), and the use of exosomes as cargo delivery vehicles.

Secretomes or exosomes isolated from certain cell types are being used to stimulate tissue healing in several regenerative applications. For example, purified exosome products derived from allogeneic platelets are being studied as agents promoting cell growth and formation of new blood vessels in a variety of tissues, with indications for wound healing, acute myocardial infarction, female stress urinary incontinence, and more.[82] In addition to delivering regulatory factors to promote healing, exosomes isolated from healthy stem cells may contain fully functional proteins or mRNAs encoding functional versions of genes that are rendered nonfunctional in some genetic disorders. For example, exosomes isolated from human bone marrow-derived MSCs (BM-MSCs) contain functional COL7A1 mRNA and its corresponding protein COL7, which are responsible for normal collagen production. Use of these exosomes in patients with the severe skin disease dystrophic epidermolysis bullosa (DEB), caused by a mutation in COL7A1, may represent a novel therapeutic avenue.[83] Further, secretomes isolated from allogeneic MSC banks are being investigated for wound healing, anti-inflammatory, and neuroprotective properties that can be applied to indications such as rare ocular surface disease and degenerative retinal disease.[84]

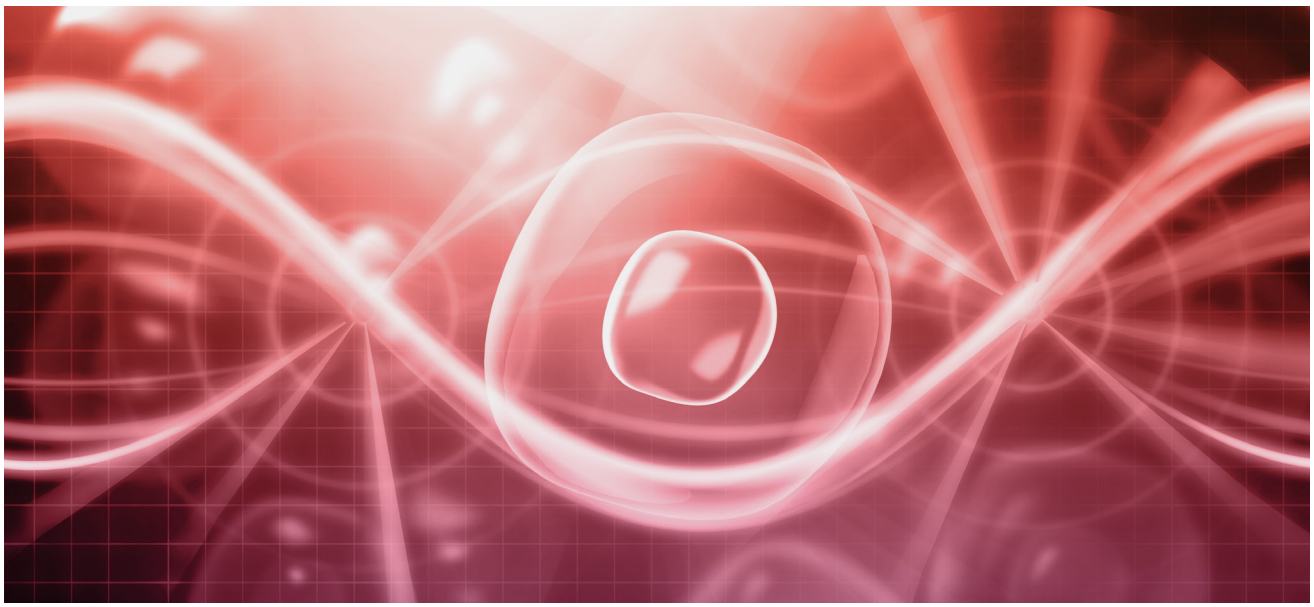
Immunomodulatory secretomes or exosomes in development seek to address several inflammatory conditions. For example, secretomes isolated from partially differentiated human stem cells are being developed into injectable therapeutics to treat a variety of age-related muscle atrophy conditions.[85] Additionally, exosomes isolated from the secretomes of human BM-MSCs are being investigated as therapeutic agents to facilitate paracrine signaling to reduce inflammation associated with COVID-19-induced Acute Respiratory Distress Syndrome (ARDS).[86] BM-MSCs are a highly studied source of exosomes, and exosomes purified from these secretomes are known to carry more than 1,000 regulatory proteins, such as anti-inflammatory cytokines and anti-apoptotic proteins. In addition, BM-MSC-derived exosomes can transport mRNAs and miRNas that can regulate inflammatory signaling pathways from cell-to-cell.[87]

Tumor-derived exosomes are a fascinating area of development based on research showing that exosomes secreted by tumors can carry with them cell-surface neoantigens (presented on MHC molecules) or costimulatory molecules that can activate immune responses against the parental tumor cells.[88] Some therapies in development use modified tumor-derived exosomes depleted of immunosuppressant miRNAs that may subdue cell-mediated anti-tumor immunity to supply tumor neoantigens to immune cells. These developing modified exosome therapies are derived from cancer cell lines and designed for use in combination with standard checkpoint inhibitor immunotherapies.[89]

Exosomes are also being leveraged as delivery vehicles for various cargoes. Therapies in development seek to deliver proteins embedded on the exosomes' surface as precision therapeutics and vaccines. For example, in research settings, lung stem-cell derived exosomes have been engineered to express the receptor-binding domain of SARS-CoV-2 to function as an inhalable COVID-19 vaccine.[90] Other therapies use engineered exosomes to encapsulate various biological molecules, such as mRNAs, proteins, and even AAV gene therapy vectors. Since exosomes are naturally taken up and released by cells, they are associated with lower immunogenicity, more specific cell-targeting, and more efficient delivery of cargo compared to other gene therapy delivery vehicles like viral vectors and synthetic lipid nanoparticles (discussed further in the "Nonviral Delivery of Gene Therapies" section).[91,92]

03 Looking ahead

As secretome and exosome therapies surpass preclinical stages of development, it will be important to assess their mechanisms of action on human cells in the body. Currently, the exact mechanism of action underlying secretome and exosome therapies are not very well-defined. Therefore, a critical aspect of their translation will be to identify the exact biological composition of secretomes and exosomes to determine which components mediate their biological effects. Moreover, secretome and exosome products do not have standardized protocols for large-scale manufacturing, purification, or quality control, which will be important to ensure batch uniformity and uphold safety requirements.[81]



in vivo CAR-T cell engineering

01 Background

Both approved autologous and developing allogeneic cell therapies require isolation of cells from a donor, followed by *ex vivo* culture, expansion and engineering, and subsequent infusion into patients. This process requires a complex and highly coordinated manufacturing process that may hinder the broader applicability of these therapies. Moreover, in the case of allogeneic cell therapies, recipients are at risk of immune rejection and GvHD in response to the introduction of donor cells. Further, introduced cells do not always persist in the body or impart sufficient efficacy. To bypass these manufacturing and persistence constraints, many developers are optimizing the *in vivo* engineering of CAR-T cells, whereby the CAR transgene is delivered directly to patient T cells *in situ.* This impactful approach aims to provide the durability benefits of autologous CAR-T cell therapies without the associated manufacturing complexity of *ex vivo* cell therapies.[93,94]

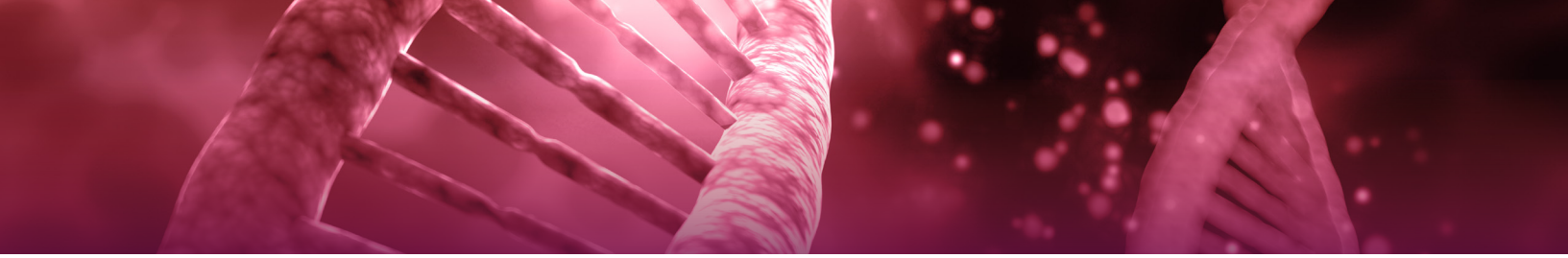
02 Areas of active therapeutic development

Therapies in development aim to deliver the CAR transgene to target T cells *in vivo* via a variety of vectors, including lipid nanoparticles[95], liposomes[57], and lentiviral vectors.[96,97,98] To ensure these vectors reach target T cells, developers have engineered T cell-targeting recombinant protein binders (such as monoclonal antibodies) embedded within lipid nanoparticles[95], T cell-targeting fusogens embedded in the membranes of liposomes ("fusosomes")[57], and T cell-specific targeting molecules on the surface of lentiviral vectors,[97] among other strategies.

