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**Concise Review**

# Compliance in Non-Clinical Development of Cell-, Gene-, and Tissue-Based Medicines: Good Practice for Better Therapies

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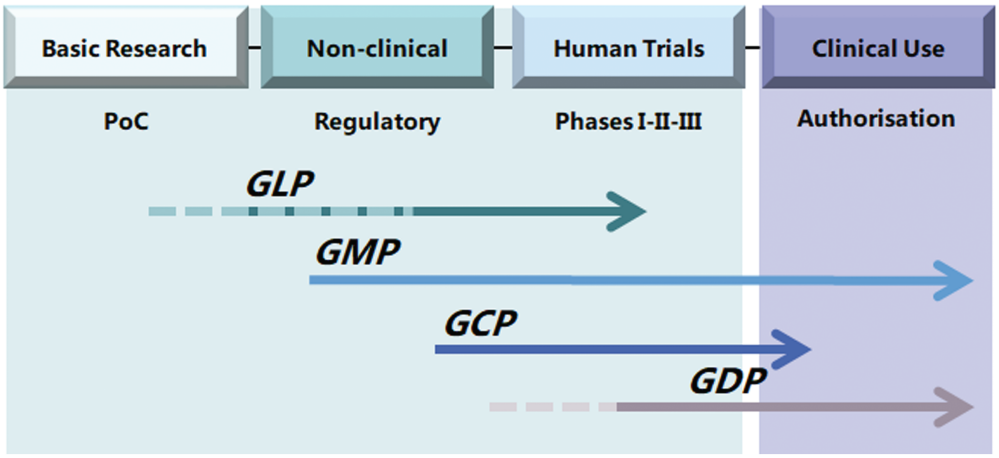
## Abstract

The development of cell-, gene- and tissue engineering (CGT)-based therapies must adhere to strict pharmaceutical quality management standards, as for any other biological or small-molecule drug. However, early developments often failed to fully comply with good laboratory practices (GLP) in non-clinical safety studies. Despite an upward trend of positive opinions in marketing authorization applications, evidence of adherence to the principles of GLP is not openly reported; therefore, their relative impact on the overall quality of the product development program is unknown. Herein we investigated the actual degree of GLP implementation and the underlying factors impeding full compliance in non-clinical developments of CGT-based marketed medicines in the EU and USA, including (i) the co-existence of diverse quality management systems of more strategic value for small organizations, particularly current Good Manufacturing Practices n(GMP); (ii) lack of regulatory pressure to pursue GLP certification; and (iii) the involvement of public institutions lacking a pharmaceutical mindset and resources. As a final reflection, we propose conformity to good research practice criteria not as a doctrinaire impediment to scientific work, but as a facilitator of efficient clinical translation of more effective and safer innovative therapies.

**Key words:** advanced therapy medicinal products; good laboratory practice; quality compliance; regulatory guidelines; product development; preclinical assessment; non-clinical safety.

## Graphical Abstract




## Quality Compliance in Non-Clinical Development

Despite the mandatory application of pharmaceutical quality management (QM) systems in non-clinical safety assessment program involving cell-, gene- and tissue engineering- (CGT-) based medicinal products (Box 1), it may surprise the reader that not all known developments actually meet this requirement. In fact, no metrics exist to monitor QM enforcement

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in the non-clinical setting and the consequent impact on the success of marketed drugs.

The principles of good laboratory practice (GLP) of the Organisation for Economic Co-operation and Development (OECD) provide guidelines for assuring the quality of the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, reported, and archived.[1] Although GLP rules were not originally established to guarantee scientific significance, they build a QM framework to ensure reliable and reproducible data with a regulatory purpose. However, the adaptation of GLP to the particularities of CGT-based therapies is challenging due to the living nature of the test items and the complexity of specific methodologies and test systems involved, which lack standardization in most cases. As an illustrative example, the first cell-based medicinal product that successfully completed the entire development track from research through clinical development to European regulatory approval, receiving Marketing Authorisation as Advanced Therapy Medicinal Product (ATMP) in October 2009 (ChondroCelect ; TIGenix NV), was also the first ATMP that failed to produce safety data in accordance to GLP. The European Public Assessment Report (EPAR) for ChondroCelect clearly states that non-clinical studies, consisting of combined pharmacodynamics (PD), pharmacokinetic (PK) (biodistribution), and toxicological (Tox) studies in the ectopic mouse (*nu/nu* model) and in orthotopic models in sheep and goats, "were non-GLP, which is not in conformity with pharmaceutical standards".[2] However, the Committee for Medicinal Products for Human Use (CHMP) accepted the deficiencies in quality compliance in view of the specificity of the development program for this particular product. In addition, human data were supported by adequate clinical studies and did not raise safety concerns. Although EPAR are extremely useful for extracting this type of information, it is also clear that developers of CGT-based products follow different strategies regarding the dissemination of their non-clinical data, therefore making it difficult to assess the level of compliance by the broad scientific community.[3] In contrast, other examples also exist from developers who have performed and published in the scientific literature their GLP-compliant studies of similar marketed CGT-based medicines, as it is the case of co.don chondrospheres.[4]

