CELL AND GENE THERAPY

# Overview, current landscape and future trends

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# Overview

**Many diseases are caused by alterations in the genome that impact a specific cell type(s). Such mutations can be either inherited, such as the faulty cystic fibrosis transmembrane conductance regulator (CFTR) gene that causes cystic fibrosis, or acquired, as in the case of many cancers. Whether inherited or acquired, the result of these disease-causing mutations is generally the same: a loss of the normal function of the proteins encoded by them. The ability to restore gene function through cell and gene therapies has transformed medicine and is now at a turning point in our ability to treat diseases with a personalized medicine approach.**

Cell and gene therapies are in many ways overlapping modalities that aim to treat (and potentially cure) diseases caused by genetic alterations, including some cancers that arise through aberrant genetic activity. Both involve the transfer of new genetic material to patients' cells to either restore the normal expression and function of proteins affected by genetic alterations, or to direct immune cells against the patient's cancer. In this way they differ significantly from conventional therapeutic approaches.[1]

Gene therapy generally involves the use of a vector to deliver specific genetic sequences to a cell, either in vivo or ex vivo, to replace, disrupt, or change a faulty gene.[1] In contrast, cell therapy involves the transfer of whole, functioning, 'living' cells that supplement or replace the activity of the original cells.[2] These cells may originate from the patient themselves (autologous cells), or from a donor (allogeneic cells), and are also genetically modified in a specific manner.[1, 2]

### Table 1. Key features of cell and gene therapies

| Cell therapy | Gene therapy |
|---|---|
| Involve transfer of whole, functioning, 'living' cells | Deliver genetic information to correct a well-defined genetic defect |
| Generally include CAR T-cells, TILs, TCR, NK, DC and stem cell derived products (induced pluripotent, mesenchymal, and hematopoietic stem cells) | Gene replacement, addition, or disruption, silencing using viral or non-viral delivery methods |
| Restore normal function & impact underlying cause of disease; enhance immune response to cancers | Restore normal function and expression & impact underlying cause of disease |
| Risks: Cytokine release syndrome (CRS); generation of replication competent retro- or lentivirus | Risks: Insertional mutagenesis; immune response to vector |
| Key biomarkers to assess cellular kinetics, phenotypic characterization and PK/PD | Neutralizing antibodies; PK/PD dosing considerations |
| Approved examples: Kymriah®, Yescarta®, Tecartus™ | Approved examples: Luxturna®, Zolgensma® |

**In practice, cell and gene therapy techniques are often considered together because of a certain level of commonality, but they differ in their specific applications.[3]**

For example, cancer therapies utilizing cells that have been extracted from a patient, genetically modified and are then returned back to the patient result in an innovative therapy such as CAR (chimeric antigen receptor) T-cells. These cells are genetically engineered to express an antigen-specific, non–major histocompatibility complex (MHC)-restricted receptor, which can engage antigens on target cells and initiate mechanistic signaling pathways. This technology harnesses the power of T-cells, the workhorses of one's immune response, and has elicited remarkable therapeutic effects in patients with hematological cancers and holds great promise for the future.[4, 5] The key features of adoptive cell and gene replacement therapies are outlined in Table 1.

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**After some early setbacks, the last decade has yielded some remarkable advancements in these technologies,[2] and a number of new cell and gene therapies have recently been approved.[6] Over 1,000 active trials are ongoing,[7] and drug delivery and patient profiling techniques are rapidly improving.[1, 8] Cell and gene therapies are pillars of precision medicine, and have the potential to transform the lives of those affected by genetic diseases and many types of cancer–therapies for many of which have so far been unattainable.[2]**

Some of the earliest conceptual studies into the use of cells and genes to correct disorders were published nearly 50 years ago.[1] While these early reports identified several theoretical considerations that would be necessary for successful gene therapy,[9] it was not until the early 1990s that technological advances made clinical studies possible.