For traditional *ex vivo* CAR-T cell therapy, patients must undergo lymphodepletion to remove lymphocytes that may compete with the expansion and proliferation of introduced CAR-T cells. However, for *in vivo* CAR-T cell engineering, lymphodepletion cannot be used without also removing the target T cells themselves. Therefore, alternative strategies for the induction and expansion of *in vivo* CAR-T cells without lymphodepletion include using T cell-targeting lentiviruses that encode for additional signaling proteins[97] or drug-inducible cytokine receptors[98] to provide proliferation signals and costimulatory molecules, respectively, to *in vivo* CAR-T cells.

03 Looking ahead

Several obstacles must be overcome for *in vivo* CAR-T cell therapies to enter the clinic. The first is the lack of a lymphodepletion step prior to *in vivo* CAR-T cell treatment, which is necessary for sufficient proliferation of *ex vivo* CAR-T cells after infusion. While this feature is in some ways a benefit, as it bypasses secondary health complications associated with lymphodepletion, it remains to be determined whether *in vivo* CAR-T cell therapies– even those engineered with additional stimulatory measures– will be able to persist without lymphodepletion. Moreover, it will be important to ensure that the viral vector or alternative transgene delivery vehicle is able to target the right cells with high specificity–to prevent any unintended gene modification– and with high efficiency–to ensure sufficient transduction. This can be achieved by experimentally determining the degree of T cell selectivity achieved by each candidate vector. Likewise, in the absence of *ex vivo* process monitoring and quality control to validate the specificity of *in vivo* T cell gene editing, it is imperative to ensure CAR gene insertion in the correct target location to avoid genotoxicity. Finally, a robust safety profile must be established through rigorous monitoring of antibody-mediated immune responses against vector particles, particularly those derived from viruses, as well as immune reactions commonly experienced using *ex vivo* CAR-T therapies, like cytokine release syndrome and neurotoxicity.[94]



Non-double-stranded break-inducing Gene Therapies

01 Background

A variety of gene therapies have been approved in the United States to address several heritable genetic conditions. Currently, all approved gene therapies rely on the *in vivo* or *ex vivo* delivery of a healthy version of a gene to cells in patients lacking a functional copy (Figure 5A). However, gene therapies applying CRISPR-Cas9 technology are in advanced clinical stages and could gain FDA approval by the end of this year.

During CRISPR-Cas9-mediated gene editing, a sequence-specific guide RNA (gRNA) directs a nuclease (Cas9) to a target site in host DNA where it induces a double-stranded break (DSB). After a DSB, natural DNA repair processes, such as non-homologous end-joining (NHEJ) or homology-directed repair (HDJ), are activated. NHEJ results in the addition or deletion of a few base pairs, which disrupts the original gene sequence and can cause inactivation. Further, if two gRNAs are used to induce DSBs at either end of a target sequence, the entire gene can be deleted. When a corrected DNA template designed with cut sit homology is provided, homology-directed repair (HDR) can insert the corrected (or additional) gene in the deleted gene's place. Many companies are developing gene therapies that employ DSBs to edit regions of the genome, whether by gene inactivation (disruption), gene deletion, or gene insertion (Figure 5B). Beyond the use of Cas9 as the nuclease, other nucleases (or nuclease combinations) are under investigation to enhance the efficacy of gene editing.[99,100,101] For example, the Cas-CLOVER platform uses two deactivated Cas9 proteins and two flanking gRNAs to direct the nuclease domain of the Clo051 enzyme (CLOVER) to the target gene. CLOVER can cut DNA targets only as a homodimer and therefore requires both pairs of Cas9:gRNA complexes to cut DNA, thus enhancing its cutting fidelity.[102]

While revolutionary, DSB-inducing gene editing comes with several obstacles, including concerns about potential off-target effects, chromosomal aberrations associated with homologous recombination after nuclease cleavage[103], the imprecision of NHEJ and the low frequency of native HDR, and DSB byproducts such as indels, translocations, and rearrangements.[104,105] Therefore, gene editing that does not rely on DSB breaks is an area of active therapeutic development.

Several technologies are being developed to manipulate gene expression without inducing DSBs in patient DNA. Strategies for gene editing that do not rely on cutting the genome include epigenetic editing, transposon-mediated editing, prime editing, and base editing.

Epigenetic editing refers to the pioneering technology that manipulates transcriptional levels of target genes through the addition or removal of epigenetic markers on the backbone of DNA or on histones that comprise chromatin (Figure 5C). The epigenome refers to the culmination of these reversible small molecule marks that modulate the accessibility of transcription factors to target genes by blocking recognition sites or influencing chromatin packaging.[106]

A separate mechanism, transposon-mediated editing, leverages naturally occurring DNA sequences called transposons, which encode for a transposase enzyme that "cuts and pastes" the transposon sequence (the target gene) from one location in the genome to another, enabling the insertion of transgenes into genomic DNA (Figure 5D).[107]

Prime editing is a unique editing system that relies on two components: (1) an engineered prime editor (PE) protein comprised of a mutated Cas9 enzyme domain ("nickase") fused to a reverse transcriptase (RT) domain and (2) a prime editing guide RNA (pegRNA) that contains an RNA "find" template complementary to the target gene next to an edited RNA "replace" template. When delivered into cells via lipid nanoparticles, the pegRNA directs the PE protein to the target gene, where the nickase domain induces a single-stranded cut of the non-complementary DNA strand. This cut exposes a DNA flap with a 3'OH group that binds to the primer binding site (PBS) of the RNA "replace" template, serving as a primer for the RT domain of the PE protein. The RT domain then copies the "replace" sequence to edit the DNA sequence. The edited strand then replaces the original mutated strand by strand invasion. DNA repair enzymes remove the original mutated strand and correct the mutation on the unedited strand (Figure 5E).[108]

Base editing is the most clinically advanced non-DSB-inducing technology. This technology relies on a catalytically inactive Cas9 variant (dead Cas9, dCas9) or other nuclease that is fused to an adenosine or cytidine deaminase, which converts their respective nucleotides into other DNA bases. These adenosine base editors (ABEs) and cytidine base editors (CBEs) are guided to the target gene by a gRNA where either the adenine or cytosine are deaminated to either inosine or uracil, respectively. Since inosines are read by DNA polymerase as guanines and uracils are read by DNA polymerase as thymines, an overall A $\rightarrow$ G or C $\rightarrow$ T change is achieved, respectively. Base editors can thus be used to (1) repair point mutations underlying genetic diseases (Figure 5F)[109] or (2) insert a premature stop codon into the target gene, preventing the translation of a protein associated with disease.[110]

CBEs also contain inhibitors of base excision repair enzymes to ensure the base edit is stable, while ABEs do not require this additional domain since adenine deaminases and their cognate repair enzymes do not exist in nature.[106] Importantly, base editing does not rely on DSBs, thereby limiting the generation of potential indels at target and off-target sites.

