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Advanced therapy medicinal products development - from guidelines to medicines in the market

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ABSTRACT

In Europe, Advanced Therapy Medicinal Products (ATMPs) include medicines based on gene therapy, somatic-cell therapy, tissue-engineered products, and combined ATMPs. ATMPs constitute an emerging and innovative class of medicines used to treat multiple pathologies and are particularly relevant in pathologies where therapeutic options are limited and require high medical needs. These therapies act, among others, through the insertion of recombinant nucleic acids, including genes, to promote a therapeutic effect and through the restoration of cell functions, and repairing or replacing damaged cells and tissues impaired in pathological conditions. Despite their unique potential, these therapies face challenges related to scientific complexity, production processes, regulatory approval, and market access that hinder their development and availability.

Based on official European guidelines, the present review explores the current regulatory framework for the non-clinical and clinical development of advanced therapies. We aimed to discuss the regulations applied to the different types of ATMPs, as well as the challenges associated with their development until these therapies reach the market. Accordingly, topics such as the implementation of proof-of-concept studies to provide evidence supporting the potential clinical effect; biodistribution studies to evaluate tissue distribution and persistence; and toxicology studies to assess potential undesirable effects, integration potential of viral vectors, tumorigenicity, and germline transmission, are discussed. This work also covers some of the ATMPs available to patients on the EU market.

1. Introduction

In the European Union (EU), ATMPs are classified into three main groups, gene therapy medicines, somatic cell therapy medicines, and tissue engineered products. Additionally, some ATMPs may include one or more medical devices as part of the medicine, being classified as combined ATMPs (Iglesias-Lopez et al., 2019). The Committee for Advanced Therapies is the main committee responsible for providing scientific recommendations for the classification of ATMPs and for evaluating the quality, safety, and effectiveness of these medicines. This committee's main responsibility is to prepare an opinion on each application for an ATMP submitted to the European Medicines Agency (EMA) before the Committee for Medicinal Products for Human Use (CHMP) issues a final opinion on the granting, amendment, suspension, or revocation of marketing authorization (MA) for the medicinal product in question (CAT, 2022; EC., 2007; Farkas et al., 2017; Hanna et al., 2016a).

Gene Therapy Medicines (GTMs) are medicines that contain an

Abbreviations: ATMPs, Advanced Therapy Medicinal Products; EMA, European Medicines Agency; CHMP, Committee for Medicinal Products for Human Use; MA, Marketing Authorization; GTMs, Gene Therapy Medicines; SCTMs, Somatic Cell Therapy Medicines; TEMs, Tissue Engineered Medicines; CNTF, ciliary neurotrophic factor; LPL, lipoprotein lipase; AAVs, adeno-associated virus; ZFNs, Zinc finger nucleases; PEI, polyethyleneimine; PoC, proof-of-concept; NAAT, nucleic acid amplification tests; GM-CSF, granulocyte-macrophage colony-stimulating factor; CAR-T Cells, chimeric antigen receptor T-cells; TCR, Engineered T cell receptor therapy; HLA, human leukocyte antigen; iPSCs, induced pluripotent stem cells; GTMP, gene therapy medicinal product; CBMP, cell-based medicinal product; HD, Huntington's disease; PSCA, Prostate stem cell antigen; GMP, Good manufacturing practices.

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active substance that consists of a recombinant nucleic acid to be inserted into the body and used to regulate, repair, replace, add, or delete a genetic sequence. The vectors used in these medicines are designed to target specific cells/tissues and to ensure the safety of GTMs by eliminating genes associated with virulence, pathogenicity, immunotoxicity, or replication (EMA/CAT/80183/2014) (CAT, 2018). GTMs may have a prophylactic, diagnostic, or therapeutic effect, and this effect is closely related to the nucleic acid sequence they contain or to the product resulting from the genetic expression of the sequence (CD, 2009). GTMs might include viral and non-viral vectors to deliver the recombinant nucleic acid, such as genetically modified viruses, lipid- and polymer-based nanoparticles, and cells (Nóbrega et al., 2020a, 2020b).

Somatic Cell Therapy Medicines (SCTMs) are biological medicinal products that consist of or contain cells or tissues that have been subjected to substantial manipulation to alter their biological and physiological characteristics or structural properties relevant to the clinical situation in question, or cells or tissues which are not intended to be used for the same functions in the recipient and the donor (CD, 2009; EC., 2007; Luria and Schmidt, 2016).

Tissue Engineered Medicines (TEMs) contain or consist of modified cells or tissues and are intended to regenerate, repair, or replace human tissue. They may contain cells or tissues of human or animal origin, or both. They may contain additional substances such as cellular products, biomolecules, biomaterials, chemicals, supports, or matrices (Bonferoni et al., 2021; Luria and Schmidt, 2016).

SCTMs and TEMs may have similar components, such as cells and matrices. These medicines are differentiated from each other by the purpose for which they are used. SCTMs are used to prevent, treat, or diagnose a disease through a pharmacological, immunological, or metabolic action on cells or tissues. TEMs on the other hand, aim to regenerate, repair, or replace human tissue (EC., 2007).

A product that falls within both the SCTMs and TEMs definitions, under Article 2 of Regulation (EC) No. 1394/2007 is a TEM. However, a product that simultaneously fits within the definition of SCTMs, TEMs, and GTMs is considered a GTM (EC., 2007).

Combined ATMPs incorporate one or more medical devices, containing cells or tissues, capable of acting on the human body through a mode of action that can be considered primary to said devices. For a product that contains viable cells or tissues, the pharmacological, immunological, or metabolic action of these cells or tissues must be considered as the main mode of action of the product (EC., 2007; Luria and Schmidt, 2016).

Many ATMP-based treatments require a single administration and often lead to a radical improvement in the patient's health condition. Thus, although ATMPs are significantly more expensive medicines, by reducing absenteeism, preventing premature death, and decreasing the high costs of specialized health care, in the long term these products might result in a favorable economic impact on health systems (CAT, 2022; EC., 2007; Farkas et al., 2017; Hanna et al., 2016a).

The main objective of this work is to distinguish the different types of ATMPs, as well to review the main current regulatory framework for the non-clinical and clinical development of these medicines. Therefore, a search was carried out primarily on the EMA website to gather official guidelines and reports about the regulatory framework of these medicines. Additionally, PubMed database was also used. The key terms searched, on the websites cited, included terms describing advanced therapies (“advanced therapies”, “cell-based therapy”, “somatic cell therapy”, “CAR-T cells”, “gene therapy”, “oncolytic virus”, “tissue engineered medicines”, “combined ATMPs”), product development (“non-clinical development”, “proof-of-concept”, “clinical development”, “quality and safety standards”) and approved ATMPs.

2. Cell therapy and tissue engineered medicines

2.1. Non-clinical development of cell-based medicinal products

During the development of these medicines, non-clinical evaluation must allow the establishment of therapeutic dose, route of administration, cell biodistribution, toxicity, and potential side effects (EMEA/CHMP/410869/2006) (CHMP, 2008a; Detela and Lodge, 2019; McBlane et al., 2018). When possible, these studies should be carried out in two different animal models, ideally from different species (one rodent and one non-rodent) (EMA/CHMP/ICH/731268/1998) (ICH, 1998).

The safety issues of cell-based products are mainly related to the origin and manipulation of the cells. For example, potential malignant transformation must be monitored in the case of stem cells, which is justified by the ability of these cells to differentiate into different cell types and because they are often obtained from cell sources with cellular pluripotency. It is also necessary to surveil immunological rejection of transplanted cells in the case of xenogeneic and allogeneic cells, monitoring long-term rejection and evaluating possible inflammatory reactions (EMEA/149995/2008 rev.1) (CHMP, 2008a; McBlane et al., 2018).

2.2. Clinical development of cell-based medicinal products

In the case of ATMPs, for ethical reasons, most Phase I trials are carried out in a small number of patients. Often, Phase I/II studies are carried out, in which the safety and efficacy of the product are simultaneously assessed. Later, in Phase III, additional safety and efficacy data are collected to demonstrate that the ATMP under study has a beneficial therapeutic effect (Detela and Lodge, 2019; Hanna et al., 2016a; Pizevska et al., 2022). Biological effects can be influenced by the patient's immune system; therefore, clinical development must include the standardization and optimization of these products (EMEA/CHMP/410869/2006) (CHMP, 2008a).

