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Emerging technologies and regulatory agency guidance for CGTs

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Advances in cellular and gene therapies (CGTs) – referred to as advanced therapy medicinal products (ATMPs) in the EU – offer opportunities for treating and potentially curing diseases for which no other treatment options were previously available. This article examines three areas of these groundbreaking technologies: gene therapies and the search for safe and optimized vectors in genome editing; cellular therapies and their programmable synthetic gene circuitries; and organoids for tissue-engineered therapies and as possible personalized companion diagnostics. The article also addresses current guidance on ATMPs and CGTs from the European Medicines Agency (EMA) and US Food and Drug Administration (FDA), respectively, and the steps they have taken to deliver these new therapies to patients safely and speedily.

Keywords – CAR T cells, ethics, organoids, synthetic gene circuits, vector delivery systems

Introduction

As we enter the era of precision medicine, CGTs are at the center of biomedical developments and aspirations. There are currently more than 35 FDA-approved CGTs[1] and 18 EMA-approved ATMPs from a total of 25 approvals of which 7 were market authorization withdrawals or not renewed.[2]

The EMA uses specific terminology to define the CGTs under the umbrella term ATMPs, which incorporates the same three areas as CGTS: gene therapy medicinal products, somatic cell therapy medicinal products, and tissue-engineered therapies. This article is divided into three sections, each addressing specific emerging technologies in the three fields.

Gene therapies: The search for safe and optimized vectors

Gene therapies treat a genetic disease by introducing genetic material with specific cell-altering function into a patient.[3] The success of such therapies relies on having an efficacious means for transferring the proper genetic material into

the correct target tissue.[4] That breakthrough came in the 1990s when Kotin and colleagues noticed that the adeno-associated virus (AAV), previously discovered by Atchison and colleagues in 1965, could easily integrate into the long arm of the human chromosome 19 (19q13.3).[5-7] Since those findings, site-specific recombinant AAVs have become the most popular viral vector gene delivery system, with more than 300 Phase 1, 2, and 3 clinical trials reported and market approval of numerous AAV-based gene therapy drugs.[4,8]

Despite the success of AAV vectors, it has become clear that the first generation of vectors is not quite optimal and is associated with costly production, poor transduction efficiency in specific tissues, low organ specificity, preexisting humoral immunity to AAV capsids, and vector dose-dependent toxicity.[4,8] In fact, the relatively large vector doses that are needed to achieve clinical efficacy has been shown to provoke host immune responses that culminate in serious adverse events, with the known death of 10 patients.[8] Thus, there is an urgent need to develop new gene therapy vectors that are safer and more effective and human tropic.

Lentivirus and herpes simplex virus type 1 (HSV-1) have also been successfully used as vectors, and HSV-1 has shown to be valuable because of its ability to carry large amounts of DNA compared with AAVs.[9] In fact, HSV-1 vectors have transgene payload capacities exceeding 30 Kb (compared with 5 Kb of AAV vectors) and are nonintegrating and episomal, which means they are particularly suitable for in vivo direct gene transfer because they do not pose any insertional mutagenesis risk.[10] In 2023, both the EMA and FDA approved Vyjuvek, a unique topical HSV-1 vector-based gene therapy for treating wounds in patients aged six months or older who have dystrophic epidermolysis bullosa and who carry mutations in the collagen type VIIα1 chain gene.[11,12]

Vyjuvek, with its HSV-1 vector, fulfills a long-standing goal of off-the-shelf direct gene therapy that can be applied in a local outpatient setting and repeatedly dosed on demand.[10] Such novel treatment has far-reaching implications with the potential to transform the gene therapy field. It suggests that topical delivery to mucosal surfaces such as the oropharynx, esophagus, and eyes, or even treatments that are able to cross the blood-brain barrier, could be a possibility. There are still issues with the manufacturing of HSV-1 vectors that need to be addressed and improved (e.g., contamination with helper viruses and temporary expression in replicating cells),[9] but the ability to host large amounts of foreign DNA offers an opportunity to deliver large or multiple genes and provide a level of gene-expression control elements, such as cell-specific and inducible promoters, to the development of gene therapies.[13]