Figure 5: Current and developing gene therapy mechanisms

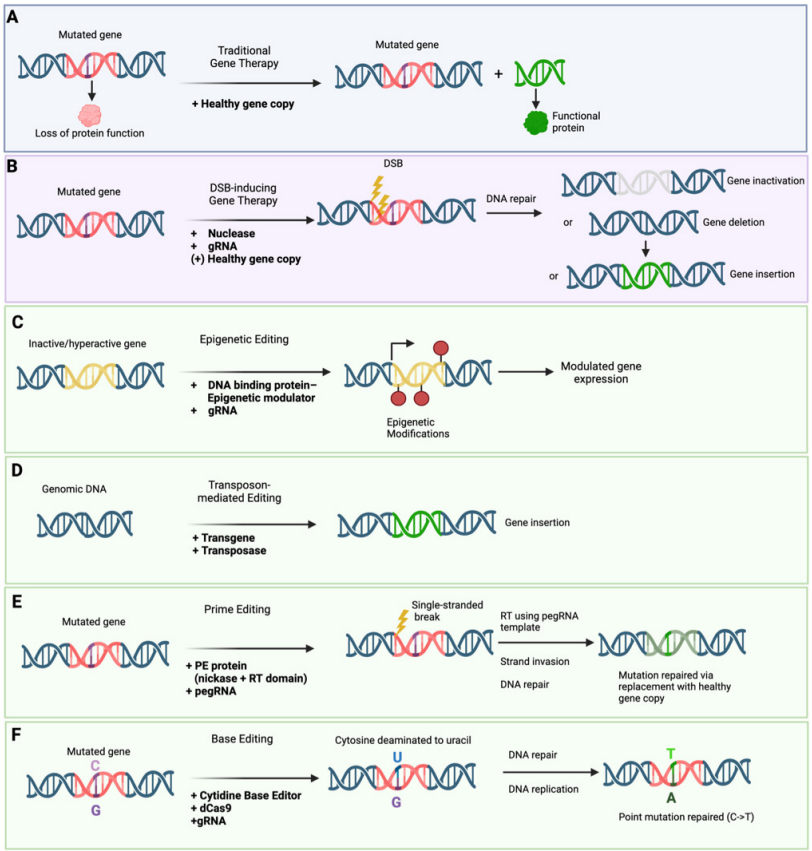


Figure created by Catalyst Healthcare Consulting with biorender.com

02 Areas of active therapeutic development

Several epigenetic editing technologies are in development for gene therapy applications. Each of these technologies delivers one or more gRNAs and one or more epigenetic modulator proteins fused to a DNA-binding protein, which can be either a zinc finger protein (ZFP), a modified Cas protein, or transcription activator-like effector nucleases (TALENs), to target cells. The gRNA directs the DNA-binding protein to the target gene, where its binding enables the activity of the epigenetic modulator (**Figure 5C**). Currently, a toolkit of histone modifiers (which add or remove methyl or acetyl groups on histones), DNA (de)methylators, and chromatin remodelers are being developed to achieve desired epigenetic editing.[111–113] Benefits of epigenetic editing include the ability to target several genes simultaneously through epigenetic modification of complex signaling cascades. This is in direct contrast to most DSB-inducing gene therapies that function to insert or remove a single gene underlying a disease. Therefore, the disease indications for epigenetic editing are broader compared to available DSB-inducing strategies. Moreover, epigenetic editing can fine-tune the levels of expression of the target gene, rather than acting as a simple on/off switch like other gene therapy strategies.[113]

Transposon-mediated gene therapy applications in development, which leverage natural transposon-transposase systems, function by delivering two vectors: one encoding for the transposase and the other encoding for the therapeutic transgene (flanked by transposon-derived sequences that are recognized by the transposase) (**Figure 5D**). These vectors are typically delivered into cells via electroporation. Currently, the most promising transposons in development for gene therapy are derived from the Sleeping Beauty (SB) transposon and the piggyBac (pB) transposon.[107] Some gene therapy developers have designed "next-generation" versions of these natural transposons that stably integrate large target genes into the genome.[102] Importantly, these transposon systems don't require viral vectors for delivery and are therefore associated with less complicated and expensive manufacturing regimens.

Several gene therapies are in development that use prime editing, which has benefits over traditional CRISPR-Cas9 editing because it only uses a single pegRNA construct as opposed to both an sgRNA and a DNA donor template. Less RNA means more cargo can be packaged into space-constrained gene therapy delivery vectors. This is particularly beneficial for large genes that are too big to be packaged into traditional gene therapy vectors, like the dystrophin gene that is mutated in Duchenne Muscular Dystrophy (DMD), an indication for which prime editing solutions are currently being developed.[114] Moreover, unlike traditional DSB inducing gene editing approaches, which most commonly induce gene deletions, prime editing can be used to correct all types of point mutations, insertions, and deletions, significantly expanding the scope of gene editing to include ~89% of known genetic variants associated with human disease.[115]

Therapies that use base-editing to correct genetic mutations underlying diseases, such as sickle cell disease and beta thalassemia, are likely to gain approval earlier than the 3-10 year time frame of the horizon scan. However, an approval of this editing technology will likely open the floodgates for other base-editing gene therapies to enter clinical stages within this timeframe.[116]

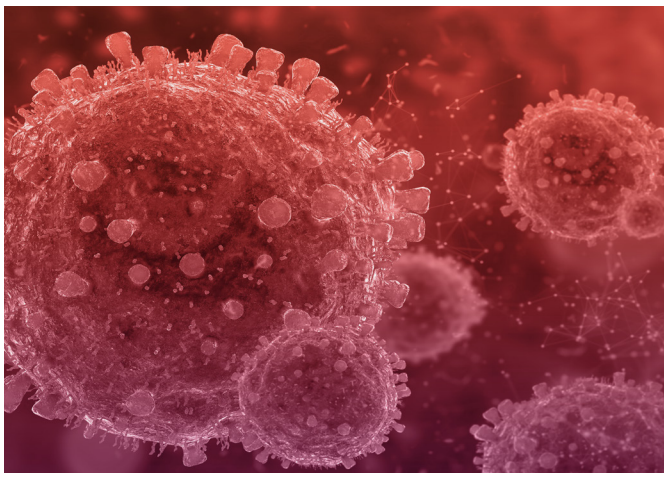
Gene coding, an investigational gene editing mechanism, relies on the lipid nanoparticle-mediated cell delivery of mRNA coding for a mammal-derived enzyme called Saliogase, which can integrate DNA sequences into defined regions of the genome without inducing DSBs.[117]

03 Looking ahead

The path ahead for novel gene editing strategies will need to consider the risk of off-target effects with the benefit of the therapy, the degree of gene-level changes, and the specificity of cell delivery mechanisms.

Capsid Engineering

01 Background



Viral vectors for gene therapy, such as adeno-associated viruses (AAVs) and lentiviruses, leverage their parent virus's function of host cell receptor binding and entry via endocytosis or fusion, respectively, to deliver gene editing machinery into target cells. A major area of development for optimizing these viral vectors is refining their tissue tropism, which is determined by which cellular receptors are bound by surface antigens on the viral vector capsid. Only target cells with receptors that recognize the vector capsids can be penetrated and therefore receive the cargo. Tropism is highly important for ensuring that gene therapy-mediated modifications occur only in desired cell types and tissues. Optimized tropism may also reduce the need for high gene therapy doses, reducing the risks of toxicity.[118]

02 Areas of active therapeutic development

Many gene therapy developers are interested in optimizing and refining the tropism of their viral vectors. These optimized capsids may allow for ideal tropism due to enhanced cell-specificity, tissue penetration, immune evasion, intracellular trafficking, or other functional avenues.[118] Developing technologies in this space rely on *in vivo* screening of capsids using directed evolution, *in silico* screening of capsids using platforms such as artificial intelligence (AI) and machine learning (ML), or modification of existing capsids with cell-targeting ligands.[119,120]