The first successful use of cell and gene therapy as a therapeutic approach in humans occurred in 1990, in which patients with advanced melanoma in the study were administered tumor-infiltrating lymphocytes. The treatment persisted in the patients' circulation and tumor deposits for up to several months and they experienced no side effects, demonstrating the clinical feasibility of administrating genetically modified cells.[10] This first attempt was quickly followed by a number of other attempts to address specific diseases, including to treat ADA-SCID,[11, 12] and familial hypercholesterolemia.[13]

However, the initial enthusiasm for gene therapy diminished following the death of a patient owing to an immune response to the vector delivering the gene therapy in a clinical trial for ornithine transcarbamylase deficiency in 1999.[14] This event, and the discovery that several individuals who received gene therapy for X-linked SCID had subsequently developed leukemia, led the FDA to review the ethical concerns and safety risk associated with gene therapy trials.[15, 16]

Despite these early setbacks, the new millennium saw renewed interest and growth in the development of cell and gene therapies, primarily due to the rapid advancements in drug delivery technology, genetic engineering, and synthetic biology, as well as in our understanding of genomics and biology of disease. This progress has led to the development of a variety of techniques to manipulate genes and refinements in ways to deliver genetic information to cells.

Over the last decade, **research has accelerated** and resulted in several approvals for gene and cell therapies for a broad variety of indications.

# Current landscape

**More recently, these include the FDA approvals for brexucabtagene autoleucel for mantle cell lymphoma (MCL; Tecartus™), axicabtagene ciloleucel for B-cell lymphoma, (BCL; Yescarta®), tisagenlecleucel for acute lymphoblastic leukemia (ALL; Kymriah®), onasemnogene abeparvovec-xioi for spinal muscular atrophy (SMA; Zolgensma®), and voretigene neparvovec-rzyl for inherited retinal diseases (Luxturna®).[6]**

All of these therapies offer significant clinical benefit and show great promise in the treatment of these diseases. For example, in a Phase 2 trial involving 75 children and young adults with pre-treated CD19+ ALL, a remarkable 81% of patients were in remission at 3 months following a single infusion of tisagenlecleucel (Kymriah); a preparation of CAR T-cells expressing an anti-CD19 receptor. Event-free survival was 73% and 50% at 6 and 12 months, indicating durable efficacy in this difficult-to-treat population.[4] Similarly, in an open-label study involving 22 children (mean age 3.7 months) with Type 1 spinal muscular atrophy, patients received onasemnogene abeparvovec-xioi (Zolgensma), an adeno-associated virus (AAV) vector-based gene therapy carrying a functional copy of the human survival motor neuron (SMN) gene. After a single infusion, over 90% of patients were still alive without permanent ventilation two years later. Without treatment, only around 25% remain alive without permanent breathing support at age 14 months.[17] The potential of these therapies in either preventing disease progression or providing a curative benefit in certain disorders highlights the clinical value compared to existing therapies that are often geared towards symptomatic treatment.

These recent successes illustrate how interest in cell and gene therapy has increased exponentially over the past few years, due in part to the rapid advancements in vector biology, drug delivery technology and precision medicine: areas which have until recently limited the application of cell and gene therapies.[1] Additionally, cell and gene therapies are typically developed for smaller, underserved patient populations with rare diseases and cancers, and the potential for substantially enhancing these patients' lives is high. As such, shorter development timelines and accelerated regulatory approvals are possible.[18] Indeed, so far the FDA has issued 44 Regenerative Medicine Advanced Therapy (RMAT) designations, designed to streamline and expedite the approval process for promising cell and gene therapy products for serious or life threatening disorders.[19] These accelerated pathways are shifting the clinical trials paradigm by enabling innovative designs including novel surrogate endpoints and potential for registrational approval as early as Phase II.