**Box 1. Good pharmaceutical practices (GxP) applicable to the development, production, distribution, and testing of advanced therapy medicinal products (ATMP).**

*Good laboratory practice* (GLP) focuses on the organizational process and the conditions under which non-clinical *safety studies* are planned, performed, monitored, recorded, reported, and archived. Compliance with GLP contributes to ensuring the credibility and traceability of the generated data, thereby addressing non-reproducibility issues in biopharmaceutical experiments.

*Good manufacturing practice* (GMP) describes the minimum standard that a manufacturer must meet in the *production* of clinical-grade medicinal products for human use throughout their entire life cycle. Compliance with GMP helps to minimize the risks involved in the production of pharmaceutical products that cannot be eliminated through testing of the final product.

*Good clinical practices* (GCP) is an international ethical and scientific quality standard for designing, recording, and reporting *clinical studies* that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and wellbeing of trial subjects are protected and that clinical-trial data are credible.

*Good distribution practices* (GDP) ensure that the quality and integrity of medicinal products are maintained throughout the *supply chain*. Compliance with the GDP requirements is recognized as a proof of a company having successfully established the relevant controls in the supply chain and it is, therefore, able to deliver products in accordance with the requirements of pharmaceutical manufacturers.

## Does Quality Compliance Impact Better Therapies?

The experience in hematopoietic stem cell transplantation (HSCT) with the quality management scheme promoted by the Foundation for the Accreditation of Cellular Therapy (FACT)-Joint Accreditation Committee of the ISCT-Europe & EBMT (JACIE) offers a clear example of the confirmed correlation between the occurrence of new center accreditation with FACT-JACIE standards and significant improvements in patient survival and reduction of procedural mortality, therefore demonstrating the clinical benefits of adoption of quality standards.[5] Consistently, centers in advanced phases of FACT-JACIE accreditation are linked to significantly higher survival rates, independent of other risk factors.[6] Similarly, adherence to good pharmaceutical practices (GxP) is a tool to ensure that products are fit for their intended use, establishing acceptable safety and efficacy profiles, and providing a framework to streamline the revision and approval processes. Indeed, the main goal of GxP compliance is to guarantee consistency, reproducibility, and traceability in all steps of the development, production, distribution, and clinical testing of ATMP and this should be considered independently on a case-by-case assessment by the competent Regulatory Authorities that may result in apparent inconsistencies (eg, ChondroCelect). Herein we investigated the actual degree of GLP implementation and the underlying factors impeding full compliance in non-clinical safety developments of CGT-based marketed medicines in the EU and USA.

From a regulatory perspective, GxP is the cornerstone for developers that research, produce, store, transport, or sell CGT-based medicines (Box 1). It is important to highlight that GxP is of mandatory application and span beyond GLP, including (i) Good Manufacturing Practice (GMP), which are required in the production of medicines for human use, from clinical testing throughout their entire life cycle; (ii) Good Clinical Practices (GCP), referring to the design, recording, and reporting of trials that involve the participation of human subjects; and (iii) Good Distribution Practices (GDP), ensuring that the quality and integrity of medicines is maintained throughout the supply chain (Fig. 1).

The laws and regulations are specific to each country and typically include a national or international authority, such as the Food and Drug Administration (FDA) in the USA, European Medicines Agency (EMA) in the European Union, or Swissmedic in Switzerland, all coordinated by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). In addition to official regulations, a number of organizations issue guidance documents supporting and detailing