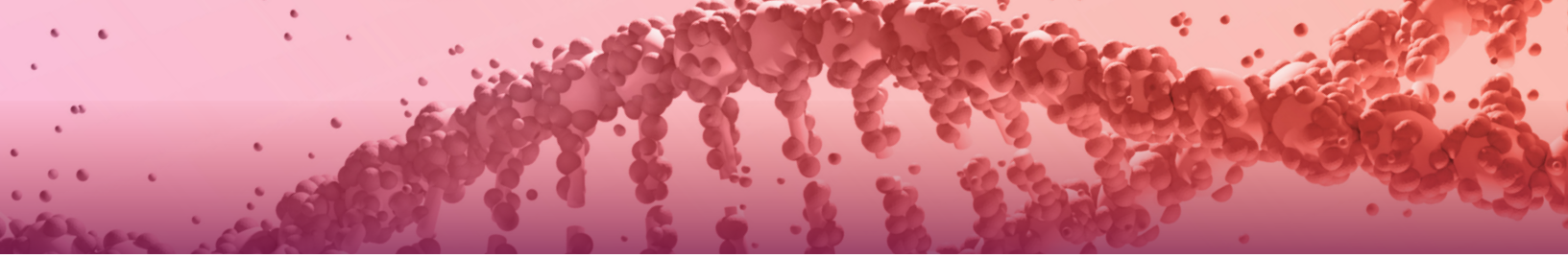
Directed evolution refers to the screening of capsid libraries under selective pressure *in vivo* to identify variants that most effectively target tissues of interest.[121,122] For example, in order to achieve penetration of the restrictive blood-brain barrier (BBB), AAV variants were generated at the DNA level by inserting degenerate nucleotides, which allow a mixture of nucleotides to be synthesized at the specific degenerate position, into the coding sequences of certain viral capsid proteins, generating over 30,000 capsid variants. This capsid library was then passaged in marmosets and viral DNA was isolated from multiple tissues, with the capsids best at targeting the brain being selected as neurotropic candidates.

The top candidates were then cloned and assessed for neurotropic targeting in rhesus macaques, resulting in the identification of an engineered AAV9 that was highly targeted to the CNS.[121] Other *in vivo* screening approaches rely on identifying fully functional capsids using cell type-specific fluorescent reporters as a readout, simplifying the *in vivo* screening process.[123] Other developers are bypassing *in vivo* screening by harnessing the developing power of AI and machine learning for capsid design. To train the machine, millions of capsids are designed and experimentally assessed in a variety of cell types *in vitro*. The algorithm then learns which sequences predict fitness in specific cell types for the ultimate goal of *in vivo* testing and capsid commercialization for gene therapy applications.[119,122,120]

An additional mechanism for enhancing tissue targeting and payload delivery is the covalent conjugation of rationally designed ligands, such as small molecules or peptides, onto the vector surface. These ligands provide cell-specific moieties for enhancing the specificity of vector targeting.[124]

03 Looking ahead

The use of modern tools, such as AI and ML, are advancing the capabilities of rational design for gene therapy capsids. However, it remains to be seen whether these *in silico* applications can produce a viable viral vector for enhanced targeting *in vivo*. However, *in vivo*-based directed evolution, *in silico* approaches, and cell-specific ligand conjugation are avenues for enhancing the efficacy and reducing the risks of viral vector-mediated gene therapy. Strategies that reduce the immunogenicity of capsids may ultimately enable the possibility for redosing of gene therapies.



Nonviral Gene Therapy Delivery

01 Background

While all currently approved gene therapy products in the United States use viral vectors for delivery of cargo, there are several risks associated with these vectors. These include a risk of liver toxicity, immunogenicity to viral antigens, and potential lentiviral-associated oncogenicity. Due to associated immunogenicity, AAV vectors can typically only be dosed once, resulting in a narrow window of error for perfecting the dosing strategy to ensure a therapeutic effect. Moreover, viral vectors can only package a small amount of genetic material, limiting the size of genes that can be delivered to cells. Finally, the scalability of viral vectors is limited as the process requires the expression and assembly of multiple recombinant proteins. These risks and limitations have stimulated study of alternative mechanisms for delivery of gene therapy cargo.[125]

02 Areas of developing technology

Several alternative, nonviral-based gene therapy delivery strategies are currently in development, including lipid-based vectors, synthetic nanoparticle-based vectors, and physical disruption.

Various iterations of lipid-based vectors are currently in development. A major developing class of lipid-based vectors is lipid nanoparticles, which are comprised of a single, spherical phospholipid membrane that engulfs the gene therapy cargo. These lipid nanoparticles are associated with large cargo capacities, transient expression, redosing capabilities, low immunogenicity, and scalable manufacturing potential. Lipid nanoparticles in development are comprised of a combination of synthetically produced lipid types, including (1) ionizable lipids that enable endosome escape once engulfed by the target cell, (2) helper lipids that promote binding to the target cell surface, (3) neutral lipids, like cholesterol, that fill in gaps in the lipid layer, and (4) stealth lipids, like polyethylene glycol (PEG), that prevent detection of the LNP by the host immune system.[126,102,127] Despite their associated benefits, developers have struggled with directing LNPs to specific target cell types.

Other gene therapy developers are therefore incorporating cell-specific engineered molecules, such as fusogens, into the membranes of lipid-based vesicles to better target gene therapy cargo to target cells.[57] Fusogens are natively found on the surface of endogenous membrane vesicles and some membrane-bound viruses.

Rather than synthesizing lipid nanoparticles in the lab, some developers are using lipid vesicles that are naturally derived from cells, such as red blood cell extracellular vesicles (RBCEVs) or exosomes, as primary or secondary cell delivery vehicles, respectively.[92,128] As secondary vehicles, exosomes act to engulf AAV particles carry the gene therapy cargo in order to shield the AAV particle from detection by the host immune system.[92] Other developers are leveraging whole, enucleated cells as lipid-based delivery vehicles.[129]

Another class of nonviral gene therapy delivery vehicles are synthetic nanoparticles, which leverage polymeric nanocarriers consisting of ionizable chemical compounds, called polycations, that interact with nucleic acids to promote condensed self-assembly. The composition of the polymers used to create the nanoparticles can then be adjusted to govern cell-specificity.[130]

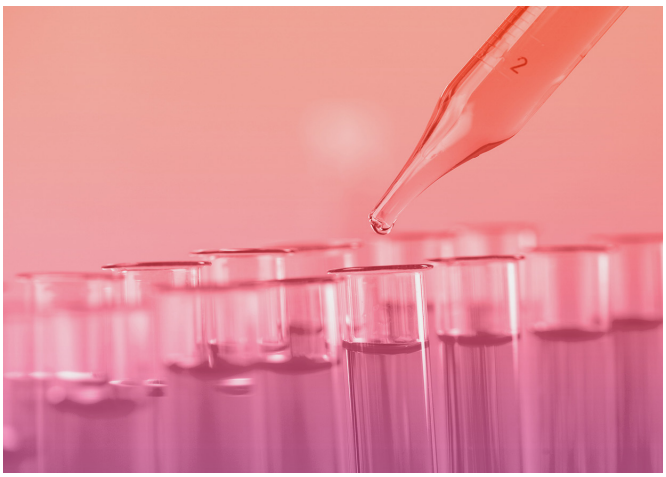
Other nonviral delivery platforms do not rely on a vehicle for cargo delivery, but rather deliver naked transgenes to the target cell cytoplasm by first disrupting the target cell membranes through physical means, including the use of disruptive ultrasound-induced microbubbles ("sonoporation").[131]

03 Looking ahead

As these novel strategies for gene therapy delivery are developed, it will be important to characterize their target cell specificity, gene delivery efficiency, and *in vivo* toxicity to ensure safe and effective translation into the clinic. Moreover, each nonviral delivery method will come with its own set of manufacturing considerations as protocols for their production will need to be scaled to meet demands for dosing clinical patients while maintaining identity, purity, and potency. Despite these challenges, the decreased spatial limitations for packaging cargo and the increased opportunities for broad tissue targeting provided by nonviral delivery vehicles will likely enable progress towards gene therapies for complex, multigenic diseases.