Although the mechanism of action is often not completely known or is multifactorial, the main undesirable effects of the product under study must be known. To evaluate the pharmacodynamic activity of these medicines, the tests carried out will depend on the purpose of the therapy and various techniques can be used. Furthermore, when any cell-based product includes a non-cellular component, it must also be evaluated for its compatibility, degradation rate, and functionality (EMEA/CHMP/410869/2006) (CHMP, 2008a; El-Kadiry et al., 2021).

For the evaluation of the pharmacokinetic activity of cell-based products, conventional absorption, distribution, metabolism, and elimination (ADME) studies are not relevant, nor applicable. On the other hand, studies that monitor the viability, proliferation, differentiation, distribution, and functionality during the intended viability of the ATMP under study are particularly important (EMEA/CHMP/410869/2006) (Aijaz et al., 2019; CHMP, 2008a).

Dose determination should be based on potency and evidence obtained during ATMP development. The objective is to identify the lowest dose to obtain the therapeutic effect (minimum effective dose), or an optimal effective dose range defined as the dose range necessary to obtain the intended effect based on clinical results for efficacy and tolerability. Often, in these medicines, the dose is related to the individual characteristics of each patient and the patient's disease stage (EMEA/CHMP/410869/2006) (Bravery et al., 2013; CHMP, 2008a).

As cells can react to the new environment and change their phenotype, migration pattern, or other characteristics, it is possible to observe significant differences in the effectiveness and toxicity of a given ATMP, namely TEM, when tested on two different species, for example between rodents and humans. It is sometimes necessary to resort to structural/histological images to evaluate the organization of the implanted tissue/artificial organ, especially when part of the product is integrated and/or degradable (EMA/CAT/573420/2009) (EMA, 2014; Lee et al., 2010).

Depending on the intended therapeutic effect of TEMs,

Table 1
Examples of cell-based medicines approved by EMA.

Name	ATMP	Composition	Therapeutic indication	Authorization	Comment
ChondroCelect®	TEM	Viable autologous cartilage cells expanded *ex vivo* (EMA, 2012).	To repair damage to the cartilage in the knee, more specifically defects in the cartilage of the femoral condyle that are causing symptoms (EMA, 2012).	10/2009 (CAT, 2024a)	MA withdrawn in July 2016 (CAT, 2024a)
MACI® (*matrix-applied characterized autologous cultured chondrocytes)*	TEM	Patient's own cartilage cells on 14.5 cm^2 collagen membranes (EMA, 2013b).	To repair cartilage defects at the knee joint; to repair full-thickness defects with a surface area of between 3 and 20 cm^2 in adults experiencing pain and problems moving the knee (EMA, 2013b).	06/2013 (CAT, 2024a)	MA not renewed (MA ended in June 2018) (CAT, 2024a)
Holoclar®	TEM	Autologous cells from the limbus (at the edge of the cornea) expanded *ex vivo* (*EMA*, 2015).	Adult patients with moderate to severe limbal stem-cell deficiency due to burns (including chemical burns) (*EMA*, 2015).	02/2015 (CAT, 2024a)	_
Spherox®	TEM	Spherical aggregates (10–70 spheroids/cm^2) of *ex vivo* expanded human autologous chondrocytes and self-synthesized extracellular matrix (EMA, 2021a).	Symptomatic articular cartilage defects of the femoral condyle and the patella of the knee with defect sizes up to 10 cm^2 in adults and adolescents with closed epiphyseal growth plates in the affected joint (EMA, 2021a).	07/2017 (CAT, 2024a)	_
Alofisel®	SCTM	Expanded human allogeneic mesenchymal adult stem cells extracted from adipose tissue (EMA, 2018a).	Treatment of complex perianal fistula in adult patients with non-active/mildly active luminal Crohn's disease (EMA, 2018a).	03/2018 (CAT, 2024a)	_
Ebvallo®	SCTM	T cells obtained from a donor and then mixed with B cells infected with the Epstein-Barr virus, from the same donor, so that the T cells learn to recognize the infected B cells as foreign. These T cells are given to the patient and kill the patient's own infected B cells (EMA, 2022a).	Treatment of children from 2 years of age and adults diagnosed with blood cancer Epstein-Barr virus-positive post-transplant lymphoproliferative disease (EBV+ PTLD) after receiving an organ- or a bone marrow-transplantation (EMA, 2022a).	12/2022 (CAT, 2024a)	_

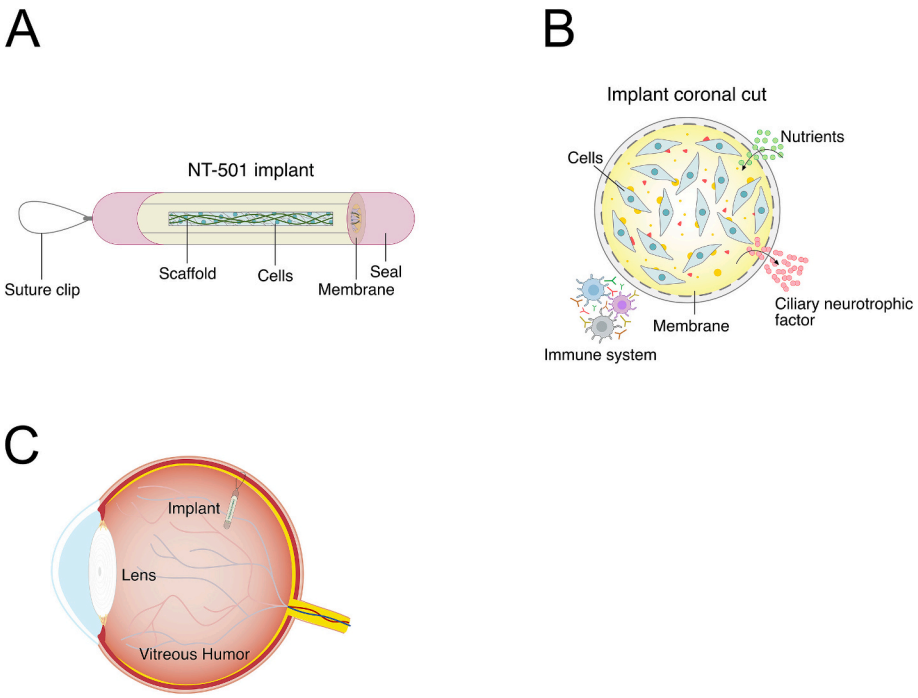

Fig. 1. Schematic illustration of the NT-501 implant. (**A**) The implant contains human retinal pigmented epithelial cells that produce ciliary neurotrophic factor (CNTF) released through a semipermeable membrane. The membrane capsule is closed at both ends, with one end having a suture clip for attachment to the sclera. (**B**) The membrane allows diffusion of oxygen and nutrients, as well as diffusion of CNTF out of the implant to the eye. (**C**) The implant lies outside the visual axis of the eye when anchored to the sclera.

pharmacokinetic studies should reflect the persistence and biodistribution of functional cells and/or other constituents of the product (EMA/CAT/573420/2009) (EMA, 2014; Khalil et al., 2020). The dose to be administered is defined considering the characteristics of the tissue injury to be regenerated, repaired, and/or replaced. However, the limitation of the number of cells/tissues available in TEMs can lead to the use of variable doses (EMA/CAT /573420/2009) (EMA, 2014).

In 2009 was approved ChondroCelect® (Table 1), the first TEM, a product based on autologous chondrocytes for implantation in the treatment of focal chondral defects of the knee (EMA, 2009; Joyce et al., 2023). Like ChondroCelect®, MACI® is a TEM based on autologous chondrocytes, indicated for the repair of focal chondral defects (EMA, 2013a; Joyce et al., 2023). These two medicines are no longer available on the market. Currently, examples of TEMs on the EU market are Holoclar® and Spherox®.