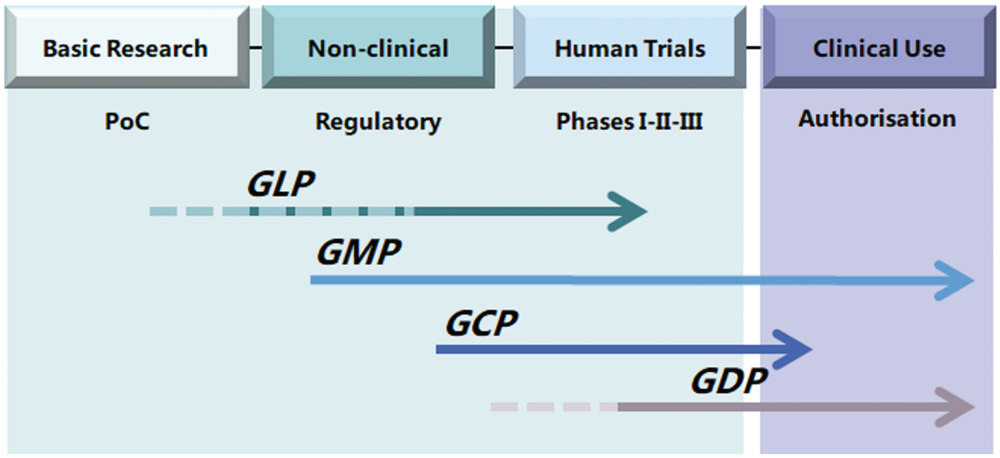


**Figure 1.** Roadmap for compliance with pharmaceutical quality management standards in the development of cell-, gene-, and tissue-based medicines in Europe. Cell-, gene- and tissue-based medicines (excluding vaccines) in Europe are regulated as advanced therapy medicinal products (ATMP) and their regulatory development has similarities to small-molecule drugs and biologicals, with the particularity of centralized authorization by the European Medicines Agency (EMA) and some considerations regarding the living nature of the drug substance (ie, conditional batch release) or non-industrial manufacture (ie, hospital exemption).[20-23] Research stages are shaded in green.
Abbreviations: GCP, good clinical practice; GDP, good distribution practice; GLP, good laboratory practice; GMP, good manufacturing practice; PoC, proof of concept.

the regulations and their application in specific situations. These include the International Society for Pharmaceutical Engineering (ISPE), the United States Pharmacopeia (USP), the Parental Drug Association (PDA), and the World Health Organization (WHO), amongst others. The regulatory framework is constantly evolving with new laws becoming effective and new guidance documents being published along with scientific and technical progress.

Due to the growing interest in CGT-based therapies, the number of manufacturing sites that comply with GMP for clinical-grade production is increasing along with productivity needs. Most of these studies fail in the early clinical phase (ie, first-in-man pilot studies, phase I/II trials), because of the involvement of academic laboratories and small biotech companies with very limited funding and immature QM structures. In this context, a priority is often given to GMP certification, to receive regulatory authorization for production and proceed with clinical testing, whereas it has been shown that substantiation of an adequate justification and risk assessment is sufficient to support non-GLP non-clinical safety studies. Indeed, it is recognized that it is not always feasible to conduct non-clinical safety studies in conformity with GLP due to the unique characteristics of CGT-based medicines. This provides flexibility to developers not holding GLP certification to perform studies following GLP guidelines, when these studies are carried out in the context of other QM frameworks (ie, ISO9001, ISO17025, GMP) or in the early stages of GLP implementation. Developers of authorized CGT-based products relied largely on non-GLP studies in their submissions, and the reasons provided by developers were accepted by the regulators. On another note, the application of GMP has facilitated the implementation of pharmaceutical standards beyond manufacturing procedures including non-clinical aspects of product development, particularly the characterization of the test item or including safety in vitro tests (ie, proto-oncogene expression, the occurrence of senescence, telomerase activity) in validations, otherwise replacing the expected GLP-compliant studies by GMP data in new product applications. Although it is difficult to associate solely GMP implementation with the success of novel developments, the upward trend of initial marketing authorization applications (MAA) observed in the CGT field may reflect some extent a change of regulatory and quality mindset both by developers and National Competent Authorities (NCA), which is confirmed by a reduction in the number of negative opinions and applicant withdrawals in Europe since 2013[7]. On this subject, novel initiatives for the development of CGT-based products at regional and national levels foster regulatory and quality compliance, as well as scale-up opportunities in the production of innovative medicines (eg, Cell and Gene Therapy Catapult, Andalusian Network for the design and translation of Advanced Therapies).[8,9] Remarkably, collaborative projects raising awareness of regulatory aspects in product development may help to overcome challenges in the implementation of GLP and difficulties to access funding to cover, amongst others, the costs of GLP studies (specifically for academic developers). One proposal in this regard is the EU-funded Coordination and Support Action (CSA) on the Strengthening Training of Academia in Regulatory Science (STARS; www.csa-stars.eu) with NCAs from 22 European countries participating and the EMA. The project aims to reach out to medicinal product innovators in academia, bridge the regulatory knowledge gap, and enhance the dialogue between academia and regulatory authorities.

The regulatory context has not evolved at the same pace as the rapid scientific progress in the CGT field and schemes like the Mutual Acceptance of Data (MAD), which offers the possibility of accepting data from non-clinical safety studies conducted in other OECD countries, are not sufficient incentives to facilitate to speed up developments toward the clinical arena. If data resulting from GLP studies are readily shared between countries this may also make these developments cost-effective and meet expectations to reduce the use of animals in experiments. As described previously, compliance with GMP for the characterization of test items may be appropriate in some cases, considering that identity, purity, and potency are critical quality attributes (CQA) of the CGT-based medicinal products to be considered in both

non-clinical and clinical assessment. This approach brings consistency to data generated along with the product development programme. However, specific safety assessment falls beyond the scope of GMP, which are focused on the routine manufacture and quality control of medicines. On the contrary, the focus of GLP is the study itself, focusing on its design, execution, reporting, and archiving, so it can be reconstructed in all detail at any time.