Nonviral vectors or delivery systems, such as lipid nanoparticles (LN) and nanoparticles made of other materials such as proteins or polymers, are emerging as safer for patients and cheaper alternatives for CGTs since they have

reduced risk of immunogenicity in comparison with viral vectors.[14] They can also circumvent the liver – the body scavenger of exogenous and endogenous macromolecules – to reach their target organs.[14] The most advanced of these nonviral vectors is RCT110. This engineered, double-stranded, linear, covalently closed-ended DNA construct is formulated in a cell-targeted LN delivery system transferred through a nebulizer. One manufacturer is currently recruiting participants for a Phase 1 trial with this nonviral vector for the treatment of primary ciliary dyskinesia, a rare genetic disorder that affects the structure and function of cilia, the microscopic hairlike structures that protrude on cell surfaces.[15] Development of these therapies is rapidly gaining momentum, particularly after the success of the COVID-19 mRNA vaccines, which were delivered through LNs.

The FDA's and EMA's regulatory view on gene therapies

The latest guidance from the FDA on human gene therapy products incorporating human genome editing addresses the delivery method component of genome editing.[16] Specifically, the agency emphasizes that, when determining the optimal delivery method into the cells, it is essential to consider the advantages and limitations of each potential method (e.g., the amount of nucleic acid the delivery vector can contain, the efficiency and specificity of targeted delivery, and genome-editing component persistence and stability).[16] Furthermore, for in vivo genome editing using vectors or nanoparticles, the FDA states that it is important to consider the ability of the delivery vector to target the cells or tissue of interest and to minimize distribution to nontargeted tissue. As such, consideration should also be given to the ability to control the expression of vector-delivered genome-editing components (e.g., tissue-specific promoters or small-molecule inhibitors). The guidance states that viral vectors may support the sustained expression of the gene-editing component transgenes, whereas nanoparticles may allow the temporal delivery of genome-editing components such as DNA, RNA, or proteins. However, the potential for vector-mediated toxicity, as well as preexisting immunity to the genome-editing components and vector, should always be considered from the FDA's regulatory point of view, and developers should select the appropriate delivery method based on the specific intended use and patient population.[16]

In March 2024, the EMA released an updated draft guideline on the quality, clinical, and nonclinical requirements for investigational ATMPs in clinical trials for better alignment with FDA requirements on certain ATMP-related concepts and terminologies.[17] The guideline advises a risk-based approach revised by the developer throughout the product lifecycle as new data become available. It states that vectors are considered a critical starting material and should be fully validated before the start of a clinical trial.[17] In addition, EMA scientists have summarized insights from 16 scientific advice procedures for nine genome-editing medicinal products (GEMPs) presented by developers between 2019 and 2022.[18] Specifically, carriers of genome-editing tools were considered by EMA as excipients; and if

they were considered novel, then guidance requirements for novel excipients was advised.[19] Product-and-process-related impurities should also be well characterized, because they could affect clinical safety by causing off-target effects, for example.

The EMA stresses that off-target toxicity is the most important safety issue for GEMPs. There is currently no gold standard for analytical tools to assess genome-editing errors, although it is crucial to minimize the risks using a risk-based approach throughout the product lifecycle.[17,18] Developers are asked to describe in detail the relevant methodology that led to the identification of the off-target site(s), and the approach used must provide information about the potential biological consequences and the associated safety concerns.[18] From a patient safety perspective, even a low frequency of off-target events merits consideration because they could have significant biological consequences, such as mutagenicity. The EMA advises that the use of the final product for identifying off-target toxicities as well as for pivotal nonclinical safety and toxicity studies is recommended, using the dose, mode, and schedule of administration as intended for the clinical studies to generate data that could inform its safe use in patients.[17,18] Furthermore, developers seeking to market their products in Europe should use tailored support from the EMA, such as innovation task force meetings; the priority medicines, or PRIME, scheme; and designation and scientific advice.[18]

Cellular therapies and their programmable synthetic gene circuitries

Viral vectors are crucial for ex vivo manipulation of autologous cellular therapies, such as chimeric antigen receptor (CAR) T cells – in which T cells from individual patients are removed and then synthetically engineered to recognize and kill specific cancer cells before being re-administered to the same patient.

