CELL & GENE THERAPY INSIGHTS

Clinical Trends

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EXPERT INSIGHT

Clinical applications of gene and cell therapies: case studies for the relevance of precision medicine

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Precision medicine, a medical modality focusing on tailoring medical decision-making to individual patients, is changing the way we think about, prevent, treat, and monitor many diseases, including those requiring gene and cell therapies. Both gene and cell therapies involve the therapeutic transfer of new genetic material into a target cell with the goal of treating disease. The fields of gene and cell therapies are growing, but there are many unknowns and reasons to be cautious remain. Selecting the right patient for the right therapy and monitoring that patient's response to the therapy is imperative. Biomarkers are tools that can facilitate selection and monitoring of gene and cell therapies, and their proper identification and application allows patients to be treated accurately, effectively, and safely. Several biomarkers of disease, immune, cellular, and molecular responses to gene and cell therapies are available, and the role of biomarkers will expand as gene and cell therapies continue to develop. With the rapid growth of gene and cell therapies, biotechnology and pharmaceutical companies face a call to action: we must establish proper selection and monitoring protocols to provide patients with the safest and most effective therapeutic options for genetic diseases. This article presents two case studies from a biopharmaceutical company's clinical programs for gene and allogeneic cell therapies and provides a primer for the relevance of precision medicine applications.

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INTRODUCTION

Precision medicine is a medical modality that focuses on tailoring medical decision-making to individual patients, and it offers an innovative, individualized approach to health care by considering a patient's genetics, lifestyle, and environmental exposures to tailor disease prevention and treatment [1]. Gene and cell therapies are part of precision medicine, and they are changing the way we think about, prevent, treat, and monitor many diseases [2]. Several modalities of gene and cell therapies involve the therapeutic transfer of new genetic material into a target cell. With gene therapy, only genetic material is transferred to a patient. The new genetic material changes how a cell expresses a gene and makes a targeted protein. This approach may include making more disease-fighting protein, less disease-causing protein, or an entirely new protein. With cell therapy, whole cells are transferred to a patient. The new cells restore or alter cells in the body or carry therapy to specific organs or tissues. Terminology related to gene and cell therapies is listed in Table 1 [3–9].

The fields of gene and cell therapies are growing at unprecedented rates and will change the future of health care, but there are many unknowns and reasons to be cautious remain. For gene and cell therapies to be effective, the body is challenged to do something that it does not normally do, such as express a new gene that synthesizes a protein or interacts with a foreign cell, or to do what it normally does in a different way or in a different quantity, such as producing more of a naturally occurring protein. These changes may result in immune response and toxicity concerns. Therefore, it is imperative to first select the right patient for the right therapy and, second, to monitor that patient's response to the therapy. Biomarkers are tools in the arsenal of selection and monitoring of precision medicine, and their proper identification and application allows patients to be treated accurately, effectively, and safely with gene and cell therapies. An expanded role for biomarkers is emerging as gene and cell therapies continue to develop.

Precision medicine is a fast-changing and variable field, and this article is not intended to be a comprehensive review of its relevance for gene and cell therapies. Instead, this article offers two representative case studies of gene and allogeneic stromal cell therapies targeting diseases with unmet clinical needs where precision medicines are essential components. Many clinical trials of gene and cell therapies are underway around the world and several comprehensive review papers of gene and cell therapies have been published; readers are encouraged to learn more about precision medicine applications by accessing these resources.

CLINICAL DEVELOPMENT OF GENE & CRISPR THERAPIES

Gene therapy involves transferring a new gene to a patient with the goal of treating a disease [10,11]. The new gene may be an addition to the host genome, replace a disease-causing gene, or correct or inactivate a defective gene. For example, hemophilia A is a monogenic hereditary disorder (meaning that it is caused by a single defective gene) that leads to deficient production of factor VIII, a key blood-clotting protein. The genetics of hemophilia A are well understood and, as such, hemophilia A has become a target for gene therapy that corrects the defective gene.