Alternative RNA therapeutics

01 Background



Many available RNA therapeutics, including RNA aptamers, antisense oligonucleotides (ASOS), RNAi (e.g., small interfering RNA (siRNA) and miRNA), and mRNA therapeutics (e.g., mRNA vaccines) rely on the intracellular delivery of RNA species that alter or influence gene expression. Briefly, RNA aptamers function to bind and inhibit a variety of target molecules in a sequence-independent manner via their tertiary structure; both ASOS and RNAi function to target complementary regions of mRNA to modulate expression; and mRNA therapeutics function to provide instructions for the expression of the proteins they encode.[132]

Many available RNA therapeutics, including RNA aptamers, antisense oligonucleotides (ASOS), RNAi (e.g., small interfering RNA (siRNA) and microRNA (miRNA)), and mRNa therapeutics (e.g., mRNA vaccines) rely on the intracellular delivery of RNA species that alter or influence gene expression. Briefly, RNA aptamers function to bind and inhibit a variety of target molecules in a sequence-independent manner via their tertiary structure; both ASOS and RNAi function to target complementary regions of mRNA to modulate expression; and mRNA therapeutics function to provide instructions for the expression of the proteins they encode.[132]

A unique class of RNA therapeutics is emerging, which for the purpose of this paper, are called "alternative" RNA therapeutics. These therapies influence protein expression at the transcriptional or translational level in manners distinct from the existing RNA therapeutics described above. Mechanisms of developing alternative RNA therapeutics include exon editing via trans-splicing, tRNA therapeutics, and RNA editing.

Cellular RNA is encoded by the transcription of genes in DNA. During splicing of pre-mRNA, the spliceosome removes non-coding introns and combines exons from pre-mRNA species to form mature mRNAs for protein translation.[133] The mature mRNA species are then translated into proteins by ribosomes and a cellular pool of tRNAs. tRNAs are molecules that recognize mRNA codons via their complementary anticodon and transfer the corresponding amino acid to the growing polypeptide chain during translation.[134] Alternative RNA therapeutics seek to edit the final protein at the steps of pre-mRNA splicing, tRNA coding, and reading of the final mRNA sequence.

02 Areas of developing technology

Restoring protein function at the pre-mRNA splicing step of transcription requires innovative exon editing technology that provides the durability of gene therapy without the associated risks of direct DNA editing or gene replacement. To achieve this, developers are using synthetic and computational biology to design exon editing RNAs, comprised of wildtype RNA exons attached to a synthetic RNA strand encoding domains that bind to a specific site in the target pre-mRNA and engage with the cellular spliceosome. These editor RNAs are delivered to the cell via viral or nonviral delivery vehicles in the form of DNA constructs. Once in the cell, the DNA is transcribed into the designer exon editor RNAs that bind to target pre-mRNA and interact with the spliceosome to induce trans-splicing, allowing for the replacement of mutated exons with healthy exons in the mature mRNA.

Restoring protein function at the pre-mRNA splicing step of transcription requires innovative exon editing technology that provides the durability of gene therapy without the associated risks of direct DNA editing or gene replacement. To achieve this, developers are using synthetic and computational biology to design exon editing RNAs, comprised of wildtype RNA exons attached to a synthetic RNA strand encoding domains that bind to a specific site in the target pre-mRNA and engage with the cellular spliceosome. These editor RNAs are delivered to the cell via viral or nonviral delivery vehicles in the form of DNA constructs. Once in the cell, the DNA is transcribed into the designer exon editor RNAs that bind to target pre-mRNA and interact with the spliceosome to induce trans-splicing, allowing for the replacement of mutated exons with healthy exons in the mature mRNA.This strategy bypasses the need for delivery of a healthy copy of a DNA-encoded gene, which are often too large for packaging as gene therapies, and instead delivers only the healthy exons required to restore the mutated region. It also ensures the repaired exons are expressed at the same levels as the wildtype gene, as the edited pre-mRNA will still be under endogenous transcriptional controls.[133]

Restoring protein function at the level of translation requires engineering of tRNA therapeutics. Many developers are using engineered tRNA molecules to suppress nonsense mutations, which are single point mutations that result in premature stop codons. Premature stop codons ultimately result in the translation of shorter, often nonfunctional proteins. These nonsense mutations account for 10-15% of all inherited diseases, including DMD, certain forms of cystic fibrosis, and some forms of Dravet syndrome.[135,136,137] Some tRNA therapeutics function to modify tRNAs to be able to recognize nonsense mutations and add the correct amino acid to the chain, thereby rescuing functional protein production.[135,136,138,137] These engineered "suppressor" tRNA molecules can be delivered to cells as therapeutics via viral or lipid nanoparticle vectors.

Importantly, a single suppressor tRNA therapeutic has the potential to treat thousands of diseases that share the same nonsense mutation that results in production of disease-associated proteins.[135]

Changes to the composition and abundance of tRNA pools in the cell can alter the levels of protein expression.[134] Developers are leveraging this knowledge to treat certain diseases that are caused by haploinsufficiency, where the abundance of a functional protein is limited by the presence of only one healthy gene copy. By delivering combinations of endogenous tRNAs to target cells using viral vectors, developers are hoping to increase the amount of healthy protein produced by the single wildtype copy of the gene to restore normal cellular function.[136]

Other tRNA-based platforms in development seek to reduce protein expression, rather than enhance it. Since some diseases are caused by unwanted levels of certain proteins, developers are introducing engineered tRNA molecules that carry modified amino acids that mark the translated protein for destruction by the cell.[138]

To directly edit mRNA sequences that encode for disease-associated proteins, some developers are delivering guide RNAs that can recruit the cell's own enzymes to directly edit target RNA. In particular, these guide molecules recruit adenosine deaminase acting on RNA (ADAR) enzymes to convert adenosines to inosines (which are ultimately read as guanosines) at precise locations in the RNA sequence, resulting in the translation of a functional protein. This system bypasses the need for the delivery of an entire CRISPR system and provides the ability to edit RNA without permanently altering the genome.[139 ,140,141]

03 Looking ahead

Bringing alternative RNA therapeutics to fruition will require innovative manufacturing approaches and careful clinical trial design. Additionally, since RNA molecules are transient, preclinical research should address how long these therapeutics persist in the body in addition to assessing any associated off-target effects. For suppressor tRNA therapeutics specifically, since multiple disease indications can be treated by a single tRNA, regulatory guidance will be needed for determining the requirements for clinical assessment. Moreover, since tRNA has long been untapped as a therapeutic option, research is still ongoing to understand tRNA stability and abundance towards the development of future therapeutics.

CONCLUSIONS

These top emerging technologies in C&GT are powered by ground-breaking scientific innovations that have the immense potential to change the industry and patient lives. However, whether these technologies reach the market within the next 10 years will depend on how the companies developing them navigate the scientific, clinical, regulatory, and financial aspects of commercialization. Moreover, a successful shift from research-scale to industrial-scale manufacturing is imperative for successful production of C&GTs.

Clearly, this finite list does not represent an exhaustive collection of viable, promising emerging technologies. Technologies may win or lose favor based on scientific developments. Readers may argue that other technologies are more deserving, especially if other criteria or methodologies had been used. Our research team recognizes that there are many other technologies in development that could be added to this list in the future and warrant periodic horizon scan activities.

Given the speed at which C&GT advancements are progressing, regulators and stakeholders will need to be ready to work together to develop modernized frameworks to facilitate promising therapies reaching patients expeditiously.

ACKNOWLEDGEMENTS

We would like to thank Dr. Taby Ahsan, Dr. Matt Porteus, Chris Schilling, Dr. Christopher Mason, Dr. Bob Tepper, Dr. David Smith, Dr. Phil Vanek, Dr. Catherine Bollard, Oleg Nodelman, Dr. Matthew Hewitt, Dr. Andy Holt, and Dr. Laura Sepp-Lorenzino for their time and expert input.