Challenges also exist for the definition of the scope of the studies carried out under GLP, given that the study types originally designated in the GLP guidelines do not fit current CGT-based developments and are often included under type ix ("others") where they do not match any of the following topics: (i) physical-chemical testing; (ii) toxicity studies; (iii) mutagenicity studies; (iv) the environmental toxicity studies on aquatic and terrestrial organisms; (v) studies on behavior in water, soil and air; bioaccumulation; (vi) studies to determine pesticide residues in food or animal feedstuffs; (vii) studies on effects on mesocosms and natural ecosystems; and (viii) analytical and clinical chemistry testing.

## Scientific Challenges for GLP Compliance

A proper balance of benefits and risks is key for marketing authorization of any new drug and failure of an insufficient safety assessment could entail terrible consequences for patients and, ultimately, may also result in a loss of confidence in this new class of therapies. Therefore, in vitro and in vivo studies conducted with CGT-based medicines to establish their safety should be done in compliance with GLP.[10] Altogether, the studies conforming to the non-clinical safety package are critical to determining whether the benefits of the investigational product outweigh its risks in the group of patients for whom the medicinal product is intended and therefore provide relevant information for decision-makers on whether to proceed with human testing. In particular, these types of studies are described in the International Conference on Harmonization (ICH) Safety ("S") guidelines, as (i) carcinogenicity, (ii) genotoxicity, (iii) toxicokinetics and pharmacokinetics, (iv) toxicity testing, (v) reproductive toxicology, (vi) biotechnological products, (vii) safety pharmacology studies, (viii) immunotoxicology, (ix) non-clinical evaluation for anticancer pharmaceuticals, and (x) photosafety evaluation. (https://www.ich.org/page/safety-guidelines). The many challenges encountered in the assessment of human cell products in xenogeneic test systems require careful consideration of all relevant information, which will be key to generating the overall risk profile of the CGT-based therapy product candidate. This is particularly relevant provided that xenotransplants of human cells, genes, and tissues may lead to misleading observations in animals. Indeed, the high species specificity of gene therapies makes it difficult the establishment of adequate animal models mimicking the tissue tropism, immune response, and cellular specificity expected in humans for Tox and PK/biodistribution studies.[3] For this reason, isolation of species-specific CGT-based medicines can be considered, although this approach entails the need to consider whether the results with one product can be applied to support the clinical use of a product from a different source.[11] In addition, the lack of strong primary pharmacological targets significantly complicates the design and robustness of the proof-of-concept animal studies.[12] Although tumorigenicity, biodistribution, and persistence to non-target locations are often cited as specific theoretical concerns for CGT-based therapies, and indeed they must be addressed, the routine goals of toxicology testing cannot be ignored, and the principles of non-clinical safety evaluation are the same as for all biopharmaceuticals.[10]

## Evidence of GLP Compliance in Marketed CGT-Based Products

Despite human CGT-based products being highly heterogeneous and governed by different regulations, a number of developments have successfully reached marketing authorization in a few countries.[13,14] Availability of information on non-clinical studies conducted in the development of any marketed drug is rarely reported in the scientific literature and most often found in public documents released by the regulatory authorities. Although this is a clear limitation to analyzing the actual degree of compliance with pharmaceutical quality standards in the non-clinical development of CGT-based medicines, information extracted from public documents provided us with an illustrative picture of the situation for marketed products in Europe and the USA (Tables 1 and 2, respectively). It is common to observe that safety studies typically performed on small-molecule drugs (eg, genotoxicity, carcinogenicity, toxicokinetics and pharmacokinetics, safety pharmacology, and immunogenicity) were not conducted in the non-clinical assessment of CGT candidates due to their nature and the characteristics of the patient population; this type of medicinal products are not used as a first line of treatment. Illustrative examples of gene therapy, somatic cell-based, and tissue engineering products listed in Tables 1 and 2 are presented and further discussed next.