Combined ATMPs represent just 1 % of all ATMPs presently under

development in the EU. NT-501 is a successful example of combined AMTPs, consisting of encapsulated human retinal pigmented epithelial cells that have been genetically modified to release therapeutic doses of ciliary neurotrophic factor (CNTF) into the back of the eye. The cells are encapsulated in an intravitreal implant (medical device), which consists of a semipermeable external capsule, where cell growth occurs in a controlled manner, as well as the release of CNTF in effective doses (Fig. 1) (Emerich and Thanos, 2008; Ghasemi et al., 2017; López-Paniagua et al., 2021). In 2012, the EMA classified this implant as an orphan drug for the treatment of type 2 macular telangiectasia and in 2013 for the treatment of retinitis pigmentosa (López-Paniagua et al., 2021).

3. Gene therapy medicines

In 2012, EMA granted the first market authorization for a GTM – Glybera®. Glybera® was intended for patients with lipoprotein lipase (LPL) deficiency with multiple or severe pancreatitis. LPL deficiency is a rare autosomal recessive disease and is estimated to affect 1–2 people per million. The underlying genetic alteration of this disease is reflected in the insufficient production of LPL, the enzyme responsible for the hydrolysis of triglycerides (CHMP, 2012; EMA, 2021b).

Glybera® uses adeno-associated virus (AAVs) as a vector for delivering functional copies of the LPL gene into muscle cells, resulting in the production of the LPL enzyme with a single administration, thereby reducing the severity of the disease. Before the introduction of this GTM, the patients' treatment consisted of a reduction in lipid intake, which was very difficult to comply with, resulting in episodes of recurrent pancreatitis. In 2017, this GTM was withdrawn from the market due to commercial reasons (CHMP, 2012; EMA, 2017).

In February of 2024, another GTM was approved by the EMA, the Casgevy (exagamglogene autotemcel) which was approved for the treatment of β thalassemia and Sickle cell disease and contains, as its active substance, genetically modified stem cells (cells edited by CRISPR/Cas9 technology that can develop into blood cells) taken from the patient's own blood (CAT, 2024a; EMA, 2024a).

The vector systems more used in GTMs are viral vectors and DNA plasmids. DNA plasmids can be employed as naked DNA (in a simple saline solution) or complexed in a delivery system, such as lipid nanoparticles. Nonviral vectors and physical methods for the delivery of DNA plasmids are also available (EMA/CAT/80183/2014) (CAT, 2018; Nóbrega et al., 2020a). Over the last few years, there has been an expansion in the diversity of gene therapy tools allowing genome editing. These tools include, for example, the CRISPR/Cas system and Zinc finger nucleases (ZFNs). Each genome-editing nuclease has unique properties that must be taken into consideration at the time of selection, such as gene disruption and transcriptional/epigenetic regulation of gene expression (EMA/CAT/80183/2014) (CAT, 2018; Xu and Li, 2020).

In GTMs, the choice of vector will depend on several factors, namely the clinical indication, mechanism of action, route, and frequency of treatment administration. Furthermore, the selectivity and efficiency of transduction/transfection of the vector in target cells, and the expression and functional activity of the therapeutic sequence(s) must be considered (EMA/CAT/80183/2014) (CAT, 2018; Xu and Li, 2020).

When viral vectors are used, additional issues must be considered, namely, the vector pathogenicity and virulence both in humans and other animal species. Moreover, it may be necessary to eliminate characteristics related to the vector virulence by minimizing non-essential components. The vector tropism has also to be considered regarding the target cells/tissues, as well as specific tissue replication, germline transmission, persistence of viral sequences, and transduction efficiency. The potential vector integration into the host cell genome must also be evaluated, as well as the risk of insertional mutagenesis and oncogenesis (EMA/CAT/80183/2014) (CAT, 2018; Ghosh et al., 2020).

For the treatment of diseases requiring long-term gene expression, the use of integrating vectors is the more frequent option. Although integrating vectors have a higher probability of insertional mutagenesis, the vast majority of these are clinically silent (EMA/CAT/190186/2012) (EMA, 2013c). Nevertheless, if some safety problems are identified in non-clinical studies, it may be necessary to carry out integration studies in the clinical assays, to monitor patient safety and evaluate potential adverse effects (EMA/CAT/190186/2012) (EMA, 2013c).

During the development and production of GTMs, information on the control and stability of the vector and therapeutic sequence(s) must be provided. Replication systems must guarantee the integrity and homogeneity of the nucleic acids to be expressed in cells. It is also necessary to demonstrate that the correct sequence of nucleic acids is delivered and that this is maintained in a stable form, ensuring that the therapeutic sequence remains unchanged during the period in which the vector is designed to act. The cells used to produce the vector and/or genetic material must also be completely characterized for identification, origin, passage number, potential viral contaminants, and mycoplasma. Appropriate process validation studies must be carried out to demonstrate genetic stability during production (EMA/CAT/80183/2014) (CAT, 2018).

3.1. Non-clinical development of gene therapy medicines

The non-clinical studies to be carried out vary on the type of GTM and vector used, namely on whether plasmids, viral vectors, non-viral vectors, or genetically modified somatic cells are used (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b).

For **plasmids and naked DNA**, integration studies may be requested depending on the intended clinical use and the administration method used. Transmission and integration studies must also be carried out in the germline when plasmids are designed to have integration capacity (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b; Ryu et al., 2023).

In the case of **viral vectors** that are unable to replicate (incompetent replication), the possibility of replication after combination with a wild-type virus must be investigated. In the case of vectors with complete replicative capacity (replication-competent virus) or conditional replication (replicating only under specific conditions), the behavior of these vectors in different types of tissues and cells and the influence of possible concomitant medication must be evaluated. Integration, germline transmission, and carcinogenesis studies must be carried out if the vector has integration capacity. Furthermore, if the vector originated from a virus with latency capacity or if is designed to have this capacity, it must be investigated whether latency is restricted to specific tissues and whether the vector has reactivation capacity. If the viral vector triggers a significant immune response, may be difficult to re-administer it, and therefore the impact of the immune response must be assessed. The virulence of the original virus strain must be considered as well as the ability for recombination events to occur that could inadvertently restore virulence (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b; Nóbrega et al., 2020b).

There are several types of **non-viral vectors**, namely polymers, lipids, inorganic particles, and even combinations of these materials. This type of vector is increasingly attracting researchers due to its low cytotoxicity, immunogenicity, and mutagenesis. However, they present some challenges in terms of gene transfer efficiency, specificity, and duration of gene expression (Nyamay'Antu et al., 2019; Zu and Gao, 2021).

Although non-viral vectors represent a small percentage in gene therapy clinical trials, polymer polyethyleneimine (PEI) is increasingly used in oncological treatments and immunotherapy (Nyamay'Antu et al., 2019; Zu and Gao, 2021). Moreover, the two mRNA vaccines approved in several countries to prevent COVID-19, Comirnaty™ (Pfizer/BioNTech) and Spikevax™ (Moderna) (Al-Jighefee et al., 2021; Eroglu et al., 2021; Sheridan, 2021), use lipid nanoparticles as a vector to deliver mRNA encoding for the virus's spike protein, protecting the mRNA from enzymatic degradation and facilitating cell transfection and the delivery of the nucleic acid to the cells.

The *in vivo* use of naked DNA, genetically modified viruses, and viral or non-viral vectors may be associated with a risk of germline transmission. Therefore, non-clinical studies to evaluate germline transmission are mandatory for non-cellular gene therapy products at the marketing authorization stage (EMEA/273974/2005) (EMA, 2006).

Genetically modified somatic cells must be evaluated for their morphology, phenotype, function, and behavior (for example, unwanted differentiation, immortalization, or induction of an inappropriate phenotype). The possibility of cells releasing the vector or plasmid *in vivo*, intentionally or unintentionally, must be investigated, including the potential for interaction with infectious agents or medications. When cells with replicative potential are transduced with integrating vectors, the integration regions must be characterized in terms of the identity of adjacent genes and their functions, especially when activation of oncogenes and/or inactivation of tumor suppressor genes may occur. Studies must demonstrate the distribution, localization, and persistence of cells and gene products *in vivo*. The therapeutic effect of the transduced cells and/or genes must be demonstrated, as well as the limitation to the intended organ/tissue. In non-clinical studies, pharmacodynamic studies of proof-of-concept (PoC) must be carried out in appropriate animal models for the demonstration that the gene/nucleic acid reaches the intended target and exerts the intended function (level of expression and functional activity) (EMA/CAT/80183/2014) (CAT, 2018). Evidence must also be provided that the transduced cells do not provoke an unwanted immune response (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b).