Currently, many clinical studies of gene therapy for hemophilia A use an adeno-associated viral (AAV) vector to deliver genes that encode production of factor VIII directly into target cells in the liver. The liver cells, in turn, become 'protein factories' that secrete factor VIII into the body's circulation. With this gene therapy technology, the host cell primarily retains the transgene sequences as episomes; that is, the AAV vector exists as extrachromosomal material and is able to synthesize protein independently from the host chromosomes (Figure 1). It is uncommon for episomes to integrate into the host

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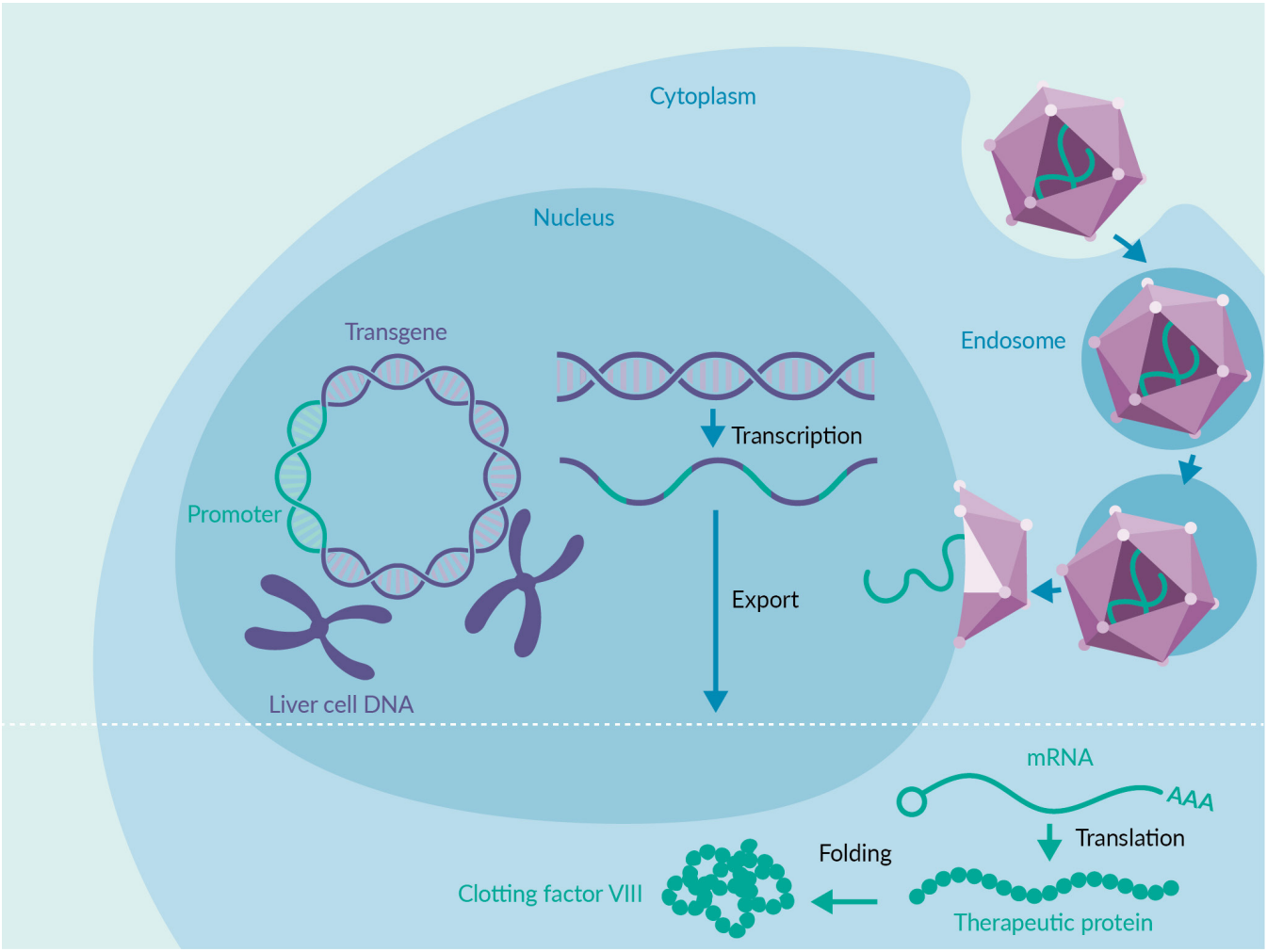
TABLE 1

Terminology used in cell and gene therapies [3–9].