### Alofisel

Alofisel (darvadstrocel, Takeda) is a somatic cell therapy product used as a second-line therapy for the treatment of complex perianal fistula in adult patients with non-/mildly-active luminal Crohn's disease, where fistulas are refractory to conventional or biologic agents, or in patients intolerant to conventional treatments. Alofisel contains the active substance darvadstrocel, which comprises adult allogeneic expanded Adipose-derived Stem Cells (eASC) from liposuctions.[15] Alofisel was designated an "orphan medicine" (a medicine used in rare diseases) in October 2009 because the number of patients with anal fistula is low. It was not approved for commercialization until March 2018. Several non-clinical studies supported its MAA, one of them was conducted in accordance with GLP (in vivo single-dose toxicity) whereas in vitro studies were non-GLP (assessment of the immunogenic potential of eASC, evaluation of crosstalk between Natural Killer cells and eASC, and T-cell recognition of eASC). This is an advance in GLP compliance given that the first marketed product (ChondroCelect ) failed to produce non-clinical safety data in adherence to pharmaceutical quality standards.

### Gintuit

Gintuit (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen; Organogenesis Inc.) is a Tissue Engineering Product (TEP) consisting of cellular sheets of

**Table 1.** List of CGT products approved by the EMA in Europe.

| Name (MA holder) | Indication | Type | Date of MA | No. studies | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Total | GLP | % GLP | In vitro | In vivo |
| Skysona (Bluebird bio B.V.) | Children (<18 y.o.) with early cerebral adrenoleukodystrophy (CALD) | GTP | 2021/07 | 6 | 1 | 17 | 4 | 2 |
| Libmeldy (Orchard Therapeutics B.V.) | Children with metachromatic leukodystrophy (MLD) | GTP | 2020/12 | 7 | 1 | 14 | 3 | 3 |
| Tecartus (Kite Pharma EU B.V.) | Adult patients with mantle cell lymphoma | GTP | 2020/12 | 7 | 0 | 0 | N/A | N/A |
| Zolgensma (Novartis Gene Therapies EU Ltd.) | Spinal muscular atrophy (SMA) | GTP | 2020/05 | 8 | 3 | 38 | N/A | N/A |
| Zynteglo (Bluebird bio B.V.) | β-Thalassemia | GTP | 2019/05 | 4 | 0 | 0 | 0 | 4 |
| Luxturna (Novartis Europharm Ltd.) | Adults and children with loss of vision due to inherited retinal dystrophy caused by mutations in the gene *RPE65* | GTP | 2018/11 | 2 | 0 | 0 | 0 | 2 |
| Kymriah (Novartis Europharm Ltd.) | Pediatric and young adult patients (<25 y.o.) with B-cell acute lymphoblastic leukemia (ALL) that is refractory, in relapse post-transplantation or in second or later relapse, and for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy | GTP | 2018/08 | 3 | 0 | 0 | 0 | 3 |
| Yescarta (Kite Pharma EU B.V.) | Adult patients with relapsed or refractory DLBCL and primary mediastinal large B-cell lymphoma (PMBCL), after two or more lines of systemic therapy | GTP | 2018/08 | 2 | 0 | 0 | 1 | 1 |
| Alofisel (Takeda Pharma A/S) | Complex perianal fistulas in patients with Crohn's disease | CTP | 2018/03 | 6 | 1 | 17 | 4 | 2 |
| Spherox (CO.DON AG) | Knee cartilage defects | TEP | 2017/07 | 3 | 2 | 67 | 0 | 3 |
| Strimvelis (GSK Trading Services Ltd.) | Severe combined immunodeficiency due to adenosine deaminase (ADA) deficiency | GTP | 2016/05 | 2 | 1 | 50 | 1 | 1 |
| Imlygic (Amgen Europe B.V.) | Unresectable melanoma | GTP | 2015/12 | 22 | 5 | 23 | 3 | 2 |
| Holoclar (Chiesi Farmaceutici S.p.A.) | Severe limbal stem cell deficiency | CTP | 2015/02 | 1 | 1 | 100 | 0 | 1 |
| Provenge* (Dendreon) | Metastatic prostate cancer | CTP | 2013/09 | 2 | 0 | 0 | 0 | 0 |
| MACI* (Aastrom Biosciences,Inc.) | Knee cartilage defects | TEP | 2013/06 | 14 | 3 | 21 | 3 | 11 |
| Glybera* (uniQure biopharmaBV) | Adult patients with familiar lipoprotein lipase deficiency | GTP | 2012/10 | N/A | 5 | N/A | N/A | 3 |
| Chondrocelect* (TIGenix) | Knee cartilage defects | CTP | 2009/10 | 0 | 0 | 0 | 0 | 3 |
| Abecma(Celgene Corp.) | Adult patients with relapsed or refractory multiple myeloma after 4 or more prior lines of therapy including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody | GTP | 2021/08 | 8 | 1 | 13 | 4 | 3 |

*Withdrawn products.
Abbreviations: CTP, cell therapy product; EMA, European medicines agency; GTP, gene therapy product; MACI, matrix-associated autologous chondrocyte implantation; TEP, tissue engineering product; N/A, information not available.