CAR T cells have now revolutionized the treatment of various blood cancers, with six such therapies currently approved by the FDA and three by the EMA.[20,21] However, despite the enthusiasm around these therapies, fewer than half of the patients treated achieve long-term survival.[22] In addition, this is a costly treatment (e.g., a course of treatment with a recently approved CAR T-cell therapy costs more than $450,000).[20] Furthermore, the infusion of engineered CAR T cells into a patient can start a nonstop chain of immunologic reactions that kill the cancer target cell as expected; however, these reactions might continue in an uncontrolled way, resulting in a series of immunologic off-target toxicities that can lead to different symptoms of cytokine release syndrome, which affects a range of organs with severe outcomes.[23] Therefore, the drawbacks of such initial cell therapy designs are a concern for regulatory agencies, and the developers of such technologies are now trying to overcome them.

Cells use networks of interacting molecules to combine and process molecular signals into appropriate output responses – the basis of molecular biology.[24] As such, the ultimate aim of synthetic biology and CGTs is to manipulate this molecular biology process with precise, temporal, and context-specific control of therapeutic cellular activity. This process requires "platforms" that can drive robust functional changes in vivo and can be fit for clinical use. In 2022, Li and colleagues developed a compact synthetic transcriptional regulator platform based on human-derived proteins (synthetic zinc finger transcription regulators), on which gene switches and circuits were engineered to allow precise, user-defined control over therapeutically relevant genes in T cells.[24] The researchers used orthogonal, FDA-approved, small-molecule inducers to instruct T cells to sequentially activate multiple cellular programs, such as proliferation and antitumor activity, in order to drive synergistic therapeutic responses.[24]

The advantage of this platform system is that the desired molecular activities can be induced and titrated with different FDA-approved drugs, such as the antiviral protease-inhibiting drug grazoprevir, the breast cancer drug tamoxifen, or the plant hormone abscisic acid.[24,25] As such, the drug's "on" and "off" switches could be induced at specific time points by programming the zinc fingers (i.e., small DNA-binding motifs) to recognize the drug's chemical motifs in a time-dependent manner, where the proliferation of primary human T cells could be switched "on" before CAR expression is induced.[25] Furthermore, such switches can also be used to enhance CAR T-cell functions by regulating the expression of immunoregulatory cytokines, such as interleukin-2 (IL-2, an autocrine T-cell mitogen), and IL-12 (a promoter of T-cell activity typically produced by innate immune cells).[24] This raises the possibility of manipulating the local immune microenvironment so that such treatments can be used beyond blood malignancies and to treat solid tumors.

Indeed, overcoming the suppressive tumor microenvironment has been a significant barrier to treat solid tumors by CAR T cells, because the solid tumor immunosuppressive microenvironment blocks T-cell infiltration, activation, and proliferation. The inhibition of T-cell receptor signaling, together with the consumption of inflammatory cytokines, is a major tumor suppression mechanism that has hindered the use of such therapies in solid tumors.

Recombinant cytokines such as IL-2 can be administered to pass through the immunosuppressant environment in the treatment of solid malignant diseases.[25] But such systemic IL-2 treatment has proven prohibitively toxic, causing severe adverse effects, including capillary leak syndrome and end-organ dysfunction.[26] With this in mind, Allen and colleagues engineered T cells bearing a tumor-specific synthetic notch (synNotch) receptor that drives IL-2 production in situ (synNotch→IL-2).[26] The researchers observed that the engineered synNotch to IL-2 induction circuit drove a potent infiltration of the therapeutic to the tumor cells, without being toxic to the model.[26] These engineered

autocrine therapeutic cells established an effective foothold in the tumors, cooperatively enabling the initiation of CAR-mediated T-cell expansion and subsequent killing of cancer cells, but in a manner that evaded critical points of tumor immune suppression. These types of engineered delivery circuits might provide a potential general strategy to drive effective T-cell activities against immune-suppressed solid tumors.