Term	Definition
Adeno-associated virus (AAV) vector	An adenovirus that is used as a vehicle for genes, whose core genetic material has been removed and replaced by the dysfunctional gene
Antibody	Proteins that help fight infections
Biomarker	A measurable indicator of a physiologic state of an organism
Chromosome	A DNA molecule stabilized by proteins that carries hereditary (genetic) information (genes) of an organism
Cellular therapy	Transferring intact cells into a patient to cure a disease
CRISPR	Stands for clustered regularly interspaced short palindromic repeats; a gene-editing technique that is used to identify and modify specific DNA sequences in the genome of an organism
DNA	Deoxyribonucleic acid. One of two types of nucleic acids made by cells (the other being RNA); the molecules inside cells that carry genetic information and pass it from one generation to the next
Decidua stromal cells (DSCs)	Maternal stromal cells derived from the fetal membrane, more immunosuppressive than other types of stromal cells
Gene	The pieces of DNA that are passed from parent to offspring; genes contain instructions for making a specific protein
Genome	All the genetic information of a cell or organism
Gene deletion	The loss of all or part of a gene
Gene duplication (gene amplification)	An increase in the number of copies of a gene
Gene editing	The use of biotechnological techniques to make changes to specific DNA sequences in the genome of a living organism
Gene substitution	A type of mutation where one nucleotide is substituted for another
Gene therapy	A type of treatment in which altered genetic material is inserted into a person's cells to prevent or treat disease
Gene transfer	The insertion of genetic material into a cell
Genetic mutation	A permanent change or alteration in the DNA sequence that makes up a gene; it can be harmful, beneficial, or have no effect
Hematopoietic stem cells	Cells that can replenish themselves and produce cells that develop into a variety of mature types of blood cells
Hepatocytes	Liver cells
Immune response	The action of the immune system against foreign substances (antigens)
Mesenchymal stromal cells (MSCs)	Multipotent, non-hematopoietic stem cells that are present in adult and fetal tissues; capable of differentiating into various cell types, including adipocytes, osteocytes, chondrocytes, and cells in connective tissues
Protein	The major macromolecular constituent of cells; it is required for structure, function, and regulation of the body's cells, tissues, and organs
RNA	Ribonucleic acid. One of two types of nucleic acids made by cells (the other being DNA); contains information that has been copied from DNA. Several types of RNA exist, each with diverse functions that are important to normal cellular processes
Transgene	A gene that has been transferred from the genome of one species into that of another
Transcription	The process of synthesizing messenger RNA from DNA
Transduction	The process of transferring foreign DNA into a host cell using a virus or viral vector
Translation	The process by which the information from a sequence of messenger RNA is used to produce a protein
Virus	A simple microorganism that infects cells and may cause disease; can multiply only inside infected cells, so they are not considered to be alive
Vector	Viral DNA that is used to transmit genetic material to another cell or organism

FIGURE 1

Gene therapy for hemophilia A.



genomic DNA [12,13]. A key limitation of episomal genetic material is the inability to be maintained during cell division. This impacts the use of gene therapy in target cells in organs that continue to grow and develop through childhood. Therefore, gene therapies targeting liver cells are indicated only for adults.

Gene therapy is limited by high costs and challenges in the large-scale manufacturing of vectors, vector quality control and assay standardization, and immunologic barriers to gene delivery through viral vectors. Additionally, the purification of recombinant AAV particles is difficult and batch-to-batch variations in vector potency limit consistency [14]. One particular concern of AAV-based delivery is $CD8^+$ T-cell–mediated immune responses. These cells are able to eliminate vector-transduced cells, which induces an inflammatory response in the target organ and diminishes the potential benefit of the gene therapy. In AAV-based delivery in hemophilia A, $CD8^+$-mediated response has been identified against the viral capsid, which causes the loss of hepatocytes that express the therapeutic transgene [15–17].