Integration studies may be necessary even when the GTM is not expected to have integration capacity, to confirm the lack of integration. If integration occurs, the possible risks associated with integration must be determined (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b).

When nucleic acid amplification tests (NAAT) are used to quantify the number of copies of integrating vectors, the limits of detection and quantification should preferably be expressed in the number of copies of the vector/genome. For episomal vectors, it must be expressed as the number of copies/μg of host cell DNA analyzed. The specificity of these methods depends on the choice and design of primers and probes, as well as the reaction conditions and detection method. Detection of vector or transgene DNA within cells/tissues can also be performed through *in-situ* nucleic acid amplification and hybridization techniques (EMA/CAT/80183/2014) (CAT, 2018).

Safety studies should be considered on a case-by-case basis, depending on the route of administration and biodistribution, pre-existing knowledge of the vector class, and the product's mechanism of action (EMA/CAT/80183/2014) (CAT, 2018).

Biodistribution studies should provide data not only of target organs but also of organs where accumulation is likely to occur, for example, the liver and lung in intravenous administration. This data should include the duration of gene expression and activity and periods for which there is no signal detection (if applicable). The route of administration, treatment regimen (frequency and duration), and dosage should be representative of clinical use (EMA/CAT/80183/2014, EMEA/CHMP/GTWP/125459/2006, and EMA/CHMP/ICH/318372/2021) (CAT, 2018; CHMP, 2008b; ICH, 2023).

Time points and sampling frequency should be chosen to allow determination of the maximum level of administered GTM present at target and non-target sites, as well as clearance over time. The study should continue until there is no signal detected or until a long-term plateau phase is reached (EMA/CAT/80183/2014) (CAT, 2018).

A GTM may present toxicity due to the number of vector particles and the expression and/or integration of the genes delivered. Therefore, the proportion of infectious viral particles (with the capacity to express the gene) to the number of total viral particles must be determined (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b).

Toxicity assessment should allow the evaluation of the entire GTM construction (virus or vector particle and/or delivery system, expression vector including the cassette and gene/transgene), considering its intracellular positioning and the number of expression vectors/copies of the gene. Furthermore, these studies must also evaluate the gene to determine the consequences of its overexpression and/or immunogenicity or adverse effects (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b).

Depending on the type of GTM, the oncogenic and tumorigenic potential must be investigated in relevant *in vivo/in vitro* models, which allow the evaluation of signs of neoplasia, activation of oncogenes, and cell proliferation index (EMA/CAT/80183/2014) (CAT, 2018).

Pre-existing immunity to the transgenic product (product transcribed from the therapeutic sequence) must be characterized, as well as anti-vector immunity after multiple administrations of the viral vector (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b). In some cases, animal models may not be representative of the clinical situation and therefore may not provide relevant data for immunotoxicity studies. In these cases, eligibility criteria and immunogenicity studies must be carefully planned at a clinical level (EMEA/CHMP/GTWP/125459/2006) (CHMP, 2008b).

Excretion assays make it possible to assess the spread of the virus/vector through the patient's secretions. Normally, infectivity assays and PCR tests are carried out (EMEA/CHMP/ICH/449035/2009) (ICH, 2009a).

Concomitant administration of other drugs should be investigated on a case-by-case basis when they may affect transfection/transduction/infection, tropism, efficacy of the vector, expression of the therapeutic gene, biological activity of the expressed proteins, and tissue distribution of the vector. Furthermore, inflammation or cytokine release in the liver can affect the hepatic metabolism of pharmaceutical products administered simultaneously (EMA/CAT/80183/2014) (CAT, 2018).

3.2. Clinical development of gene therapy medicines

The pharmacokinetic studies carried out in the clinical phase will differ from case to case, depending on the specific characteristics of the GTM. However, some tests are required, for example, when possible, studies should be carried out to assess the persistence, clearance, and biodistribution of the gene therapy vector. Biodistribution studies must address the risk of germline transmission. Excretion studies and the risk of transmission to third parties must include an environmental risk assessment (EMA/CAT/80183/2014) (CAT, 2018). If the assay carried out to evaluate excretion does not allow distinguishing the intact virus/vector from the degraded or non-infectious one, then it is not possible to understand the risk associated with transmission. When the tests do not allow distinguishing the state of the virus/vector (intact, degraded, or non-infectious), it is assumed that the excreted material is infectious (EMEA/CHMP/ICH/449035/2009) (ICH, 2009a).

In pharmacokinetic studies, if possible, the minimum effective concentration and half-life of the therapeutic product should be determined. Furthermore, the correlation between levels and duration of expression and clinical efficacy/safety should be investigated. Dose selection should be based on the non-clinical development of the product and should be related to the potency. When it is not possible to determine the dose, the minimum effective dose and the maximum tolerable dose can provide information on the relationship between exposure and effect, and the proposed dose must be justified by scientific data (EMA/CAT/80183/ 2014) (CAT, 2018; Parra-Guillén et al., 2010).

Before starting a GTM-based therapy, the immune response to the therapeutic gene and pre-existing immunity to the vector must be assessed, as previous infections and vaccination may affect the safety and efficacy of GTMs and determine whether it is necessary to use immunosuppression (EMA /CAT/80183/2014) (CAT, 2018).

Studies must demonstrate efficacy in the target population, support the proposed dosage, and evaluate the duration of the therapeutic effect of the medicine. (EMA/CAT/80183/2014). The duration of follow-up should reflect the duration of the treatment, *i.e.*, the persistence and functionality of the therapeutic gene. In some cases, follow-up can only

Table 2
Examples of gene therapy medicines approved by EMA.

Name	Description	Therapeutic indication	Authorization	Comment
Glybera® (alipogene tiparvovec)	Contains a variant of the human *LPL* gene in a vector derived from adeno-associated virus serotype 1 (AAV1) (EMA, 2021b).	Lipoprotein lipase deficiency who has severe or multiple pancreatitis episodes despite maintaining a low-fat diet (EMA, 2021b).	10/2012 (CAT 2024a)	MA not renewed (MA ended in October 2017) (CAT, 2024a)
Imlygic® (talimogene laherparepvec)	Attenuated herpes simplex virus type-1 (HSV-1) obtained by functional deletion of 2 genes (ICP34.5 and ICP47) and insertion of the coding sequence for human granulocyte-macrophage colony-stimulating factor (GM-CSF) (EMA, 2016a).	Unresectable melanoma that is regionally or distantly metastatic (Stage IIIB, IIIC, and IVM1a) with no bone, brain, lung, or other visceral disease (EMA, 2016a).	12/2015 (CAT, 2024a)	_
Strimvelis®	Autologous $CD34^+$ cells transduced with a retroviral vector that encodes for the human adenosine deaminase (ADA) cDNA sequence from human hematopoietic stem/progenitor ($CD34^+$) cells (EMA, 2016b).	Adenosine deaminase deficiency (ADA-SCID) for whom a suitable matched family stem cell donor is not available (EMA, 2016b).	05/2016 (CAT, 2024a)	_
Luxturna® (voretigene neparvovec)	Adeno-associated viral vector serotype 2 (AAV2) encoding for the human retinal pigment epithelium 65 kDa protein (hRPE65) (EMA, 2018b).	Patients with vision loss due to inherited retinal dystrophy caused by confirmed biallelic RPE65 mutations and who have sufficient viable retinal cells (EMA, 2018b).	11/2018 (CAT, 2024a)	_
Zolgensma® (onasemnogene abeparvovec)	Non-replicating recombinant adeno-associated virus serotype 9 (AAV9) encoding for the human *SMN* gene (EMA, 2020).	Patients with 5q spinal muscular atrophy (SMA) with a bi-allelic mutation in the *SMN*1 gene and a clinical diagnosis of SMA Type 1, or with 5q SMA with a bi-allelic mutation in the *SMN1* gene and up to 3 copies of the *SMN2* gene (EMA, 2020).	05/2020 (CAT, 2024a)	_
Upstaza® (eladocagene exuparvovec)	Non-replicating recombinant AAV2-based vector containing the cDNA of the human *DDC* gene (EMA, 2022b).	Severe aromatic L-amino acid decarboxylase (AADC) deficiency with a genetically confirmed diagnosis, in patients aged ≥18 months (EMA, 2022b).	07/2022 (CAT, 2024a)	_
Hemgenix® (etranacogene dezaparvovec)	Recombinant AAV5 vector encoding a functional copy of the human coagulation Factor IX gene (EMA, 2023).	Severe and moderately severe Hemophilia B (congenital Factor IX deficiency) in adult patients without a history of Factor IX inhibitors (*e.g.*, auto-antibodies) (EMA, 2023).	02/2023 (CAT, 2024a)	_

Table 3
CAR-T cell therapies approved by EMA.