allogeneic cultured neonatal foreskin-derived keratinocytes and fibroblasts in bovine collagen for topical (non-submerged) application to a surgically created vascular wound bed in the treatment of mucogingival ulcers in adults. In vivo transplantation of Apligraf (an identical sister product of Gintuit , manufactured using the same process and approved as a medical device in 1998) onto full-thickness cutaneous wounds in nude mice resulted in graft integration with the host tissue and persistence of the human keratinocytes and fibroblasts for one year. Biocompatibility testing of Apligraf was conducted in conformance to ISO-10993 standards, given that it was approved prior to Gintuit for wound healing by the FDA's Center for Devices and Radiological Health, and not as a biologic, as it is the case of Gintuit. Some of the tests performed include general safety, cytotoxicity, sensitization, intracutaneous reactivity/irritation, systemic Tox (acute and subacute), subchronic Tox, and hemocompatibility. Subcutaneous administration of Apligraf into rabbits resulted in implantation site reactions, likely due to the xenogeneic immune response. In contrast, the toxicology study designs proposed in the ICH Safety ("S") guidelines (consisting of PK, acute and chronic Tox, genotoxicity, carcinogenicity,

**Table 2.** List of cell-, gene-, and tissue engineering-based products approved by the FDA in the USA.

| Name (MA holder) | Indication | Type | Date of MA | No. studies | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Total | GLP | % GLP | In vitro | In vivo |
| Provenge (Dendreon Corporation) | Asymptomatic or minimally symptomatic metastatic castrate-resistant (hormone-refractory) prostate cancer | CTP | 2010/04 | 6 | 0 | 0 | 2 | 4 |
| Laviv (Fibrocell Technologies Inc.) | Moderate-to-severe nasolabial fold wrinkles in adults | CTP | 2011/06 | 0 | 0 | 0 | 0 | 0 |
| Gintuit (Organogenesis Inc.) | Mucogingival conditions in adults | TEP | 2011/11 | 30* | 4 | 13 | 17 | 12 |
| Hemacord (New York Blood Center Inc.) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | CTP | 2012/03 | 0 | 0 | 0 | 0 | 0 |
| HPC, cord blood (Clinimmune Labs, University of Colorado Cord Blood Bank) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2012/05 | 0 | 0 | 0 | 0 | 0 |
| Ducord (Duke University School of Medicine) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2012/10 | 0 | 0 | 0 | 0 | 0 |
| Allocord (SSM Cardinal Glennon Children's Medical Center) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2013/05 | 0 | 0 | 0 | 0 | 0 |
| HPC, Cord Blood (LifeSouth Community Blood Centers Inc.) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2013/06 | 0 | 0 | 0 | 0 | 0 |
| HPC, Cord Blood (Bloodworks) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2015/10 | 0 | 0 | 0 | 0 | 0 |
| Clevecord (Cleveland Cord Blood Center) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2016/01 | 0 | 0 | 0 | 0 | 0 |
| HPC, Cord Blood (MDAnderson Cord Blood Bank) | Disorders affecting the hematopoietic system that are inherited, acquired or result from myeloablative treatment | | 2016/09 | 0 | 0 | 0 | 0 | 0 |
| Imlygic (Amgen Europe B.V.) | Local treatment of unresectable cutaneous, subcutaneous and nodal lesions in patients with melanoma recurrent after initial surgery | GTP | 2016/12 | 52 | 8 | 15 | 8 | 44 |
| MACI (Vericel Corporation) | Repair of single or multiple symptomatic, full-thickness cartilage defects of the knee with or without bone involvement in adults | TEP | 2017/08 | 18 | 0 | 0 | 12 | 6 |
| Kymriah (Novartis Europharm Limited) | Pediatric and young adult patients up to 25 y of age with B-cell ALL that is refractory, in relapse post-transplantation or in second or later relapse, and for the treatment of adult patients with relapsed or refractory DLBCL after two or more lines of systemic therapy | GTP | 2017/10 | 4 | 0 | 0 | 2 | 2 |
| Yescarta (Kite -Pharma EU B.V.) | Adult patients with relapsed or refractory DLBCL and PMBCL, after two or more lines of systemic therapy | GTP | 2017/12 | 4 | 0 | 0 | 3 | 1 |
| Luxturna (Spark Therapeutics Inc.) | Biallelic RPE65 mutation-associated retinal dystrophy | GTP | 2018/06 | 19 | 2 | 11 | 8 | 11 |
| Zongelsma (AveXis Inc.) | Patients in pediatric care less than 2 years of age with spinal muscular atrophy (SMA) with bi-allelic mutations in the survival motor neuron 1 (SMN1) gene. | GTP | 2019/05 | 7 | 2 | 29 | 3 | 4 |
| Tecartus (Kite Pharma Inc.) | Adult patients with relapsed/refractory mantle cell lymphoma (r/r MCL) | GTP | 2020/07 | 7 | 0 | 0 | 4 | 1 |