CRISPR (which stands for clustered regularly interspaced short palindromic repeats) is an effective gene-editing tool for targeted gene therapies. The CRISPR technology requires two key components:

1. An RNA guide that identifies the target sequence; and
2. An enzyme that cuts DNA (usually Cas9).

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There are three different approaches currently in clinical development to treat monogenic diseases: *ex vivo*, *in vivo* gene deactivation, and *in vivo* gene replacement [3,18]. In contrast to standard gene therapies, CRISPR does not retain genes in the cell nucleus as episomes. With CRISPR, the gene is integrated into the host DNA and preserved during replication, meaning that it can be used in cells that are growing and dividing, such as the liver cells of children. For example, in hemophilia A, CRISPR has been used to insert the B-domain that is deleted from the *FVIII* gene, which directs factor VIII production, to restore factor VIII expression [19].

Despite its promise, CRISPR is associated with potential off-target effects. The consequences of the off-target effects are variable and depend on many factors. The risks of off-target effects, though also variable, may limit future uses of CRISPR technology [20–22].

A key consideration in the clinical development of gene therapies is defining the single therapeutic dose to be administered to patients. Initially, preclinical studies are conducted in animal models, and a starting dose is evaluated and adjusted. After the dose is established, the findings can be translated to dose-finding and safety trials (Phase 1/2). Next, large-scale Phase 3 clinical trials can be conducted to demonstrate safety and efficacy for the target population.

Biomarkers for patient selection & monitoring in gene therapies

Gene therapy for hemophilia A is limited by potential neutralization or inhibition of the transgene or vector by antibodies and cell-mediated immune responses. Additionally, a variety of patient characteristics can impact the distribution, uptake, and response to therapy. By identifying biomarkers that indicate potential safety or efficacy concerns, one can optimize therapy for patients with the highest likelihood of successful outcomes.

Neutralizing antibodies of the viral vector

Neutralizing antibodies to specific AAV serotypes are prevalent due to natural infection with wild-type AAV during childhood [4,23–26]. By neutralizing the vector, these antibodies reduce the efficacy of gene therapy for hemophilia A. Unfortunately, no tests for detecting anti-AAV antibodies have been standardized [13] and no strategies for overcoming the antibodies have proven effective so far [27].

INHIBITORS TO THE TRANSGENE PRODUCT

Traditional hemophilia A treatment consists of factor VIII replacement [28,29]. A primary complication to this approach is the development of inhibitors, which are antibodies that neutralize the replacement factor [29]. To date, there have been no reports of inhibitors to factor VIII in clinical studies of AAV gene therapy for hemophilia A treatment. However, clinical studies excluded patients who had any history of inhibitor presence and, therefore, have included only patients with a low risk of antibody formation [30]. Studies of patients with active factor VIII inhibitors are ongoing to determine the impact on safety and efficacy [26].

Functional biomarkers

Liver enzymes can serve as functional biomarkers of response to gene therapy. Asymptomatic increases in alanine aminotransferase (ALT) levels can be observed in patients receiving gene therapy for hemophilia A. Most ALT elevations are no more than 1.5- to 2-fold above the upper limit of normal and are transient in nature; as such, the ALT elevations are unlikely to be clinically relevant. However, hepatocyte death can occur after ALT increases [26].

Clinical data in hemophilia A show that the increase in ALT after gene therapy is

dependent on vector dose and, possibly, the number of CpG motifs (a cytosine linked to a guanine by a phosphate bond), but is independent of the AAV capsid, genome configuration, transgene promoter, and method of manufacture [25]. Long-term assessment of the health and function of liver cells is critical to understanding the safety and efficacy of gene therapy.

