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GUIDELINES

EMA commentary on the guideline on quality, nonclinical and clinical aspects of medicinal products containing genetically modified cells

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Great advances have been made in the knowledge of development and regulatory approval of medicinal product containing genetically modified cells. Although a guideline has been available in the EU since 2012, the current updated version provides a useful guide to developers and professionals involved in the regulatory process of these medicines. This article presents the main issues communicated in that guidance, the regulators' insights and a commentary from the academic developers' point of view.

KEYWORDS
advanced therapy medicinal products, clinical, gene therapy, genetically modified cells, nonclinical, quality

1 | INTRODUCTION

The European Medicines Agency (EMA) develops guidance for advanced therapy medicinal products (ATMPs). In 2020, the guideline on quality, nonclinical and clinical aspects of medicinal products containing genetically modified cells[1] was published.

ATMPs in the European Union (EU) include the following medicinal products for human use: gene therapy medicinal products (GTMPs), somatic cell therapy medicinal products (CTMPs) and tissue engineered products (TEPs).[2,3] ATMPs are mainly developed for the treatment of diseases, including orphan diseases, for which there are no or insufficient treatment options available at present.

According to the ATMP regulation, a GTMP is a biological medicinal product that meets two characteristics: it should contain an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with the purpose of regulating, repairing, replacing, adding or deleting a genetic sequence; and its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.[4,5] Vaccines against infectious diseases are specifically excluded.

GTMPs includes two categories: in vivo gene therapies, which directly deliver the product to patients either in situ or systemically, and ex vivo gene therapies, where cells obtained from a patient or a healthy donor are genetically manipulated under GMP conditions to create genetically modified cells.[6,7] Currently, the ex vivo GTMPs are composed of CD34+ or T cells transduced with an integrating viral vector (such as a lentivirus or retrovirus). Genome editing technologies such as CRISPR-Cas9 can also be used to create genetically modified cells.

1.1 | Regulatory experience with GTMPs

From 2009 to the end of 2023, 26 ATMPs were authorized in Europe (Figure 1). Eighteen are GTMPs. Ten of these GTMPs are genetically