Name	Description	Therapeutic indication	Authorization
Yescarta® (axicabtagene ciloleucel)	Genetically modified autologous T cells transduced *ex vivo* with a retroviral vector expressing CAR composed of a mouse anti-CD19 single-chain variable fragment (scFv) linked to the CD28 costimulatory domain and the CD3-zeta signaling domain (EMA, 2018c, 2018d)	Adults with high-grade B-cell lymphoma, diffuse large B-cell lymphoma, primary mediastinal large B-cell lymphoma, or relapsed or refractory follicular lymphoma after two or more lines of systemic therapy (EMA, 2018c, 2018d)	08/2018 (CAT, 2024a)
Kymriah® (tisagenlecleucel)	Cellular immunotherapy containing autologous T cells, genetically modified *ex vivo* using a lentiviral vector encoding an anti-CD19 CAR (EMA, 2018e).	Patients ≤25 years old with B-cell acute lymphoblastic leukemia that is refractory, in relapse post-transplant, or in second or later relapse. Adults with relapsed or refractory diffuse large B-cell lymphoma and adult patients with relapsed or refractory follicular lymphoma, after two or more lines of systemic therapy (EMA, 2018e).	08/2018 (CAT, 2024a)
Tecartus® (brexucabtagene autoleucel)	Cellular immunotherapy containing autologous T cells, genetically modified *ex vivo* using a retroviral vector encoding an anti-CD19 CAR composed of an anti-CD19 scFv linked to the CD28 costimulatory domain and the CD3-zeta signaling domain (EMA, 2022c; Heelan, 2023).	Adults with mantle cell lymphoma (MCL) relapsed or refractory after two or more lines of systemic therapy including a Bruton's tyrosine kinase (BTK) inhibitor. Patients with ≥26 years old with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (EMA, 2022c; Heelan, 2023).	12/2020 (CAT, 2024a)
Abecma® (idecabtagene vicleucel)	Autologous T cells genetically modified with lentivirus encoding a CAR that recognizes the B cell maturation antigen (EMA, 2024b; Heelan, 2023)	Adults with relapsed or refractory multiple myeloma who have received at least 3 therapies, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 antibody, and demonstrated disease progression on the last therapy (EMA, 2024b; Heelan, 2023)	08/Heelan, 2023 (CAT, 2024a)
Breyanzi® (lisocabtagene maraleucel)	Autologous genetically modified CD19-targeted cells. $CD8^+$ and $CD4^+$ T cells transduced *ex vivo* with a replication-incompetent lentiviral vector that expresses an anti-CD19 CAR, which comprises a binding domain of an scFv derived from a murine monoclonal antibody specific for CD-19 and a portion of the 4-1BB co-stimulatory and signaling domains of the CD3 zeta chain and a non-functional truncated epidermal growth factor receptor (EGFRt) (EMA, 2022d; Heelan, 2023).	Adults with diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma (HGBCL), primary mediastinal large B-cell lymphoma (PMBCL), and follicular lymphoma grade 3B (FL3B), relapsed within 12 months from completion of, or are refractory to, first-line chemoimmunotherapy. Adults with relapsed or refractory DLBCL, PMBCL, and FL3B, after two or more lines of systemic therapy (EMA, 2022d; Heelan, 2023).	04/2022 (CAT, 2024a)
Carvykti™ (ciltacabtagene autoleucel)	Genetically modified autologous T cells transduced *ex vivo* with an incompetent lentiviral vector encoding an anti-B cell maturation antigen (BCMA) CAR, which includes two single-domain antibodies linked to a 4-1BB co-stimulatory domain and a CD3-zeta signaling domain (EMA, 2024c; Heelan, 2023).	Adults with relapsed or refractory multiple myeloma who have received at least one prior therapy, including an immunomodulatory agent, a proteasome inhibitor and presented disease progression on the last therapy and are refractory to lenalidomide (EMA, 2024c; Heelan, 2023).	05/2022 (CAT, 2024a)

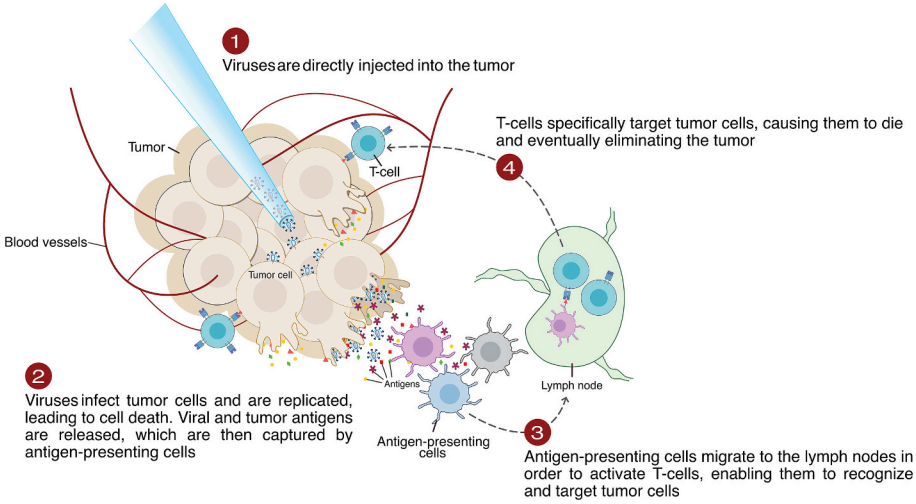

Fig. 2. Mechanism of action of Imlygic®. Imlygic® selectively infects and replicates in tumor cells and causes the cells to produce and release granulocyte-macrophage colony-stimulating factor GM-CSF. GM-CSF stimulates the patient's immune system to destroy the tumor cells.

be completed after marketing (*e.g.* when long-term therapeutic gene expression is expected) (EMA/CAT/80183/2014) (CAT, 2018).

Clinical safety must be assessed also considering the risks associated with the administration procedure (non-invasive *versus* invasive methods), use of local or general anesthesia, or the use of immunosuppressive therapy and chemotherapy. When medical devices are employed, their use must be evaluated and explained (EMA/CAT/80183/2014) (CAT, 2018).

In April 2024, there were 15 GTMs approved by the EMA, making it the class of ATMPs with more drugs approved (Table 2), including chimeric antigen receptor T-cells (CAR-T Cells) therapy represented by Kymriah®, Yescarta®, Tecartus®, Abecma®, Breyanzi®, and Carvykti™ (Table 3).

3.3. Oncolytic viruses-based GTMs

Oncolytic viruses applied to GTMs development are designed and genetically modified to selectively replicate in cancer cells destroying them without causing significant damage to normal tissues. Several modifications allow for achieving these objectives, such as mutation of viral genes essential for replication in normal cells, control of gene expression through tumor-specific promoters, change in cell tropism and/or cell entry process, and incorporation of transgenes into the viral genome (EMEA/CHMP/ICH/607698/2008) (Cristi et al., 2022; ICH, 2009b).

It is crucial to demonstrate the selectivity of the oncolytic virus for the tumor cells before use in clinical trials. Tests must be carried out to evaluate possible molecular variants of the virus, as these variants may present a replication selectivity or oncolytic profile different from the intended. Therefore, it is important to demonstrate the genetic stability of the product. Moreover, a potency test must determine the biological activity (EMEA/CHMP/ICH/607698/2008) (ICH, 2009b).

3.3.1. Non-clinical development of oncolytic viruses-based GTMs

In vitro studies must be carried out to evaluate the selectivity in normal and tumor cells, selective gene expression, cytotoxicity, and viral replication, before using animal models. Furthermore, PoC and the mechanism of action can be demonstrated in both *in vitro* and *in vivo* models (EMEA/CHMP/ICH/607698/2008) (ICH, 2009b).