Structural biomarkers

Several imaging techniques can be used for screening purposes or for comparison of gene-therapy–related anatomical changes with baseline characteristics. For example, when the liver is the target organ, as in hemophilia A gene therapy, FibroScan® (Echosens; using transient elastography) or ultrasound is often employed to assess organ structure. Such investigations can identify patients who have any indication of risk for complications to the therapy, such as preexisting or worsening fibrosis, steatosis, or cancer [31,32].

Cellular biomarkers

The overexpression of a protein in a target cell, such as factor VIII in hepatocytes, may induce cellular stress in the endoplasmic reticulum [33,34]. The unfolded protein response is designed to protect the cell from this protein accumulation and minimize cellular stress [35–37]. The unfolded protein response is a particular concern in gene therapy for hemophilia A because the hepatocytes are forced to produce a protein they do not normally produce. In addition, traditional AAV-based gene therapies for hemophilia A use a B-domain–deleted factor VIII transgene [38,39]. Because the newly expressed protein differs from naturally produced factor VIII, the risk of misfolding or overexpression is high [38]. Although unfolded protein response should ideally be measured at the cellular level, biopsy samples of the target organ have allowed the description of a serum biomarker, glucose-regulated protein 78, also called binding immunoglobulin protein [40,41], which can predict cellular stress and hepatocyte damage in response to gene therapy.

ASC Therapeutics has developed ASC618, an AAV vector-encoding B-domain–deleted factor VIII for the treatment of patients with hemophilia A. ASC618 contains two components: a liver-directed promoter that minimizes the size of the vector and a bioengineered factor VIII molecule containing 91% human and 9% porcine sequences that offers increased biosynthesis, expression, and secretion efficiency compared with standard factor VIII transgene therapies [42–47]. The design of ASC618 allows for 10- to 100-fold increased protein expression because of limited interaction with the endoplasmic reticulum and attenuated unfolded protein response. The ASC618 clinical program for patients with severe and moderately severe hemophilia A received Investigational New Drug clearance from the United States Food and Drug Administration and an interventional clinical trial is currently ongoing (ClinicalTrials.gov identifier: NCT04676048; Table 2) [48].

Several different mutations in the gene encoding factor VIII are associated with hemophilia A [49]. Depending on the mutation, patients may have different levels of naturally occurring factor VIII and may respond differently to gene therapy [13,50]. Therefore, the sequencing of a patient's genes is an important element of gene therapy. Identification of the specific mutation can help predict response to therapy [51].

CLINICAL DEVELOPMENT OF ALLOGENEIC CELL THERAPIES

Cell therapy can work through several mechanisms, such as delivering new cells to a patient to replace damaged or diseased cells or tissues [2] or provide an immunoregulatory functionality [52,53]. Several types of cells can be used for cell therapy, including stem cells and stromal cells. One of the most common cell therapies is the transplantation

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TABLE 2

Gene and cell therapy clinical trial designs for ASC618 [48] and ASC930 [64].

	Population	Selection biomarkers	Monitoring biomarkers
ASC618 gene therapy	Severe hemophilia A (FVIII activity ≤2 IU/dL)	▶ Inhibitory antibodies to FVIII protein ▶ Total and neutralizing antibodies to AAV8 ▶ Liver function tests, including imaging and liver enzymes ▶ *FVIII* gene mutations	*Monitored up to 52 weeks* **Safety** ▶ On-target liver AAV infectivity, excluding off-target in other organs and tissues ▶ Total and neutralizing antibodies to AAV8; Cellular immune response (ELISPOT) ▶ FVIII inhibitor levels **Efficacy** FVIII activity
ASC930 decidua stromal cells (DSC)	Steroid-refractory acute GVHD	▶ Immune profiling of circulating T cells and cytokines ▶ Tissue-resident immune cells in the gut and skin ▶ *In vitro* effect of steroids and ruxolitinib in DSC mixed lymphocyte response	*Monitored up to Day 56* **Safety** ▶ Multi-omics predictors of immune-related adverse events ▶ Immune profiling with mass cytometry **Efficacy** DSC phenotyping and functional tests: MAGIC biomarkers

AAV: Adeno-associated viral; DSC: Decidua stromal cells; FVIII: Factor VIII; GVHD: Graft-versus-host disease; MAGIC: Mount Sinai Acute GVHD International Consortium.

of hematopoietic stem cells, which is currently used to treat hematologic cancers and diseases and is showing promise in other conditions.