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**Together, these factors are attracting many more biopharmaceutical companies to the cell and gene technology sphere, with over 1,000 active cell and gene therapy trials ongoing. The vast majority are Phase 1 or 2 trials for oncology indications, but others span a range of therapeutic areas including metabolic diseases, ophthalmologic disorders, as well as musculoskeletal and immunological disorders.[7, 20]**

Technological advancements in vector biology along with genetic engineering platforms enabling gene editing or correction, gene addition or gene disruption have significantly impacted our ability to alter the genome. Gene editing or correction technologies based on engineered or bacterial nucleases are providing more flexible approaches to modify the genome. These techniques include CRISPR/Cas9, zinc finger, or TALEN nucleases or meganucleases that can alter a cell's DNA with expected precision at the nucleotide level, without affecting other off target sequences that may impact expression of the gene or other genes. Genome editing can be performed on cells ex vivo or the editing machinery can be delivered in vivo.[24]

In addition to gene editing, CRISPR/Cas9, zinc finger, and TALEN nucleases can also be used for gene disruption, if knockdown of a gene is desired. For example, this technology can be used to modify the T-cell genome, by knocking out negative T-cell regulators, through specific gene disruption as well as by adding transgenes. This is particularly useful for treating diseases caused by gene overexpression, including many cancers. Another application of this technology is in the modification of allogeneic donor cells wherein the immunogenic sequence can be eliminated to render these cells less immunogenic to the host receiving them. Gene editing technologies are also being applied to gene replacement therapy approaches in diseases such as sickle cell anemia, inherited retinal degenerative disorders, cystic fibrosis and others.

Another advancement in the field is the successful delivery of the genetically modified material using either a viral or non-viral based approach. The majority of the gene therapy trials utilize an engineered adeno-associated virus (AAV) or lentivirus ('lenti') vector due to the inherent ability of viruses to introduce genetic material into host cells and their ease of manipulation.[1, 31] Although these properties make viruses an obvious choice for delivery of cell and gene therapies, these delivery methods have some disadvantages including potential for immunogenicity and malignancies caused by vector-mediated insertional activation of oncogenes. Active research and focus on non-viral methods for delivery is ongoing and non-viral vectors such as the PiggyBac™ (PB) system that delivers DNA via a transposon are currently under clinical investigation.

The field of CAR T-cell therapy has also evolved with refinements in CAR design to prompt immune system activation, enhance signalling and proliferation of T-cells and improve its safety profile. The backbone of the approved CAR T-cell therapies feature a second generation CAR that consists of additional costimulatory molecules such as CD-28 or 4-1BB. Since then, CARs have evolved to third and fourth generation versions to address autoimmune issues and tumor-mediated immunosuppression. For instance, the fourth-generation CAR T, referred to as TRUCKs (T-cells redirected for universal cytokine-mediated killing) can improve CAR T-cell expansion and survival, while making them resistant to an immunosuppressive tumor environment. TRUCKs are genetically "armored" with antitumor activity maximized through additional genetic modification, including knock-out, or knock-in of different genes that can control CAR expression and activity, including transgenes for cytokine secretion and are currently being investigated to treat solid tumors. Other focus areas in the cell therapy space include induced pluripotent stem cells (iPSCs), Natural killer (NK) cells and T-cell receptor (TCR) immunotherapy approaches.

Another aspect that has played a key role in the evolution of these cell-based therapies are biomarkers that can be used to assess and monitor pharmacokinetics of the treatment dosing, level of the target cell population and other indicators of treatment efficacy and safety. In the context of CAR T-cell therapy, functional & phenotypic characterization provides insight into the potential effectiveness of the product. As these are 'a living drug', assessing the immunological fitness and the status of immune activation and differentiation, memory response and survival capacity, during both manufacturing and in the final product, is critical. In addition, incorporation of biomarker assessments in CAR T-cell clinical trials are beneficial in determining cellular kinetics, including expansion and persistence of CAR T-cells post administration, efficacy endpoints including minimum residual disease (MRD) and the impact on immune system activation. Safety related biomarkers, including inflammatory markers such as c-reactive protein (CRP) levels and cytokines such as IL-6, IL-15, IFN$\gamma$, GM-CSF, IL-8, MIP-1, among others, have provided insights in predicting cytokine release syndrome (CRS)–a condition that presents a serious and potentially life-threatening safety risk for patients. A summary of biomarker applications in CAR T-cell therapies is shown in Figure 1.