REFERENCES

1. FDA User Fee Reauthorization: Ensuring Safe and Effective Drugs and Biologics. FDA.gov. February 3, 2022. Accessed November 1, 2023. https://www.fda.gov/news-events/congressional-testimony/fda-user-fee-reauthorization-ensuring-safe-and-effective-drugs-and-biologics-02032022

2. Establishment of the Office of Therapeutic Products. FDA.gov. Accessed November 1, 2023. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/establishment-office-therapeutic-products

3. Wu G. BioPharmaDive. Cell and gene therapy manufacturing: the next generation of startups. June 20, 2023. Accessed September 12, 2023. https://www.biopharmadive.com/news/cell-gene-therapy-manufacturing-cdmo-biotech-startups/653275/

4. F D A. Establishment of the Office of Therapeutic Products. 2023. Accessed September 12, 2023. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/establishment-office-therapeutic-products

5. Schwaiger C. FDA's OTP Super Office on Track to Fill 500 Positions. BioSpace. August 10, 2023. Accessed September 12, 2023. https://www.biospace.com/article/fda-s-otp-super-office-on-track-to-fill-500-positions/

6. Mulero A. FDA Braces for Looming Boom in Cell and Gene Therapy Submissions. BioSpace. June 12, 2023. Accessed September 12, 2023. https://www.biospace.com/article/fda-braces-for-looming-boom-in-cell-and-gene-therapy-submissions/

7. Buntz B. The top 100 cell and gene therapy companies to watch in 2023. Drug Discovery & Development. March 10, 2023. Accessed September 12, 2023. https://www.drugdiscoverytrends.com/100-top-cell-gene-therapy-companies-watch-2023/

8. Gene, Cell, & RNA Therapy Landscape Report. American Society of Cell and Gene Therapy. Accessed September 11, 2023. https://asgct.org/publications/landscape-report/2023-archive

9. IPO Tracker. Life Sciences Perspectives. Accessed September 12, 2023. https://www.lifesciencesperspectives.com/ipo-tracker/

10. Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. J Clin Invest. 2015;125(6):2228-2233. doi:10.1172/JCI78088

11. Cleveland Clinic. Immunosuppressants. Accessed September 12, 2023. https://my.clevelandclinic.org/health/drugs/10418-immunosuppressants

12. Bluestone JA, McKenzie BS, Beilke J, Ramsdell F. Opportunities for Treg cell therapy for the treatment of human disease. Front Immunol. 2023;14:1166135. doi:10.3389/fimmu.2023.1166135

13. Ye B, Stary CM, Gao Q, et al. Genetically Modified T-Cell-Based Adoptive Immunotherapy in Hematological Malignancies. J Immunol Res. 2017;2017:5210459. doi:10.1155/2017/5210459

14. Mackensen A, Müller F, Mougiakakos D, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nat Med. 2022;28(10):2124-2132. doi:10.1038/s41591-022-02017-5

15. Qin C, Tian D-S, Zhou L-Q, et al. Anti-BCMA CAR T-cell therapy CT103A in relapsed or refractory AQP4-IgG seropositive neuromyelitis optica spectrum disorders: phase 1 trial interim results. Signal Transduct Target Ther. 2023;8(1):5. doi:10.1038/s41392-022-01278-3

16. Grover P, Goel PN, Greene MI. Regulatory T cells: regulation of identity and function. Front Immunol. 2021;12:750542. doi:10.3389/fimmu.2021.750542

17. Mohseni YR, Tung SL, Dudreuilh C, Lechler RI, Fruhwirth GO, Lombardi G. The future of regulatory T cell therapy: promises and challenges of implementing CAR technology. Front Immunol. 2020;11:1608. doi:10.3389/fimmu.2020.01608

18. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. Nat Rev Drug Discov. 2019;18(10):749-769. doi:10.1038/s41573-019-0041-4

19. DSG3-CAART. Accessed September 12, 2023. https://www.cabalettabio.com/pipeline/dsg3-caart

20. PLA2R-CAART. Accessed September 12, 2023. https://www.cabalettabio.com/pipeline/pla2r-caart

21. Ellebrecht CT, Bhoj VG, Nace A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science. 2016;353(6295):179-184. doi:10.1126/science.aaf6756

22. GNTI-122. Accessed September 11, 2023. https://www.gentibio.com/pipeline-programs/new-onset-t1d/

23. Opportunity in Autoimmune Disease. https://abatatx.com/our-programs/.

24. Pipeline. Accessed September 12, 2023. https://www.sangamo.com/programs/#pipeline

25. Pipeline. Quell Therapeutics. Accessed September 12, 2023. https://quell-tx.com/pipeline/

26. Prager I, Watzl C. Mechanisms of natural killer cell-mediated cellular cytotoxicity. J Leukoc Biol. 2019;105(6):1319-1329. doi:10.1002/JLB.MR0718-269R

27. Wu C, Xu Y, Zhao Y. Two kinds of macrophage memory: innate and adaptive immune-like macrophage memory. Cell Mol Immunol. 2022;19(7):852-854. doi:10.1038/s41423-022-00885-y

28. Lawand M, Déchanet-Merville J, Dieu-Nosjean M-C. Key Features of Gamma-Delta T-Cell Subsets in Human Diseases and Their Immunotherapeutic Implications. Front Immunol. 2017;8:761. doi:10.3389/fimmu.2017.00761

29. Akkaya M, Kwak K, Pierce SK. B cell memory: building two walls of protection against pathogens. Nat Rev Immunol. 2020;20(4):229-238. doi:10.1038/s41577-019-0244-2

30. Gabitova L. Anti-HER2 CAR monocutes demonstrate targeted anti-tumor activity and enable a single day cell manufaturing process. Poster presented at the: American Association for Cancer Research (AACR); October 4, 2021; Virtual.

31. Su S, Lei A, Wang X, et al. Induced CAR-Macrophages as a Novel Therapeutic Cell Type for Cancer Immune Cell Therapies. Cells. 2022;11(10). doi:10.3390/cells11101652

32. Our current pipeline. Carisma Therapeutics. Accessed September 13, 2023. https://carismatx.com/pipeline/

33. Raftery MJ, Franzén AS, Pecher G. CAR NK cells: the future is now. Annu Rev Cancer Biol. 2023;7(1):229-246. doi:10.1146/annurev-cancerbio-061521-082320

34. NKarta Therapeutics. Accessed September 13, 2023. https://www.nkartatx.com/pipeline/

35. Cytovia Therapeutics. Accessed September 13, 2023. https://www.cytoviatx.com/our-products

36. Adicet Bio. Accessed September 13, 2023. https://www.adicetbio.com/science/#gamma-delta

37. Immusoft. Accessed September 13, 2023. https://www.immusoft.com/science/

38. Walking Fish Therapeutics . Accessed September 13, 2023. https://www.walkingfishtx.com/index#our-science-intro

39. Hung KL, Meitlis I, Hale M, et al. Engineering Protein-Secreting Plasma Cells by Homology-Directed Repair in Primary Human B Cells. Mol Ther. 2018;26(2):456-467. doi:10.1016/j.ymthe.2017.11.012

40. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer

therapy. Nat Rev Drug Discov. 2022;21(11):799-820. doi:10.1038/s41573-022-00520-5

41. Bae DS, Lee JK. Development of NK cell expansion methods using feeder cells from human myelogenous leukemia cell line. Blood Res. 2014;49(3):154-161. doi:10.5045/br.2014.49.3.154

42. Regalado A. A new gene therapy based on antibody cells is about to be tested in humans September 1, 2022. Accessed September 13, 2023. https://www.technologyreview.com/2022/09/01/1058830/next-act-antibody-cells-new-form-of-gene-therapy/

43. Senti Bio. Accessed September 13, 2023. https://www.sentibio.com/index.php/pipeline

44. Garrison B. FLT3 OR CD33 NOT EMCN Logic Gated CAR-NK Cell Therapy (SENTI-202) for Precise Targeting of AML . Poster presented at the: American Society of Hematology; December 15, 2021; Atlanta, GA.