Following allogeneic hematopoietic stem cell transplantation, graft-versus-host disease (GVHD) may cause considerable morbidity and mortality [54,55]. Simply, the donor blood cells, in addition to targeting the neoplastic cells, mount an immune response against cells and tissues of the host. Acute GVHD usually appears within the first 3 months after allogeneic hematopoietic stem cell transplantation and primarily affects the skin, gastrointestinal tract, and liver with rash, secretory diarrhea, and abnormal cholestatic liver function. Chronic GVHD usually appears more than 3 months after allogeneic hematopoietic stem cell transplantation and can affect any organ system in the body through tissue-damaging inflammation and dysregulation of immune response [56]. Typically, GVHD treatment consists of steroids with or without calcineurin inhibitors, but only about half of patients respond to treatment. Many second-line therapies have been developed for steroid-refractory GVHD, with mesenchymal stromal cells (MSCs) and decidua stromal cells (DSCs) being used successfully [54].

MSCs are multipotent, non-hematopoietic stem cells that have the ability to differentiate into a variety of cell types [4,33]. MSCs are present in adult and fetal tissues, as well as adipose tissue, peripheral blood, dental pulp, the endometrium, amniotic fluid, fetal membranes, the placenta, the umbilical cord, and other tissues and secretions [57–59], and are often isolated from bone marrow [4]. MSCs have immunomodulatory and anti-inflammatory properties and have therapeutic potential across a range of diseases. They avoid immune response because they do not express human leukocyte antigen, and they secrete

immune mediators and interact with T-regulatory cells, natural killer cells, and T-helper cells [4]. Specifically, the immunosuppressive abilities of MSCs in GVHD are based on the secretion of indoleamine 2,3-dioxygenase, transforming growth factor β, and interleukin-10, among others. MSCs also stimulate and induce T-regulatory cell differentiation; inhibit T-helper 17 differentiation; inhibit B-cell activation, proliferation, and immunoglobulin secretion; inhibit T-cell and natural killer cell proliferation; inhibit interleukin-2 production; and induce T-cell apoptosis [60]. However, while MSC transplantation reduces the risk of chronic GVHD, it does not change the risks of relapse or mortality and only slightly reduces the risk of acute GVHD [4,60].

DSCs are derived from the placenta, which is composed of cells and tissues of fetal and maternal origin, and are isolated from one of its key components, the fetal membrane. They have been shown to be safe and efficacious treatments for several diseases in both *in vitro* and *in vivo* animal models. DSCs have several advantages over MSCs and other stromal cells, including decreased production of interferon gamma and interleukin-17, increased secretion of anti-inflammatory interleukin-10, and higher expression of integrins [52,53]. DSCs also suppress alloreactivity, increase expression of programmed cell death ligands 1 and 2 [61], and increase the frequency of regulatory T cells [51,60,61]. They exhibit contact-dependent suppression of allo-activated immune cells, produce indoleamine 2,3-dioxygenase, and do not upregulate human leukocyte antigen-II after interferon gamma stimulation. Furthermore, DSCs have more potent immunosuppressive properties *in vitro* and do not display any differentiation potential [54]. The lack of capacity for differentiation amplifies the immune-regulatory potential driven by a stable phenotype [62]. Together, these features make DSCs ideal candidates for treating acute GVHD and, potentially, other diseases involving a compromised immune response.

ASC930 is under development by ASC Therapeutics as an allogeneic off-the-shelf cell therapy using DSCs for the treatment of steroid-refractory acute GVHD after allogeneic hematopoietic stem cell transplantation. A Phase 1/2 clinical study of DSCs in acute GVHD reported a 100% response rate at 4 weeks among patients with steroid-refractory acute GVHD, and no major long- or short-term safety events were noted [5,6,63]. The safety and efficacy of ASC930 will be evaluated in a Phase 2b, open-label, multicenter study (ClinicalTrials.gov identifier: NCT04883918; Table 2) [64].