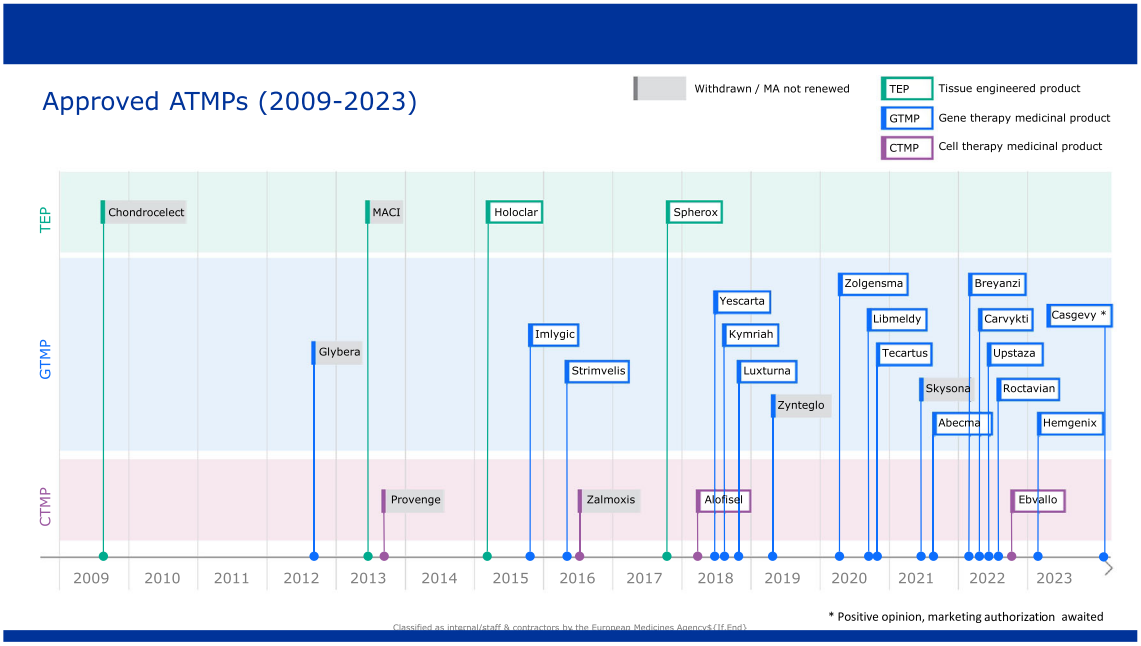

FIGURE 1 Approved ATMPs in the period 2009–2023. The approved products are divided in the three main ATMP subclasses: gene therapy medicinal products (GTMP), somatic cell therapy medicinal products (CTMP) and tissue engineered products (TEP). The grey-coloured boxes indicate products that have either been withdrawn by the applicant, or whose authorization has not been renewed (after 5 years).

modified (GM) cells: six (Yescarta, Kymriah, Tecartus, Abecma, Breyanzi and Carvykti) are chimeric antigen receptor (CAR)-T cells for the treatment of various haematological malignancies and four (Strimvelis, Zynteglo, Libmeldy, Skysona) are genetically modified CD34+ cells to treat hereditary diseases. The most recent GTMP to receive a positive opinion from the EMA Committee for Advanced Therapies and the Committee for Medicinal Products for Human Use, Casgevy, is indicated for the treatment of patients with transfusion-dependent thalasaemia and sickle cell disease. Casgevy is the first product that is based on CD34+ cells that are genetically modified using CRISPR/Cas9 genome editing technology.[8]

Developers of medicinal products, including ATMPs, can ask the EMA or the national authorities for medicines for scientific advice (SA) on appropriate tests and studies regarding the quality, nonclinical and clinical development of their product, as well as on the post-authorization evidence generation strategy. When looking at EMA SA procedures for ATMPs, most requests are for GTMPs, covering on average over 70% of all SA submissions in the last 5 years (2019: 73%; 2020: 72%; 2021: 76%; 2022: 76%; 2023: 63%).

Of the ATMPs supported under PRIME (Priority of Medicines), an EMA procedure to support the development of medicines for unmet medical needs, almost all are GTMPs (93% of all ATMPs granted PRIME between 2016 and 2023, $n = 59$). Both in vivo and ex vivo GTMPs constitute a large proportion of the ATMPs that are currently in clinical development, and the number of approved GTMPs is therefore expected to increase substantially over the next few years.

A guideline has been in place since 2012 for the development and evaluation of medicinal products containing genetically modified cells.[9] This document has since been revised, with the current version effective since June 2021.[1]

The revised version considers the experience gained since the first guidance was published and acknowledges the rapid scientific progress that has been made, such as the development of genome editing technologies, induced pluripotent stem cells (iPSC) and CAR-T cells. The guideline provides a useful guide to developers and professionals involved in the regulatory process of ATMPs (see **Box 1—Key highlights from the guideline**). In this manuscript, we present a summary, including the considerations of academic researchers, of the key elements of the revised guidance to inform early developers who could benefit from knowledge of regulatory guidance directly affecting the translation of their research.

Although the guideline provides the requirements for medicinal products containing GM cells that are submitted for marketing authorization, developers are nevertheless encouraged to, where relevant, already apply these requirements when medicinal products containing such cells are still under clinical development.

A guideline on quality, nonclinical and clinical requirements for investigational ATMPs in clinical trials is currently in development. This guideline will provide stage-specific guidance on data requirements for a clinical trial application for exploratory and confirmatory trials with investigational ATMPs.[10]

2 | QUALITY CONSIDERATIONS

To ensure appropriate product quality of GM cells, independent from the way they are manufactured, it is important that starting materials are controlled, the manufacturing process is validated, characterization and release testing of the active substance is performed using validated assays and that appropriate specifications are set.