Biodistribution studies to evaluate virus dissemination in target and non-target organs should be performed using NAAT assays. It is also important to understand the infectivity potential of the administered oncolytic virus and quantify the viral titers and/or levels of viral nucleic acids since the administered viruses are replication-competent and can therefore infect and replicate in normal tissues. The presence of viral genome sequences or transgene expression in non-target tissues at significant levels should prompt further analysis of the tissue (EMEA/CHMP/ICH/607698/2008) (ICH, 2009b).

The evaluation of viral shedding in animal models is important since one of the main concerns when using oncolytic viruses is the potential for exposure that can lead to human-to-human transmission (EMEA/CHMP/ICH/607698/2008) (ICH, 2009b; Onnockx et al., 2023).

3.3.2. Clinical development of oncolytic viruses-based GTMs

In the first stages of clinical trials, there might be questions for which there is still no answer due to the complexity of these medicines and the limitations of animal models. Due to the lack of information, it is necessary to exercise caution in initial dosages and routes of administration. Accordingly, studies often start with intratumoral injections, followed by local administration and then systemic administration (EMEA/CHMP/ICH/607698/2008) (ICH, 2009b).

Previous immunity to the virus may influence the route of administration, therapeutic regimen, and the need for successive administrations. Although immunity may confer potential safety against excessive viremia, it may also interfere with the virus-spreading ability (ICH, 2009b; Onnockx et al., 2023). Although the impact of transmission to third parties might not be well understood, caution should be taken to minimize exposure to other people including healthcare providers and family members, especially immunocompromised people. Generally, regulatory authorities require some form of barrier contraception for the duration of the clinical trial as a precaution to prevent person-to-person transmission. The potential for germline transmission should be considered (EMEA/CHMP/ICH/607698/2008) (ICH, 2009b).

In 2015, EMA approved Imlygic® (talimogene laherparepvec) the first (and only one, to date) oncolytic viruses-based medicine. Imlygic® is recommended for the treatment of adult patients with melanoma, which cannot be removed surgically and has spread to other areas of the body, without affecting the bones, brain, lungs, or other internal organs (CHMP, 2015; Ferrucci et al., 2021). Imlygic® is derived from the herpes simplex virus-1, which was genetically modified to infect and replicate in tumor cells and produce granulocyte-macrophage colony-stimulating factor (GM-CSF) (Fig. 2). GM-CSF stimulates the patient's immune system to recognize and destroy tumor cells. Each time a tumor cell dies, several copies of the virus are released into the bloodstream, allowing more tumor cells to be infected. Despite being able to enter healthy cells, Imlygic® cannot replicate in these cells or cause their death (CHMP, 2015; EMA, 2016a; Ferrucci et al., 2021).

Although there is only one approved medicine with oncolytic

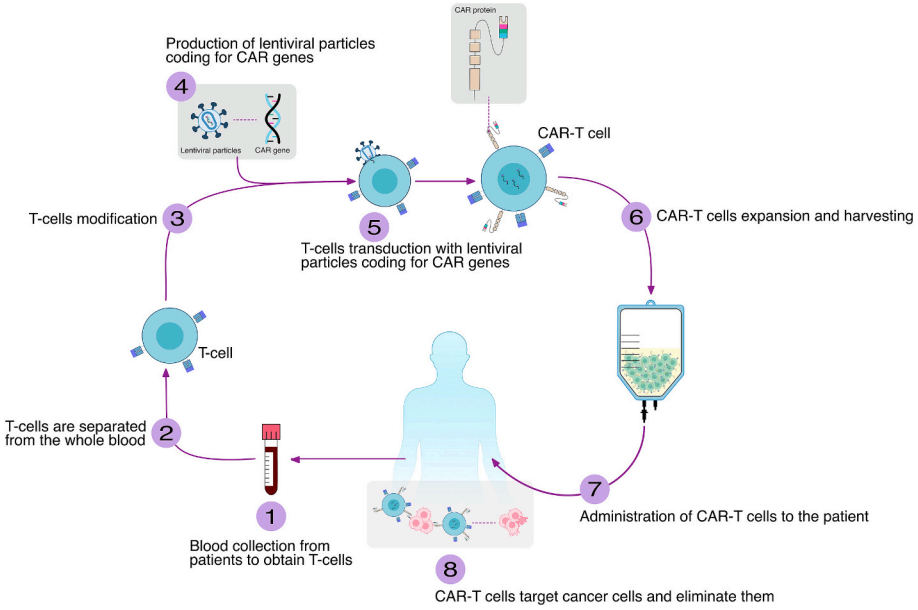

Fig. 3. Schematic illustration of CAR-T cell therapy. CAR-T cell therapy is based on the patient's T cells genetically modified to bind to specific antigens on tumor cells, consequently causing the death of the target tumor cells. 1) The first step is to collect blood from the patient to obtain the T cells; 2–5) the CAR gene is then inserted, and the CAR-T cells are produced in the laboratory; 6) cell expansion is then carried out, producing millions of CAR-T cells, 7) to be later reinjected into the patient intravenously; finally, 8) the CAR-T cells will bind to the antigens on the tumor cells promoting their elimination.

viruses, the non-clinical and clinical development of these medicines is growing. These medicines may be implemented in combination with other existing anticancer therapies (Yun et al., 2022).

3.4. Medicinal products containing genetically modified cells

These medicines include genetically modified cells for human use, regardless of whether the modification was carried out for therapeutic purposes or not. Genetic modifications can be obtained by various methods, namely through gene augmentation or genome editing tools using viral and non-viral vectors. Genetically modified cells can constitute a medicine on their own or can be combined with medical devices, and additional substances (*e.g.* matrices, biomaterials, biomolecules, and/or other components) (EMEA/CHMP/410869/2006) (EMA/CAT/GTWP/671639/2008 Rev.1 – corr. European Medicines Agency; 2020) (CHMP, 2008a; EMA, 2020b).

The production process of genetically modified cell-based medicinal products requires compliance with cell-based medicines and gene therapy guidelines. Like other ATMPs, production risks differ according to the type of product, nature, and characteristics of raw materials, and level of complexity of the production process (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

The genetic modification of cells constitutes a critical control point, as it is a step in the manufacturing process affected by several factors, such as the characteristics of the target cells (dividing or quiescent cells, cells growing adherent or in suspension, immortalized cell lines or primary cultures, number of passages of the cell line), characteristics of the cell culture system, type and amount of vector used to modify the cells, incubation time with the vector, and constituents of the cell culture medium (EMA/CAT/ GTWP/671639/2008 Rev. 1 – corr) (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b). Cell preparation and culture stages of the manufacturing and control production process must follow the described guidelines (EMEA/CHMP/410869/2006 and EMA/CAT/GTWP/671639/2008 Rev. 1 – cor) (CHMP, 2008a; EMA, 2020b).

One safety issue of cells genetically modified by viral vectors is the possibility of replication of the viral particles used to infect the cells, resulting in the production of new viral particles. Therefore, the absence of replication of competent viruses must be demonstrated throughout the production process of genetically modified cells (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

During the development of these genetically modified cell products, it may be necessary to make changes in the production process of the product itself or in the production of raw materials (*e.g.*, viral vector or cell source) that may affect the quality and safety of the final product. Therefore, all changes made during the product development must be identified and described. Comparability studies must be carried out to compare products before and after the changes in the production process and assess the impact of any differences in terms of product safety and efficacy (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

3.4.1. Non-clinical development of genetically modified cells

Ideally, non-clinical studies should be carried out with batches of genetically modified cells produced and controlled for quality following the production process guidelines for clinical studies. The genetic modification and the expected mode of action must be identified and whenever possible, dose selection and route of administration for clinical trials should be justified (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Regardless of the type of genetic modification, the expected effect must be confirmed at the cellular level. It may include studies that evaluate changes in the genome, endogenous gene expression, gene/transgene expression, or the activity of the inserted genes (EMA/CAT/GTWP/671639/2008 Rev. 1 – cor) (EMA, 2020b).