**Table 2.** Continued

| Name (MA holder) | Indication | Type | Date of MA | No. studies | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Total | GLP | % GLP | In vitro | In vivo |
| Breyanzi (Juno Therapeutics Inc) | Adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified (including DLBCL arising from indolent lymphoma), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B | GTP | 2021/02 | 31 | 2 | 6 | 22 | 6 |
| Abecma (Celgene Corporation) | Adult patients with relapsed or refractory multiple myeloma after 4 or more prior lines of therapy including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody | GTP | 2021/03 | 12 | 1 | 8 | 6 | 6 |
| Stratagraft (Mallinckrodt Pharmaceuticals Plc.) | Adults with thermal burns containing intact dermal elements for which surgical intervention is clinically indicated (deep partial-thickness burns) | TEP | 2021/06 | 18 | 0 | 0 | 14 | 4 |
| Rethymic | Immune reconstitution in pediatric patients with congenital athymia | TEP | 2021/10 | 0** | 0 | 0 | 0 | 0 |

*No specific non-clinical safety studies were performed on Gintuit, provided that extensive preclinical and clinical data existed for Apligraf (an identical sister product of Gintuit, manufactured using the same process and approved as a medical device in 1998).
**Based on the acceptable clinical safety profile of Rethymic and the lack of relevant animal species/models, no non-clinical toxicology studies were performed. In vitro or in vivo genotoxicity, carcinogenicity, or developmental and reproductive toxicity studies were not conducted with Rethymic, nor were they indicated.
Abbreviations: CTP, cell therapy product; FDA, food and drug administration; GTP, gene therapy product; HPC, hematopoietic progenitor cells; MACI, matrix-associated autologous chondrocyte implantation; MA, marketing authorization; TEP, tissue engineering product; N/A, information not available.

reproductive and developmental toxicity, safety pharmacology, and immunotoxicity) were not conducted due to the nature of Gintuit and the extensive clinical experience with Apligraf (approximately 450 000 individual treatments).[16] 'This is a clear example of noncompliance with GLP, but where convincing retrospective data obtained from nonclinical in vivo pharmacology and biocompatibility studies (nude mice and rabbits) were deemed acceptable.

## Imlygic

Imlygic (talimogene laherparepvec; Amgen Inc.) is an oncolytic viral therapy indicated for the local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after the initial surgery.[17] Imlygic is an attenuated herpes simplex virus type-1 (HSV-1) produced in Vero cells by recombinant DNA technology deleting *ICP34.5* and *ICP47* genes and insertion of the coding sequence for human granulocyte-macrophage colony-stimulating factor (GM-CSF). From the information available on the FDA website, we identified a total of 52 studies (8 in vitro, 44 in vivo), consisting of 4 studies assessing PK, 15 studies on Tox, and 33 studies evaluating pharmacology, including the intratumoral injection of the murine homologous version (OncoVEXmouseGM-CSF) into syngeneic tumor-bearing mice and evaluation of Imligic on embryo-fetal development after repeat intravenous administration during organogenesis in immunocompetent pregnant mice at dose levels up to $4 \times 10^8$ plaque-forming units (PFU)/kg. Please note that reproductive and developmental toxicity studies, although listed in the ICH Safety ("S") guidelines, (https://www.ich.org/page/safety-guidelines), are not commonly conducted in the non-clinical assessment of CGT candidates due to their nature and the characteristics of the patient population. Regarding the information released by the EMA, we identified a total of 22 studies (3 in vitro, 2 in vivo), evaluating PD, PK, and Tox.

## Abecma

Abecma (idecabtagene vicleucel, ide-cel, bb2121; Celgene Corporation) is an autologous genetically modified T-cell immunotherapy for adult patients with relapsed or refractory multiple myeloma after 4 or more prior lines of therapy including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody. Abecma consists of T-cells transduced with an anti-B-Cell Maturation Antigen (BCMA) chimeric antigen receptor (CAR) lentiviral vector (LVV) and was approved by the FDA and EMA on 26 March and 18 August 2021, respectively. BCMA is a transmembrane protein selectively expressed in both normal and malignant plasma cells and blasts. The binding of Abecma to BCMA-expressing target cells results in CAR-T-cell proliferation, cytokine secretion, and subsequent cytolytic killing of BCMA-expressing cells. From the information available on the FDA website, we have identified a total of 12 studies (6 in vitro, 6 in vivo), consisting of 1 study on pharmacokinetics, 2 on toxicology, and 9 pharmacological studies (4 in vitro and 5 in vivo), including only one GLP-compliant study on tissue cross-reactivity study of goat polyclonal anti-human BCMA antibody of normal human tissues. Interestingly, data available from the EPAR related to the EMA approval indicates a

total of only 7 studies, including the GLP-compliant study described previously.