BOX 1 Key highlights from the guideline on quality, nonclinical and clinical aspects of medicines containing genetically modified cells

- Quality requirements
  - The guideline is applicable to all types of cells, independent of the method for genetic modification (e.g. viral and nonviral vectors, mRNA, genome editing).
  - A description is provided of what is considered a raw material (reagent), starting material, excipient and active substance. This is relevant to determine the level of quality control and oversight.
  - Due to the diversity of products and manufacturing processes, the risk-based approach can be applied to the design of the manufacturing process and the characterization and quality controls to be applied.
  - Changes in the manufacturing process will trigger the need for comparability studies. The guideline provides examples of the regulatory expectations.
- Nonclinical requirements
  - Nonclinical studies should be performed in relevant in vitro, ex vivo and animal models. The 3Rs principle should be applied: any in vivo animal studies that could result in inconclusive data should be avoided.
  - To facilitate the translation of nonclinical data to the clinic, it is advised, where possible, to conduct the nonclinical studies with material that is produced and controlled according to the production process in place for clinical studies.
  - Guidance is provided on how to investigate the pharmacodynamics, pharmacokinetics and the toxicity of these products, as well as product-class-specific consideration for genetically modified immune cells, products derived from induced pluripotent stem cells and from genome editing.
  - Information is included on the environmental risk assessment of GM-based medicines.
- Clinical requirements
  - The guideline addresses the regulatory expectations for dose finding, pharmacodynamics and pharmacokinetics, clinical efficacy and safety demonstration, as well as for (long-term) patient follow-up and pharmacovigilance for GM-based medicines.
  - The design of the clinical trial(s), dose selection, pharmacodynamics, pharmacokinetics/biodistribution will depend on the distinct features of the product. A long-term follow-up of patients might be needed to establish or confirm the duration of therapeutic effect (efficacy) and the safety.
  - The current thinking of the regulators on the clinical development of CAR-T cells in haemato-oncology is provided.

A *risk-based approach* (RBA) should guide the development of a medicinal product containing GM cells. RBA can be the basis for flexible approaches applied to, for example, testing and release, control of manufacturing process and reconstitution activities. The RBA principle can also be used to guide the nonclinical and clinical development of the product.

Starting materials for GM cells are the cells themselves and the ex vivo gene transfer or genome editing tools (vector, transposon, plasmid, mRNA, guide RNA, ribonucleoprotein complex, DNA repair template). In case of combined ATMPs, the structural components can also be the starting materials.

It is important to note that, in the EU, the vectors to transduce the cells are also considered starting materials, whereas in the USA the FDA is classifying such vectors as drug substances instead. This will not impact the scientific content of the package, although it could affect the location of the information on the vector in the common technical document (CTD) module 3 of the marketing authorization application (MAA). EMA will accept a stand-alone module 3 section for the vector and for the cell starting materials and the same stand-alone document can therefore be submitted for the MAA in the EU and the biologic licence application in the USA.

Being a starting material, there is no legal requirement for vectors or other starting material for ex vivo transduction to be produced under Good Manufacturing Practices (GMP) as GMP requirements are only legally required for the manufacture of the finished product and active substance. However, an appropriate quality control is also essential for the critical starting materials. Therefore, the GMP guidelines[11] have introduced the concept of 'Principles of GMP', which is further expanded on in a recently published Q&A document.[12] Briefly, an ATMP manufacturer has the responsibility to verify that the relevant GMP requirements are implemented for the manufacturing and testing of the critical starting material (cells, vectors, genome editing tools). The components used to produce the critical starting materials (e.g. plasmids to produce the viral vectors, linear DNA used as template for mRNA or proteins) should also be controlled as starting materials.

Other materials used during the manufacture of the GM cells such as reagents or growth media are categorized as raw materials; thus, principles of GMP do not apply to such materials, but the European Pharmacopoeia (general chapter 5.2.12: Raw materials of biological origin for the production of cell-based products) should be followed.