45. ArsenalBio Announces Presentations Highlighting Preclinical Data on AB-1015 and AB-2100 at ASGCT Annual Meeting. May 16, 2023. Accessed September 13, 2023. https://www.businesswire.com/news/home/20230516005255/en/ArsenalBio-Announces-Presentations-Highlighting-Preclinical-Data-on-AB-1015-and-AB-2100-at-ASGCT-Annual-Meeting

46. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 2021;11(4):69. doi:10.1038/s41408-021-00459-7

47. Madrid M, Sumen C, Aivio S, Saklayen N. Autologous Induced Pluripotent Stem Cell-Based Cell Therapies: Promise, Progress, and Challenges. Curr Protoc. 2021;1(3):e88. doi:10.1002/cpz1.88

48. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318(5858):1917-1920. doi:10.1126/science.1151526

49. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861-872. doi:10.1016/j.cell.2007.11.019

50. Chatterjee I, Li F, Kohler EE, Rehman J, Malik AB, Wary KK. Induced Pluripotent Stem (iPS) Cell Culture Methods and Induction of Differentiation into Endothelial Cells. Methods Mol Biol. 2016;1357:311-327. doi:10.1007/7651_2015_203

51. Hildreth C. The Pipeline for iPSC-Derived Cell Therapeutics in 2023. BioInformant. September 9, 2023. Accessed September 13, 2023. https://bioinformant.com/ipsc-derived-cell-therapeutics/

52. Petrosyan A, Martins PN, Solez K, Uygun BE, Gorantla VS, Orlando G. Regenerative medicine applications: An overview of clinical trials. Front Bioeng Biotechnol. 2022;10:942750. doi:10.3389/fbioe.2022.942750

53. Bell J. Vertex begins human testing for diabetes cell therapy. BioPharma Dive. March 10, 2021. Accessed September 13, 2023. https://www.biopharmadive.com/news/vertex-semma-human-testing-diabetes-cell-therapy/596466/

54. BlueRock Therapeutics. Accessed September 13, 2023. https://www.bluerocktx.com/the-science/programs/

55. Lancaster J. Cryopreserved Cardiac Tissue Graft (MyCardia) Improved Cardiac Function in Rats With Heart Failure. Abstract presented at the: Circulation Research; February 28, 2021.

56. Intellia Therapeutics. Intellia is Leading the Genome Editing Revolution. Accessed October 20, 2023. https://ir.intelliatx.com/static-files/a57908a6-aaca-404d-9072-32471cd3d41e

57. Sana Biotechnology. Accessed September 13, 2023. https://sana.com/our-science/

58. Pluristyx, panCELLa, and Implant Therapeutics Announce Definitive Merger Agreement. panCELLa. Accessed September 13, 2023. https://pancella.com/pluristyx-pancella-and-implant-therapeutics-announce-definitive-merger-agreement/

59. Shoreline Biosciences. Accessed September 13, 2023. https://shorelinebio.com/our-science/

60. Century Therapeutics. Accessed September 13, 2023. https://www.centurytx.com/pipeline/

61. Exacis Biotherapeutics. Accessed September 13, 2023. https://www.exacis.com/

62. Fate Therapeutics. Accessed September 13, 2023. https://fatetherapeutics.com/pipeline/immuno-oncology-candidates/ft576/

63. Dahlke J, Schott JW, Vollmer Barbosa P, et al. Efficient Genetic Safety Switches for Future Application of iPSC-Derived Cell Transplants. J Pers Med. 2021;11(6). doi:10.3390/jpm11060565

64. Beyar R. Challenges in organ transplantation. Rambam Maimonides Med J. 2011;2(2):e0049. doi:10.5041/RMMJ.10049

65. Organ Procurement & Transplant Network. National Data. Accessed September 13, 2023. https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#

66. Amaeshi LC. Navigating through the complications of chronic immunosuppression in transplant patients. AIM Clinical Cases. 2022;1(8). doi:10.7326/aimcc.2022.0940

67. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng. 2011;13:27-53. doi:10.1146/annurev-bioeng-071910-124743

68. de Haan MJA, Witjas FMR, Engelse MA, Rabelink TJ. Have we hit a wall with whole kidney decellularization and recellularization: A review. Curr Opin Biomed Eng. 2021;20:100335. doi:10.1016/j.cobme.2021.100335

69. Carrier AN, Verma A, Mohiuddin M, et al. Xenotransplantation: A new era. Front Immunol. 2022;13:900594. doi:10.3389/fimmu.2022.900594

70. Montgomery RA, Stern JM, Lonze BE, et al. Results of Two Cases of Pig-to-Human Kidney Xenotransplantation. N Engl J Med. 2022;386(20):1889-1898. doi:10.1056/NEJMoa2120238

71. Kuehn BM. First Pig-to-Human Heart Transplant Marks a Milestone in Xenotransplantation. Circulation. 2022;145(25):1870-1871. doi:10.1161/CIRCULATIONAHA.122.060418

72. OrganaMet Bio. Accessed September 13, 2023. https://organametbio.com/our-technology/

73. MiroMatrix. Accessed September 13, 2023. https://www.miromatrix.com/our-technology-1

74. eGenesis. Accessed September 13, 2023. https://egenesisbio.com/xenotransplant/

75. Arabi TZ, Sabbah BN, Lerman A, Zhu X-Y, Lerman LO. Xenotransplantation: current challenges and emerging solutions. Cell Transplant. 2023;32:9636897221148772. doi:10.1177/09636897221148771

76. Gwam C, Mohammed N, Ma X. Stem cell secretome, regeneration, and clinical translation: a narrative review. Ann Transl Med. 2021;9(1):70. doi:10.21037/atm-20-5030

77. Heslop JA, Hammond TG, Santeramo I, et al. Concise review: workshop review: understanding and assessing the risks of stem cell-based therapies. Stem Cells Transl Med. 2015;4(4):389-400. doi:10.5966/sctm.2014-0110

78. Daneshmandi L, Shah S, Jafari T, et al. Emergence of the stem cell secretome in regenerative engineering. Trends Biotechnol. 2020;38(12):1373-1384. doi:10.1016/j.tibtech.2020.04.013

79. Xu Z, Zeng S, Gong Z, Yan Y. Exosome-based immunotherapy: a promising approach for cancer treatment. Mol Cancer. 2020;19(1):160. doi:10.1186/s12943-020-01278-3

80. Muthu S, Bapat A, Jain R, Jeyaraman N, Jeyaraman M. Exosomal therapy-a new frontier in regenerative medicine. Stem Cell Investig. 2021;8:7. doi:10.21037/sci-2020-037

81. Zhang K, Cheng K. Stem cell-derived exosome versus stem cell therapy. Nat Rev Bioeng. April 12, 2023:1-2. doi:10.1038/s44222-023-00064-2

82. Rion Therapeutics. Accessed September 13, 2023. https://riontx.com/pipeline/

83. Aegle Therapeutics Receives Rare Pediatric Disease Designation from the Food and Drug Administration (FDA) for AGLE-102 for Patients with Dystrophic Epidermolysis Bullosa. October 6, 2020. Accessed September 13, 2023. https://www.prnewswire.com/news-releases/aegle-therapeutics-receives-rare-pediatric-disease-designation-from-the-food-and-drug-administration-fda-for-agle-102-for-patients-with-dystrophic-epidermolysis-bullosa-301146053.html

84. Kala Pharmaceuticals. Accessed September 13, 2023. https://www.kalarx.com/our-technology.htm

85. Immunis Biomedical. Accessed September 13, 2023. https://immunisbiomedical.com/technology/

86. Bari E, Ferrarotti I, Saracino L, Perteghella S, Torre ML, Corsico AG. Mesenchymal Stromal Cell Secretome for Severe COVID-19 Infections: Premises for the Therapeutic Use. Cells. 2020;9(4). doi:10.3390/cells9040924

87. Direct Biologics. Accessed September 13, 2023. https://directbiologics.com/technologies/

88. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Commun Signal. 2022;20(1):145. doi:10.1186/s12964-022-00959-4

89. EV Therapeutics. Accessed September 13, 2023. https://www.evtherapeutics.com/about-us

90. Wang Z, Popowski KD, Zhu D, et al. Exosomes decorated with a recombinant SARS-CoV-2 receptor-binding domain as an inhalable COVID-19 vaccine. Nat Biomed Eng. 2022;6(7):791-805. doi:10.1038/s41551-022-00902-5