The FDA's and EMA's regulatory view on synthetic gene circuitries of cellular therapies

The studies by Allen[26] and Li[24] and their respective colleagues represent significant steps for the development of advanced and individualized CAR T-cellular therapies, allowing, on the one hand, proliferation and activation that is spatially restricted to tumors, and on the other hand, the local expression of CAR stimulatory cytokines, or other adjuvants, that can be controlled in a titrated and time-dependent manner.[25] In fact, the latest guidance from the FDA on CAR T-cell products already addresses such possibilities.[27] Specifically, it states that "each additional functional element may affect CAR T cell safety and effectiveness [...]; as such sponsors should provide a justification and relevant data to support incorporation of additional elements [...], including an assessment of any impact that these additional elements will have on CAR T cell specificity, functionality, immunogenicity, or safety."[27] Furthermore, the guidance recommends that there should be a description in the clinical trial protocol of a detailed monitoring plan to determine the duration or persistence of the administered CAR T cells in trial subjects, including tests for the presence of CAR T cells, or the delivery vector, and for the activity of the CAR T cells, including gene expression or changes in biomarkers.[27] In case of a death occurring during the trial, the FDA requires that the sponsor should have a plan for postmortem studies to assess the cause of death and CAR T-cell persistence, toxicity, and activity. This statement in the guidance indicates that the FDA is already foreseeing that novel multidimensional toolkits of programmable synthetic gene circuitries in CAR T cells are applicable and that sponsors need to monitor CAR T-cell-derived activity closely in such patients when seeking clinical trial approval.[27]

In addition to the EMA's updated guideline on quality, clinical, and nonclinical requirements for investigational ATMPs in clinical trials,[17] the agency has also recognized that some ATMPs in Europe are being developed in academic and nonprofit settings. It has, therefore, created a specific pilot program of regulatory and scientific advice aimed at guiding noncommercial developers of promising ATMPs that address unmet medical needs as a way of facilitating the development of these products until marketing authorization.[28] The goal is to support translation of ATMPs into patient treatments through direct guidance on the best methods and study designs to generate robust information on how effective and safe a new ATMP is and whether it is eligible for the centralized authorization procedure. Throughout 2024, the EMA is looking to add another two developers to the pilot program, and initial results are expected to be published in 2025.

The FDA and EMA agree that such patients must be followed long term because of the increased risk of late onset of adverse reactions (e.g., tumorgenicity). For example, long-term monitoring of these patients can be done in a clinical trial or through enrollment in a disease registry. Sponsors should earmark sufficient funding to address long-term patient follow-up in any circumstance (e.g., acquisition).[19,27]

Organoids and 3D stem cell-derived tissue constructs

It is crucial when treating patients with CGTs or ATMPs to be able to predict patient responses to therapy before and during treatment. Patient-derived organoids of both normal and diseased tissue can serve as personalized companion diagnostics (CDx) for these predictions.[29] An organoid is a small, self-organized 3D tissue culture that mimics the key functional structural and biological complexity of an organ.[30] Compared with conventional 2D cellular cultures, organoids enable the recapitulation of in vivo tissue-like structures and functions, such as capillarization and complex cell composition. For example, Kim and colleagues have shown that, in the case of lung cancer in which there is substantial genetic and phenotypic heterogeneity across individuals, patient-derived lung cancer organoids could recapitulate the tissue architecture of the primary lung tumors and maintain the genomic alterations of the original tumors during long-term expansion in vitro.[31] Since patient-derived organoids are highly patient specific, with characteristics differing not just between patients but also between samples obtained from the same patient at different time points and sites, a personalized approach to tailor therapy to each individual improves the efficacy of the treatment and spares the patient ineffective therapies and associated toxicities.[31] Organoids can also be used for general drug screening or in vitro trials to reduce animal testing, model rare cancer heterogenicity, and test patient-specific drug response.

In addition, organoid cultures can also be leveraged for tissue-engineered therapy.[30] For example, in regenerative therapy, organoids can be a potential source of functional tissues for transplantation in human patients. Tohyama and colleagues, for example, have pioneered the production of clinical-grade cardiomyocytes from induced pluripotent stem cells in a spheroid form in patients with heart disease.[32] The researchers succeeded by optimizing the cell culture system, eliminating undifferentiated pluripotent stem cells, which can cause teratoma. By forming microtissue-like spheroids (HS-001), the viability of transplanted cells was improved in comparison with single-cell suspensions, and the ability to engraft and ameliorate the electromechanical function of the heart was achieved.[32,33] In March 2023, the first patient with severe heart failure was inoculated with HS-001 spheroids directly into the heart during a coronary bypass procedure in the LAPiS Phase 1/2 dose-escalation study.[34,35] Patient enrollment for this Phase 1/2 trial is ongoing, with the first results expected in 2025.