The *manufacture* of GM cells typically includes several steps: cell preparation and culture, genetic modification and further downstream manufacturing/purification steps. A well-controlled manufacturing process is important to avoid unintended variability. As for any other medicinal product, at the time of MAA, it is expected that the manufacturing process is fully validated. In case a manufacturing platform (e.g. same vector construct and cell type, different transgene) is used to manufacture the GM cells, process validation can consider process experience and previous validation. A similar abbreviated validation approach can be used when automated manufacturing equipment is used: validation data generated by the manufacturing of the automated equipment can be utilized in the process validation exercise.

Similar to other ATMPs, *changes to the manufacturing process* often happen during product development or even after marketing authorization. This includes upscaling, but also a change to the process itself (e.g. a different manufacturing process to generate the starting materials, change to the plasmids producing the viral vector, or a change to the downstream purification steps). All such manufacturing changes can affect the quality of the medicinal product containing GM cells and, potentially, its safety or efficacy. The developer is expected to conduct a comparability exercise, based on a risk evaluation that considers relevant aspects, such as the type of change and the stage of product development where the change(s) were implemented. Additional guidance has been prepared in the form of a question-and-answer document.[13]

Characterization studies, which are intended to identify critical quality attributes (i.e. molecular and biological characteristics that are considered necessary to ensure consistency, efficacy and safety of the product), should form the basis of setting the release specifications. For GM cells, characterization and release tests should address both the cell and the gene components of the product such as cell identity, viability, phenotype, transduction/transfection efficiency, sequence/integrity of the transgene, vector integration profile or vector copy number per transduced/transfected cell. The risk of insertional mutagenesis should consider the integration profile of the vector. The guideline allows building on prior knowledge, that is, extensive characterization data, including integration profile studies, obtained with the same vector, using the same cells and promoter, but with a different transgene sequence.

Release testing should include assays for identity (both of the cell population and the intended genetic modification), purity and potency in addition to the general pharmaceutical safety tests (e.g. sterility, endotoxin, appearance). When the cells are modified with replication-deficient viral vectors, purity testing should include an assay showing the absence of replication-competent viruses (RCV). If the absence of RCV is demonstrated at another level (e.g. at the level of the testing of the viral vector starting material) and generation of RCV during manufacturing can be ruled out, no RCV purity testing is required at release of the active substance or finished product. Potency assay(s) should provide quantitative information on the intended function of the GM cell and/or the transgene product. For release testing, this is likely to be based on an in vitro assay (e.g. cytotoxic potential of CAR-T cells in an appropriate tumour cell culture). Use of animal-based biological assays is not expected due to their inherent variability, their limited predictability for the human situation and the 3Rs principles (reduction, replacement, refinement) governing animal research.

The guideline also considers what is expected when release testing cannot be done on the actual product. If this is because of the limited product quantity (especially in case of medicinal products containing autologous GM cells), it would be acceptable to perform the tests on key intermediates. If the shelf-life of the product is too short to have all release data available before the product is released or used in patients, a two-step release testing programme could exceptionally be agreed on, based on a risk-based approach and appropriate scientific justification; missing information from the first-step release should be compensated by appropriate in-process testing and a more extensive process validation.

As mentioned in the introduction, this guideline also includes information on the quality requirement of GM cells produced using *genome editing* (e.g. CRISPR-Cas). Firstly, the guideline defines the starting materials of the genome editing machinery, which is relevant to identify the manufacturing conditions of these materials (in line with the Principles of GMP). Secondly, the generation of on- and off-target modifications should be addressed as part of process development and characterization. Characterization should include an assay to identify off-target changes in the cell type to be used therapeutically or in healthy donor cells, as well as bioinformatic tools for in silico screening of off-target changes. The possible occurrence of large deletions, chromosomal mutations and other large-scale genomic alterations should be investigated, and the on-target genome editing should be fully characterized to establish the extent of correct target site editing and whether unintended changes have occurred at the target site. Lastly, the persistence of genome editing tools in the cells should be evaluated; ideally these should no longer be present when the cells are released for clinical use.