PoC studies must be provided to assist in the evaluation of the potential clinical effect and/or prove the anticipated mode of action. Although it may not be feasible to demonstrate the PoC of this type of medicinal product in animal models (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Pharmacokinetic studies must make it possible to evaluate the biodistribution, persistence, and stability of cells. Regarding toxicological parameters, attention must be paid to toxicity related to the expression of the therapeutic gene/transgene, risk of insertional mutagenesis related to the use of viral vectors, and mobilization and recombination of viral vectors (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

3.4.1.1. Genetically modified immune system cells (CAR-T cells, engineered T cell receptor cells (TCR), and NK T-cells). Engineered T cell receptor therapy (TCR) and CAR T-cell therapy use the activated T lymphocytes to treat cancer. In both medicines, the cells can target cancer cells through the additional receptors attached to their surfaces. The two medicines differ from one another by the type of antigens they can recognize. CAR T-cells bind to naturally occurring antigens on the surface of cancer cells (Fig. 3), while the added receptors in modified TCR bind to major histocompatibility complex (MHC) proteins. Thus, TCR uses the MHC to mark cancer cells with immune system-recognizable antigens. Finally, natural killer T (NKT) cells are a subset of lipid-reactive T cells that enhance anti-tumor immunity (Korell et al., 2022; Shah et al., 2021; Zhao et al., 2021).

In this type of medicine, it is necessary to assess on-target / off-tumor toxicity, which must include in-depth analyses of the expression of the target antigen in human organs, tissues, and cells. Moreover, the expression of the tumor-specific antigen in the target cells should be confirmed and human cells with and without expression of the target antigen should be tested *in vitro* for recognition by the CAR or TCR modified cells (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Whenever TCR presents any likelihood of cross-reactivity, *in silico* tests must be carried out to evaluate the cross-reactivity. When cross-reactivity cannot be ruled out, a risk assessment should be carried out based on the expression pattern of the protein corresponding to the potentially reactive peptide and the affinity of the TCR for the potentially reactive peptide. Screening must be carried out to assess potential cross-reactions of the TCR with other alleles of the human leukocyte antigen (HLA) (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

These medicines constitute a new innovative and personalized approach for cancer patients, as the patient's cells are genetically manipulated and subsequently re-injected into the body to eliminate tumor cells (Fig. 3) (EMA, 2018c). Therapies presently approved in the EU are Kymriah®, Yescarta®, Tecartus®, Abecma®, Breyanzi®, and Carvykti™ (CAT, 2024a) (Table 3).

3.4.1.2. Cell-based medicinal products derived from induced pluripotent stem cells (iPSCs). Several safety aspects must be considered, namely, the use of iPSCs as the cell source may present a risk of insertional mutagenesis (depending on the cell reprogramming protocol) and oncogenicity. The risk of tumor formation can be reduced through the inclusion of a suicide induction mechanism, such as the insertion of suicide genes in the iPSCs. The functionality of this safety mechanism must be confirmed *in vivo*. Additionally, cell reprogramming can induce epigenetic changes in cells, and the effects of these changes are not fully understood but must be addressed. Therefore, non-clinical *in vitro* and/or *in vivo* data should be generated to demonstrate the appropriate function of the cells to be used in humans. Toxicity assays should evaluate any undesired effects caused by abnormal behavior of the administered cells. A combination of quality characterization and safety data and literature data should provide an in-depth risk assessment to ensure the patients' safety (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Several cell-based medicinal products derived from iPSCs are presently being developed (Kim et al., 2022).

3.4.1.3. Cell-based products requiring gene editing. Products that contain cells modified by genetic editing, that is, using editing techniques such as CRISPR-Cas and ZFNs, follow the same guidelines as genetically modified cells. However, due to the editing tools used, they present some particularities, for example, when genome-editing nucleases are expressed in target cells, strategies must be used to minimize expression in non-target cells. This includes transient expression of the nuclease and an appropriate design of the DNA-binding domain (in the case of ZFNs) and the sgRNA (in the case of the CRISPR/Cas system) to increase the selectivity of the enzymes. When a transient expression is desired, the absence of enzyme activity must be demonstrated (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b; Haig et al., 2023).

Genome editing at the target site must be fully characterized to establish the extent to which the target site has been edited correctly, and risk assessment will also depend on the target cells. Therefore, the activity specificity of the modifying enzyme or sgRNA for the target genomic sequence needs to be confirmed *in vitro* through on-target assessment in cells (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

There are some problems associated with genome editing, namely mutations outside the target site and unwanted chromosomal translocations associated with DNA cleavages. Furthermore, the potential for immunogenicity must be evaluated because, as genome editing enzymes are derived from non-human proteins, there is a risk of reaction by the immune system and must therefore be evaluated (Blattner et al., 2020; Yamaguchi et al., 2020).

3.4.2. Clinical development of genetically modified cells

The design of the clinical trial must include the entire therapeutic procedure, from the moment of cell collection (*e.g.*, bone marrow aspiration) to any necessary concomitant medication (*e.g.*, immunosuppressants) to assess the benefit/risk (EMA/CAT /GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

The selection of the initial dose can be complex due to uncertainties of *in vivo* non-clinical studies since several factors such as differentiation, persistence, and immunogenicity can differ between species, and that can limit the predictive value of non-clinical studies. For the dose selection, the specific characteristics of the patient (*e.g.*, etiology of the disease, age, previous treatments) and of the product (*e.g.*, cell type and origin, transduction efficiencies, cell viability, potency, biological activity, and gene/transgene expression) must be considered. The correlation between exposure and effect must be assessed to establish a range between the effective dose and the recommended dose (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Although ATMPs are not covered by the guidelines on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (EMA/CAT/123573/2024 and EMEA/CHMP/SWP/28367/07 Rev. 1) the principles to mitigate risks are applicable (CAT, 2024; CHMP, 2017). One of the principles that apply to them is compliance with adequate waiting periods between the first administration to a patient and subsequent patients, to evaluate potential situations of acute toxicity and allow the implementation of study interruption rules or prevent the recruitment of new patients (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Pharmacokinetic studies for these products include biodistribution studies, cell persistence, and assessment of the therapeutic gene/transgene levels in target and non-target tissues. Furthermore, immunogenicity tests must be carried out, as immune responses against transgenic cells and/or products may occur and consequently compromise the efficacy and safety of the product. The risk of the product triggering an immune response is closely related to the origin of the cells (allogeneic *versus* autologous), nature of the disease, location of the transgenic product (intracellular *versus* extracellular /secreted), and pre-existing immune response against the transgenic product (EMEA/CHMP/410869/2006, EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (CHMP, 2008a; EMA, 2020b). Regarding the clinical safety of the product, it must be possible to detect relevant short- and medium-term adverse events that may be associated with the procedure for using and/or applying genetically modified cells.

When lifelong persistence of genetically modified cells is possible, special attention should be paid to late effects associated with the integrated vector and its expressed products (*e.g.*, oncogenesis, immunogenicity, or vector reactivation). As with other gene therapy products, patient follow-up should allow the detection of early or late adverse

Table 4
Regulatory requirements for non-clinical and clinical development of ATMPs.