The FDA's and EMA's regulatory view on organoids and 3D stem cell-derived tissue constructs

The FDA and EMA have been clear in their support for organoid use in drug screening and evaluating the mode of action of cellular and gene therapy products. The FDA Modernization Act 2.0, approved in 2022, emphasizes the need to leverage organoids derived from human cells to reduce animal testing and improve patient clinical outcomes.[36] The EMA's updated guideline[17] and its strategic reflection for 2025 addresses leveraging organoids as nonclinical models for drug screening and evaluating the mode of action of all ATMPs.[37]

Using organoids as personalized CDx could be a strategy for the future, especially in personalized medicine. However, several challenges must be addressed regarding standardization of culture conditions, reproducibility, scaling for clinical application, and where organoids should be inserted in the regulatory framework of CDx, which now belongs to in vitro diagnostic medical devices. Currently, organoids are primarily research tools and are not yet classified as CDx in clinical settings, although the potential is there.

Besides the assessment of technical and safety-related issues, organoids are also a source of complex ethical questions. For example, clarification is needed on whether donors have property rights over them and whether they differ from cells and tissues with regard to their legal and/or moral status.[38] Moreover, the storage of organoids may create challenges for the governance of biobanks because such research may not allow for complete de-identification of data, which raises questions about informed consent, privacy, and return of results.[38] Furthermore, organoids disrupt the dualistic normative framework related to health and life-science research since it is not clear whether they should be categorized as a subject or an object. Three uncertainties must be overcome: the conceptual (ontological), the epistemological/methodological, and the regulatory.[39] The goal is to develop a conceptual and regulatory framework that will allow developers to overcome such dualism and address forms of uncertainty that cannot be evaluated through the use of statistical methods.[39]

Regulatory issues around 3D stem cell-derived tissue constructs (3D-SCTCs) are also more complex. In July 2020, the FDA released a guidance document on using human cells, tissues, and cellular and tissue-based products (HCT/Ps) to support the classification of tissue therapies.[40] According to the agency's definition, HCT/Ps contain or consist of human cells or tissues intended for implantation, transplantation, infusion, or transfer into a human recipient; but not organs, blood or blood products, body secretions, or animal tissue. Furthermore, homologous use means the tissue performs some basic function in the recipient or donor, but it may be implanted or transplanted to a different location in the body.[40,41] The FDA and institutional review boards can provide guidance on whether tissue has been minimally manipulated, although the

"many steps" necessary for cell purification and expansion of 3D-SCTCs is considered more than "minimal manipulation," which means that this guidance falls short on the proper regulation for such products.[41] In fact, 3D-SCTCs meet some of the criteria of a biological product, which means they might be subjected to additional regulations and requirements beyond those for HCT/Ps.

In the case of the EU, in July 2022 it released a draft proposal to significantly change its regulation of cells and tissues and repeal Directive 2004/23/EC, the cornerstone of such regulation for the past two decades. [41,42] Working in conjunction with Regulation (EC) 1394/2007 related to ATMPs,[43] the proposal will likely alter how 3D-SCTCs could be offered to patients. The updated guideline currently open for comment addresses some technical issues; however, it is broadly formulated rather than offering specific advice on such specific goods, and further guidance will be needed.[17,41]

Conclusion

The emerging technologies described in this article hold great promise. From the ability to host vectors with huge amounts of foreign DNA to deliver large or multiple genes in gene therapy; or to provide cell-specific inducible promoters in cancer therapy; or even the possible use of organoids as personalized CDx. These are some of the emerging technologies that might revolutinize personalized medicine soon.

Abbreviations

3D-SCTC, 3D stem cell-derived tissue construct; **AAV,** adeno-associated virus; **ATMP,** advanced therapy medicinal product; **CAR T,** chimeric antigen receptor T cell; **CGT,** cellular and gene therapy; **CDx,** companion diagnostics**; EMA,** European Medicines Agency; **FDA,** Food and Drug Administration [US]; **GEMP,** genome-editing medicinal product; **HCT/P,** human cells, tissues, and cellular and tissue-based product; **HSV-1,** herpes simplex virus type 1; **LN,** lipid nanoparticle; **PRIME,** priority medicines [EMA scheme]; **synNotch,** tumor-specific synthetic notch.

About the author

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