91. ReNeuron. Accessed September 13, 2023. https://www.reneuron.com/ctx-derived-exosomes/

92. Evox Therapeutics. Accessed September 13, 2023. https://www.evoxtherapeutics.com/

93. Xin T, Cheng L, Zhou C, Zhao Y, Hu Z, Wu X. In-Vivo Induced CAR-T Cell for the Potential Breakthrough to Overcome the Barriers of Current CAR-T Cell Therapy. Front Oncol. 2022;12:809754. doi:10.3389/fonc.2022.809754

94. Challener C. BioPharm International . Technology for In Vivo CAR T-cell Therapy Advances. February 1, 2023. Accessed September 13, 2023. https://www.biopharminternational.com/view/technology-for-in-vivo-car-t-cell-therapy-advances

95. Capstan Therapeutics . Accessed September 13, 2023. https://www.capstantx.com/#science

96. Interius Bio. Accessed September 13, 2023. https://interiusbio.com/

97. Exuma Biotechnology. Accessed September 13, 2023. https://exumabio.com/

98. Umoja Biopharma. Accessed September 13, 2023. https://www.umoja-biopharma.com/

99. Metagenomi. Accessed September 13, 2023. https://metagenomi.co/gene-editing-toolbox

100. iECURE. Accessed September 13, 2023. https://iecure.com/approach/

101. Aldevron. Accessed September 13, 2023. https://www.aldevron.com/about-us/news/aldevron-launches-eureca-v

102. Poseida. Accessed September 13, 2023. https://poseida.com/technology/

103. Turchiano G, Andrieux G, Klermund J, et al. Quantitative evaluation of chromosomal rearrangements in gene-edited human stem cells by CAST-Seq. Cell Stem Cell. 2021;28(6):1136-1147.e5.doi:10.1016/j.stem.2021.02.002

104. 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. doi:10.1038/nbt.4192

105. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells.Nat Rev Genet. 2018;19(12):770-788. doi:10.1038/s41576-018-0059-1

106. Eid A, Alshareef S, Mahfouz MM. CRISPR base editors: genome editing without double-stranded breaks. Biochem J. 2018;475(11):1955-1964. doi:10.1042/BCJ20170793

107. Wake up the Sleeping Beauty – Transposons in gene therapy: How do they compare to other genome modifying tools? BioPharma Excellence. Accessed September 13, 2023. https://www.biopharma-excellence.com/2021-5-4-wake-up-the-sleeping-beauty-transposons-in-gene-therapy- how-do-they-compare-to-other-genome-modifying-tools/#:~:text=In%20gene%20therapy%2C%20for%20safety,mRNA%2C%20or%20minicircles%20through%20electroporation.

108. Scholefield J, Harrison PT. Prime editing - an update on the field. Gene Ther. 2021;28(7-8):396-401. doi:10.1038/s41434-021-00263-9

109. Beam Therapeutics. Accessed September 13, 2023. https://beamtx.com/pipeline/

110. Verve Therapeutics. Accessed September 13, 2023. https://www.vervetx.com/our-programs/our-pipeline

111. Chroma Medicine. Accessed September 13, 2023. https://chromamedicine.com/

112. Tune Therapeutics. Accessed September 13, 2023. https://tunetx.com/the-science/

113. Epic Bio. Accessed September 13, 2023. https://epic-bio.com/science/

114. Prime Medicine. Accessed September 13, 2023. https://primemedicine.com/

115. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149-157. doi:10.1038/s41586-019-1711-4

116. FDA sets decision dates for Vertex, CRISPR gene editing drug. Biopharma Dive. Accessed October 5, 2023. https://www.biopharmadive.com/news/vertex-crispr-priority-review-sickle-cell-gene-editing/652581/

117. Saliogen. Accessed September 13, 2023. https://saliogen.com/our-science/

118. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther. 2021;6(1):53. doi:10.1038/s41392-021-00487-6

119. Shape Therapeutics. Accessed September 14, 2023. https://shapetx.com/

120. Apertura Therapeutics. Accessed September 14, 2023. https://aperturagtx.com/our-platforms/

121. Capsida Biotherapeutics. Accessed September 14, 2023. https://capsida.com/science-innovation/#approachsection

122. 4DMT. Accessed September 14, 2023. https://4dmoleculartherapeutics.com/pipeline/

123. Capsigen. Accessed September 14, 2023. https://capsigen.com/

124. CoAve Therapeutics. Accessed October 16, 2023. https://coavetx.com/

125. Knutsen A. Nonviral Platforms Streamline Gene Therapy Delivery. GEN. September 1, 2023. Accessed September 14, 2023. https://www.genengnews.com/topics/drug-discovery/nonviral-platforms-streamline-gene-therapy-delivery/#:~:text=To%20skirt%20the%20limitations%20associated,on%20physical%20or%20chemical%20mechanisms.

126. Let's talk about lipid nanoparticles. Nat Rev Mater. 2021;6(2):99-99. doi:10.1038/s41578-021-00281-4

127. Intellia Therapeutics files patent on lipid nanoparticle-based compositions for CRISPR/Cas gene editing delivery. Pharmaceutical Technology. August 22, 2023. Accessed September 14, 2023. https://www.pharmaceutical-technology.com/data-insights/intellia-therapeutics-files-patent-for-lipid-nanoparticle-based-compositions-for-crispr-cas-gene-editing-delivery/?utm_source=lgp6-patents-application&utm_medium=24-230205&utm_campaign=recommended-articles&cf-view

128. Carmine Therapeutics. Accessed September 14, 2023. https://www.carminetherapeutics.com/

129. Cytonus. Accessed September 14, 2023. https://cytonus.com/our-science/

130. Nanite Bio . Accessed September 14, 2023. https://www.nanitebio.com/

131. SonoThera. Accessed September 14, 2023. https://sonothera.com/

132. Zhu Y, Zhu L, Wang X, Jin H. RNA-based therapeutics: an overview and prospectus. Cell Death Dis. 2022;13(7):644. doi:10.1038/s41419-022-05075-2

133. Ascidian Therapeutics. Accessed October 5, 2023. https://ascidian-tx.com/

134. Anastassiadis T, Köhrer C. Ushering in the era of tRNA medicines. J Biol Chem. September 11, 2023:105246. doi:10.1016/j.jbc.2023.105246

135. Alltrna. Accessed October 5, 2023. https://www.alltrna.com/

136. Tevard Biosciences. Accessed October 5, 2023. https://tevard.com/

137. ReCode Therapeutics Presents Preclinical Data Using SORT-LNPTM and RNA Platforms to Rescue CFTR Function at the 44th European Cystic Fibrosis Conference (ECFS). ReCode Therapeutics. Accessed October 16, 2023. https://recodetx.com/recode-therapeutics-presents-preclinical-data-using-sort-lnptm-and-rna-platforms-to-rescue-cftr-function-at-the-44th-european-cystic-fibrosis-conference-ecfs/

138. hC Bioscience, Inc. Accessed October 5, 2023. https://hcbioscience.com/science-technology/

139. Taking gene therapy to the next level. Nature. Accessed October 16, 2023. https://www.nature.com/articles/d43747-022-00222-2#:~:text=ShapeTX%20uses%20a%20guide%20RNA,location%20 zon%20a%20target%20RNA.

140. Our Approach. Korro Bio. Accessed October 16, 2023. https://www.korrobio.com/our-approach/

141. Yi Z, Qu L, Tang H, et al. Engineered circular ADAR-recruiting RNAs increase the efficiency and fidelity of RNA editing in vitro and in vivo. Nat Biotechnol. 2022;40(6):946-955. doi:10.1038/s41587-021-01180-3

Alliance *for* Regenerative Medicine

Catalyst Healthcare Consulting

Questions or comments?

Contact us at **info@alliancerm.org**

www.alliancerm.org

@alliancerm

@AllianceRegenMed

@Alliance for Regenerative Medicine