Non-clinical studies
Perform a risk-based approach
Perform general safety and toxicity studies, including studies based on a risk-based approach. GTMP and Cell based-medicinal product (CBMP): Guideline on the risk-based approach (EMA/CAT/ CPWP/686637/2011). The extent of safety/toxicity studies should cover the duration of function and effect (CBMP) or gene expression and activity (GTMP) that is expected.
Carry out a proof-of-concept study / Pharmacology
Should generate **evidence supporting the potential clinical effect** or at least provide information on the related biological effect/molecular mechanism of action. Should be performed using relevant animal species and *in vitro* and/or *ex vivo* cell/tissue-based studies can also be used.
Should **support** route of **administration**, treatment **dose,** and **regimen intended in clinical use.**
Perform a potency assay study: The potency assay must relate to the mechanism of action and to the clinical outcomes. It should provide an accurate, reliable, and consistent demonstration of the biological activity of the product and must be able to detect sub-potent batches. The measure for potency should monitor the target or the outcome caused by the product. **GTMP:** The potency assay normally comprehends evaluation of the efficiency of gene transfer and the level of expression of the therapeutic sequence or its direct activity.
Pharmacokinetic studies should focus on the **biodistribution, persistence, and clearance** and should address the **risk of germline transmission**.
Biodistribution (BD): Evaluate tissue **distribution** and **persistence** (ICH S12 for GTMP). The route of administration and the treatment regimen (frequency and duration) should be representative of the clinical use. The sampling timepoints and frequency should allow the determination of the maximum level of administered GTMP present at target and non-target sites and GTMP clearance over time.
CBMP: evaluate viability, phenotype, and integration with surrounding tissue.
GTMP: evaluate **integration** to the host cell genome (*e.g.* in gonads) and risk of virus shedding into the environment.
Toxicology
Evaluate the potential **undesirable pharmacodynamic effects** of the ATMP (GTMP: consider both the vector and the transgene) on vital physiological functions (*e.g.*, central nervous system, cardiovascular system, respiratory system), and any other organ, based on the biodistribution of the product, in exposure in the therapeutic dose and above (ICH S7A). Established on a case-by-case basis.
Single dose: perform extended follow-up period (long-term effects). Repeated dose toxicity studies are to be used when multiple dosing is intended in clinics.
Integration potential of viral vectors (both intended and non-intended).
***In vivo* tumorigenicity** evaluated on a case-by-case basis. **CBMP:** analysis of proliferative capacity, dependence on exogenous stimuli, response to apoptosis stimuli, genomic stability, *etc.*
Germline transmission results of BD studies, types of target cells within the gonadal compartment. Investigate non-intended germline transmission in one specie before starting the first-in-human study.
Clinical studies
Investigate the feasibility of the administration route and pharmacokinetic characteristics such as biodistribution, persistence, and elimination
Standardize the administration procedure, also in case of surgical procedures.
Demonstrate the mechanism of action and off-target pharmacological effects
Use clinical endpoints relevant for the therapeutic indication and target population. Other study endpoints should provide mechanistic understanding and be translatable into an effect on clinical outcomes.
Investigate the effect on the disease and relevant pathophysiological systems
GTMP: investigate factors such as promotor downregulation, toxicity caused by transgene overexpression, and immune response towards transgene or vector; also investigate differences in transduction pattern within an organ and its influence in expression levels over time. **GTMP** and **CBMP**: also describe how the effect of concomitant medication can be distinguished from the ATMP effect.
Determine the starting dose of the first-in-human study and determine the dose regimen based on safety and efficacy data
Dose selection and dosing schedule should be based on the dose-response relationship data.
Investigate safety and tolerability
The duration of clinical follow-up observations should be sufficient to monitor risks that may arise from the characteristics of the product, the nature and extent of the exposure, and the manifestation of delayed adverse effects.

reactions, changes in the efficacy profile, or additional unknown risks. According to current guidelines, a 15-year follow-up is recommended (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

Pharmacokinetic studies of CAR-T cell therapies must characterize the cellular kinetics, namely their numbers, and their expansion and persistence in the blood and target tissues over relevant periods. CAR-T cells have a narrow therapeutic range and, generally after a certain dose, they cause acute toxicity due to their pharmacokinetic and pharmacodynamic properties. The underlying causes of adverse effects must be assessed, namely regarding the procedures related to the production of CAR-T cells (*e.g.*, apheresis procedure). Safety data must be obtained to identify expected and unexpected adverse effects based on non-clinical data obtained as well as clinical experience with other CAR-T cells, plan the duration of patient hospitalization when serious adverse effects are expected, define the algorithm to detect and treat life-threatening toxicity, and the duration of studies and patients' follow-up to detect late toxic effects (EMA/CAT/GTWP/671639/2008 Rev. 1 – corr) (EMA, 2020b).

4. Challenges in ATMPs development and market access

Hanna and colleagues reported that between 1999 and June 2015, 143 ATMPs clinical trials were withdrawn, terminated, or prematurely ended (Hanna et al., 2016b). In the past decade several ATMPs clinical trials were also suspended. For example, the Voyager Therapeutics' VY-HTT01 is a gene therapy experimental medicine designed to decrease the expression of the Huntingtin gene (HTT), whose protein exhibits a toxic gain of function in Huntington's disease (HD), and that the clinical trial was suspended. This therapy comprised of an adeno-associated virus capsid (AAV1) to deliver miRNA targeting HTT mRNA and promoting its levels reduction. During non-clinical phase, a widespread distribution of VY-HTT01 was demonstrated in non-human primates in striatum and cortex, relevant areas in the pathophysiological process of HD. Also, a durable reduction in *HTT* mRNA and HTT protein levels associated with phenotypic improvements were reported. Despite these promising results, in April, 2021, the clinical trial was suspended before it started due to chemistry, manufacturing, and controls issues (ClinicalTrials.gov, 2021; Ferguson et al., 2022) and the company decided to discontinue the VY-HTT01 program. Another example is the phase 1/2 clinical trial of BPX-601, which was suspended due to serious immune-mediated adverse effects observed in one patient. Bellicum Pharmaceuticals' BPX-601 CAR T cells are genetically engineered to express a CAR to target the prostate stem cell antigen (PSCA) and a rimiducid-inducible signaling domain which functions as a molecular "go-switch" to enhance activation and proliferation. The BPX-601 was used in patients with previously treated advanced prostate tumor expressing high levels of PSCA and meaningful efficacy was reported. Given the observed adverse effects, the clinical trial was halted, and the company concluded that it lacks the resources to optimize the dose and schedule of BPX-601 and rimiducid to improve the risk/benefit profile

(ClinicalTrials.gov, 2023; Stein et al., 2024).

It was also reported that market authorization success rates are substantially reduced for ATMPs compared with all medicine applications combined (59 % *vs*. 76 %) (Izeta and Cuende, 2023). Several factors contribute to this lower success rate of ATMPs. In a survey-based cohort study, the challenges indicated by ATMPs developers include complex manufacturing processes, the application of Good Manufacturing Practices (GMP) specifically for cell and gene products, complex clinical trial designs, and difficulties in complying with the regulatory framework (Ten Ham et al., 2018). Moreover, Carvalho and colleagues analyzed major objections reported in the market access authorization evaluation for GTMPs. It was indicated that clinical assessment is undoubtedly where regulators tend to find unacceptable issues, namely lack or insufficient efficacy demonstration, change or use of non-validated primary endpoints, limited safety databases and inadequately addressing immunogenicity concerns (Carvalho et al., 2019).

Accordingly, to help developers of ATMPs to navigate regulatory requirements during the non-clinical and clinical development, EMA also released EMA's guide on Advanced Therapy Medicinal Products (EMA, 2021c, 2021d, 2021e), and some points are briefly described in Table 4, including common regulatory requirements for non-clinical and clinical development of ATMPs.

5. Conclusion

Advanced Therapy Medicines have a high potential to cure diseases, by restoring the functions of affected tissues, namely through regeneration, repair, or replacement of affected cells. ATMPs aim to offer a lasting and transformative therapeutic response, often with just a single administration. However, due to the more complex nature of ATMPs, generally requiring starting materials of inherent variability (*e.g.*, cell-based products), complex biological features and manufacturing processes, some challenges prevent these medicines from reaching the market more quickly. So, the regulatory agencies are providing recommendations, guidelines, and scientific support on the required studies that ATMPs developers should carry out to guarantee the high-quality standards of ATMPs production and patients' safety.

Accordingly, this review described the current landscape of non-clinical and clinical development of advanced therapies in Europe. Additionally, some examples of ATMPs available on the EU market were described and discussed. Finally, challenges in ATMPs development and Market Access were briefly described, namely challenges pointed out by the developers and frequent objections reported in the Market Access Authorization evaluations.

In summary, ATMPs represent a class of products with the potential to cure disorders and the capability of altering the natural history of diseases that, until now, did not present effective therapeutic solutions, other than symptomatic and supportive. ATMPs continue to represent a challenge for those who develop these medicines and for regulatory authorities. Despite these challenges, there is a substantial increase in clinical trials of ATMPs and approved medicines in the EU market.

CRediT authorship contribution statement

C.F. and L.S.M conceptualized this review and drafted the manuscript. A.C. produced the Figs. A.C. and C.N. reviewed and corrected the draft. L.S.M. supervised the work. All authors read and approved the final version.

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Declaration of competing interest

The authors declare no conflict of interest.

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