Biomarkers for patient selection & monitoring in stem cell therapies

As stem and stromal cell therapies continue to be developed, more robust biomarkers are needed. Specifically, biomarkers of disease progression and response to therapy must be defined and optimized to minimize the risk and maximize the potential benefit of DSC therapy for acute GVHD.

Cell therapy biomarkers

Infused DSCs can be radiolabeled to measure their presence in various organs over time. In a study of three patients with GVHD after allogeneic hematopoietic stem cell transplantation, DSCs were labeled with ^{111}indium and the distribution of the DSCs was tracked for 48 hours. Compared with MSCs, DSCs have a higher expression of integrins, which are important for homing to inflamed and damaged tissues. However, DSCs did not show increased homing to organs affected by GVHD, including the intestine, esophagus, or skin, in the first 48 hours after treatment; instead, the DSCs traveled to the lungs, then to the spleen and liver [54]. This method of assessing the effect of DSCs should be applied to larger populations and used as a basis for further clinical study.

Immune-response biomarkers

To assess the safety of DSC therapy accurately, the patient's immune response to therapy

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must be measured. Flow cytometry is used to measure characteristics of cell populations and can be used to create a profile of immune cells and detect immunological biomarkers. Specifically, immune response to DSC therapy can be measured with mass cytometry, a variation of flow cytometry that uses mass spectrometry. Flow cytometry simultaneously identifies and quantifies cellular systems and measures cells' functional attributes at the single-cell level [65]. Additionally, proteomics, multiomics, and single-cell 'omics' are increasingly important in understanding gene expression in individual cells [66–68], and these technologies could be applied to the safety assessment of DSC therapy. The ideal biomarker will be able to identify and validate immune-related parameters to predict response and guide decision-making; standardization of immune-response biomarkers is important as the field of cell therapy continues to grow.

Disease biomarkers

Disease response in GVHD can be measured using surrogate safety and efficacy endpoints. Two biomarkers of long-term outcomes can be measured from whole blood: suppressor of tumorigenicity-2 and regenerating islet-derived protein 3-α. Both proteins have been identified in high concentrations in the blood of patients with GVHD and are predictors of increased mortality. Both biomarkers are incorporated into the MAGIC (Mount Sinai Acute GVHD International Consortium) algorithm probability [55], which is a tool for assessing mortality after GVHD treatment. In the study of ASC930, whole blood will be collected at regular intervals throughout the study and follow-up period to predict mortality and resistance to treatment [69].

CONCLUSIONS

Cell and gene therapies are extraordinarily costly and complex, and efficacy and toxicity vary according to individual patient characteristics. Therefore, it is important to select the right patients for these treatments; this is even more important than with standard therapeutic approaches. Also, comprehensive monitoring of patients is required to address inter-individual variabilities, even more variabilities than are observed with standard therapies. For example, as described in this article, for hemophilia A gene therapy, a patient's hepatocytes are forced to become 'factories' for factor VIII, and individual responses to therapy vary on immunological, cellular, and functional levels, such as quantities of naturally occurring factor VIII and patient risk factors for toxicity. When a patient's cells are repurposed through the administration of a transgene, there is little room for error. This underscores the need for careful patient selection and accurate and timely assessments of response in terms of both therapeutic benefit and adverse or unintended consequences.

With the rapid growth of gene and cell therapies, biotechnology and pharmaceutical companies face a call to action: we must establish proper selection and monitoring protocols to provide patients with the safest and most effective therapeutic options for genetic diseases. Several biomarkers of disease, immune, cellular, and molecular responses to gene and cell therapies are available, but most require further study and validation before they are routinely applied in clinical practice. As they are assessed and validated, biomarkers will continue to improve the efficacy and decrease the toxicity of gene and cell therapies. Trials are ongoing to clarify the role and utility of existing and new biomarkers and the future of precision medicine applications is strong.

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