Figure 1: Biomarker applications: CAR T-cells

- Expansion and persistence of CAR T-cells
- Phenotypic characterization of CAR T-cells
- Establish PK/PD relationship
- Mechanistic makers of efficacy
- Predictive biomarkers of safety, response, resistance or relapse
- Patient
- ID/Stratification

# Future trends

**Cell and gene therapies have progressed substantially since their conceptual beginning in the 1970s. From the first successful human trials in the early 1990s, the technologies have advanced, many reasons for their setbacks have been addressed, and this burgeoning branch of precision medicine now includes over 15 approvals and hundreds of companies globally supporting development and a significant and growing pipeline of therapeutic candidates. The numbers of clinical trials and regulatory approvals for cell and gene therapies are expected to rise over the coming years: the FDA anticipates that by 2025 it will approve 10–20 cell and gene therapy products per year,[21] and separate estimates suggest that by 2030 half a million patients in the US alone will have been treated with 40–60 approved gene or cell therapy products.[22] However, additional research and operational developments are needed if cell and gene therapies are to become more widely available to the patients who need them.**

Improvements in vector biology, manufacturing and delivery mechanisms are needed to make these therapies more broadly available and cost effective. Consortia involving government, academic and industry participants will be critical to these efforts. For example, more universal vector systems might improve the scalability of manufacturing. Such improvements may also allow enhancements in the tissue specificity of the administered therapy. The paucity of safe, effective and cost-effective delivery systems is a headwind to the clinical application of cell and gene technologies,[23] and potentially prevents some novel therapies from progressing past Phase 1.20 Advances in nanotechnology, nucleic acid engineering, and molecular biology may lead to novel non-viral vectors being developed that overcome the limitations with existing delivery methods.[23] Furthermore, reverse genetics is now possible for almost all viruses, and this has vastly expanded the virus types that can be evaluated as potential vectors. AAVs, poxviruses, herpesviruses and some non-human viruses are now being studied for their potential as vaccine and gene therapy vectors; some of which may offer safety and efficacy benefits compared with other viral vectors.[25] The ability to use allogeneic versus autologous donors will also allow for potential scalability of adoptive cell therapies.[20]

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Improvements in patient identification and stratification, through the identification of more and more therapeutic and prognostic biomarkers, may further improve the safety and efficacy of cell and gene therapies, by helping to match patients with appropriate therapies to maximize efficacy and minimize side effects.[26] A combination of methods, along with a systems biology approach for data analytics, will add considerably to our ability to use biomarkers more effectively in patient management.[27] A summary of these and other future trends is outlined in Table 2.

### Table 2. Future trends in cell and gene therapies

| Category | Detail |
|---|---|
| Improved and expanded manufacturing capabilities | • Increased scale of manufacturing addresses access and cost<br>• Industry-wide collaborations among all stakeholders to address capacity (i.e., public-private consortia)<br>• For cell therapies, use of allogeneic as well as autologous donors |
| Advances in vector biology and delivery mechanisms | • Development of universal vectors for gene transfer<br>• Improved targeting of required tissues and control of tissue specific expression |
| Expansion of indications for use | • Expansion from hematologic malignancies to applications in solid tumors for adoptive cell therapy<br>• Broadening of gene replacement therapies to more common disorders that have a genetic component |
| Role of biomarkers | • Systems biology approach to biomarker discovery and qualification<br>• Improved biomarkers of efficacy<br>• Use in long-term follow up studies |
| Regulatory support | • Global harmonization of regulatory guidelines and processes<br>• Specialized regulatory considerations for CGT |

**Advancements in these critical areas** and in our understanding of disease biology will drive the expansion of these modalities beyond current applications in rare diseases and oncology to other therapeutic areas and clinical indications.

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