## Challenges Ahead

Although the growing number of clinical trials in the CGT field is to some extent predictive of an increase in marketing authorization applications in the coming years, most of these studies are early phase, underpowered, underfinanced trials, often testing poorly characterized "me-too" CGT-based medicines (eg, 55 registered trials in ClinicalTrials.gov using mesenchymal stromal cells (MSC) for the management of Graft vs Host Disease or 458 registered trials in ClinicalTrials.gov using virus-specific T cells for adoptive cell-based immunotherapy). Further to the use of MSC, the availability of published clinical data using cells from different sources, administered through different routes and tested in a variety of pathologies greatly facilitates writing a literature-based non-clinical chapter of the IMPD rather than conducting GLP studies de novo. However, this situation provides only general information on product safety since manufacturing procedures and attributes of the resulting MSC-based products may differ between laboratories and potentially incur new risks to the patient.[18] A similar situation occurs in non-GLP non-clinical studies using poorly characterized test items, impacting reproducibility and failing to translate encouraging non-clinical observations into actual clinical success, therefore questioning (i) the comparability of products used in different studies and (ii) the validity of their inclusion in systematic reviews and meta-analyses. However, there exist tools to correct this situation. Scientific advice, for instance, is a service provided by NCAs to support the timely and sound development of high-quality, effective, and safe medicines, by asking the regulators' opinion on the most appropriate way to generate robust on a medicine's benefits and risks. Earlier (and frequent) scientific advice could help CGT developers by carefully addressing quality and non-clinical issues before entering the pivotal phase of CGT development, therefore, clearing the path for a smooth and speedy marketing authorization.[19] Better understanding of mechanisms of action and improved design of CGT alongside advances in technology may facilitate the selection of appropriate test systems, suited for this new type of medicine. This will likely facilitate the implementation of higher quality standards starting with more relevant test systems. Moreover, we envisage a convergence between GLP and GMP compliance in non-clinical safety studies for CGT-based developments, provided that GMP could be applied not only to the production of test items but also to cover some types of studies not captured in original GLP guidelines (ie, genetic stability of cells in culture, toxicity due to the occurrence of cellular senescence or spontaneous differentiation). Such studies could be easily introduced into GMP protocols for the validation of bioprocesses and the characterization of the identity, purity, and potency of the drug substance. Such an approach may help to simplify the work of Quality Assurance Units and reduce costs at the expense of either abandoning GLP certification or reformulating its scope to in vivo and histopathology studies. Conducting regulatory non-clinical studies in a timely manner may avoid duplication of animal studies in addition to the optimization of lab resources, that is, time, manpower, and budget.

In addition, original scientific publications should mention compliance with quality standards applicable to the studies presented. Otherwise, the lack of reported evidence on the outcomes of IMPDs including GLP and non-GLP safety data makes it difficult to outline a relationship between compliance and actual marketing authorization approval success. Therefore a limitation of the present study is that we based our analysis on published scientific literature and regulatory reports by EMA and FDA, as reliable sources. Importantly, regulatory decisions are supported by overall data provided by developers in the dossier, including risk assessments and therefore approved ATMP should be considered safe.

## Final Remarks

Compliance with pharmaceutical QM systems in the development of novel CGT-based medicines is manifest in our analysis with a growing number of approvals and a better understanding of potential safety issues, both by developers and regulators. However, adherence to GLP in the non-clinical development program for safety assessment is still insufficient, being substituted to some extent by other QM frameworks. GMP is the most widely implemented both in academic and pharmaceutical laboratories, while there is no evidence that GLP compliance is instrumental in getting marketing authorization. It is important to stress that rather than rules, the Principles of GLP offer a work philosophy, assuring that non-clinical safety studies generate data that are reliable, reproducible, auditable, and globally accepted, by covering all aspects of their design, execution, reporting and archiving. As a final reflection, we would like to emphasize that conformity to better research-practice criteria should not be a doctrinaire impediment to scientific work, but a facilitator of efficient clinical translation of these innovative medicines.

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## Conflict of Interest

The authors declared no potential conflicts of interest.

## Author Contributions

L.L.-N., R.S.-P., J.V.: conceived the study; L.L.-N., S.T., R.S.-P., J.V.: retrieved and analyzed data; L.L.-N., R.S.-P., J.V.: drafted the manuscript; LL.-N., S.T., R.S.-P., J.V.: revised and approved the final version of the manuscript.

## Data Availability

No new data were generated or analyzed in support of this research.

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