Academic developers commented that they often focus on therapies for orphan indications and ultra-rare diseases, which frequently require a long clinical trial recruitment period (that can be over 5 years). Long trial runtime increases the risk of supply line changes for key raw materials or critical manufacturing equipment. The stability of the starting material needs to be guaranteed for as long as a trial is recruiting. These issues are even more challenging for trials conducted with ATMPs. Additionally, for GM cells, the quantity of the viral vector can be limited, and a significant portion of it needs to be reserved for quality and stability testing, for use as a reference standard for future batches and for revalidation of the manufacturing process, if the need arises.

Academia welcomes the possibility to redirect testing to surrogate or intermediate product samples and allowing two-step release approaches in case a product needs to be administered fresh. Implementing the RBA as a basis for *all* quality control parameters, including tests now considered routine, can increase testing efficiency. Release tests should be tailored to the critical quality attributes of the ATMP and the RBA that is linked to these attributes. This ensures that relevant safety tests are performed and reduces unnecessary tests or tests performed in unsuitable models, yielding uninformative results and creating a false sense of safety. This will reduce 'loss' of finished product or of starting materials to purposes other than the production of the medicinal product. Flexibility to adjust quality control strategies to the product characteristics is considered a cornerstone in ATMP development.

The academic developers' position that quality control strategies need to be designed carefully to find the right balance between minimum testing requirements and feasibility with respect to available materials and costs, without compromising patient safety, is fully acknowledged and is in line with the spirit of the GM cell guideline.

3 | NONCLINICAL CONSIDERATIONS

Nonclinical studies are generally used to understand the proof of principle and can be necessary to predict the pharmacological and toxicological effects and to support the design of the clinical studies.

The revised guideline considers that the genetic modification of cells could have different purposes, such as the introduction of a functional copy of a disease gene or the regulation of a cellular function, and nonclinical studies should therefore be designed to clearly show the expected mode of action. Nonclinical studies should preferably be carried out using batches of genetically modified cells produced and quality controlled according to the production process in place for clinical studies.

Current regulatory thinking considers that in vivo animal studies should be carefully planned in accordance with the 3Rs principles and will be considered appropriate only if they are rigorous and of high quality. In silico tools and in vitro models are highly encouraged when appropriate. When animal models are used, homologous models or immune-deficient animal models might be advantageous, especially when a host's immune reaction to the administered modified cells is expected.

3.1 | Pharmacodynamics and pharmacokinetics studies for GM cells

Medicinal products containing GM cells carry potential risks, including adverse events, undesired immune responses and complications due to the modification of the genetic material, and it is fundamental to evaluate and estimate these risks. Pharmacodynamics (PD) and pharmacokinetics (PK) studies are important to address and demonstrate the effects of the genetic modification at the cellular level and the mode of action, addressing important aspects such as the biodistribution, homing and engraftment, as well as the stability and persistence of the modified cells in the body. A very important aspect that has to be evaluated in vivo is the duration of the transgene expression; especially for products aimed to provide long-term benefit, it is important to demonstrate the stability of the transgene expression in an appropriate in vivo model.

The new version of the guideline does not introduce any specifics regarding PD and PK studies, but it underlies the importance of those tests. As indicated in the *Guideline on nonclinical testing for inadvertent germline transmission of gene transfer vectors*,[14] the risk of germline transmission associated with the administration of GM human cells may be considered low, and omission of such studies can therefore be justifiable.

3.2 | Toxicology

The toxicology section has been deeply expanded in the revised guideline. Any adverse effects induced by GM cells have to be identified and characterized carefully by in vitro and in vivo toxicological studies in order to address potential toxicity due to the aberrant expression of a transgene, the risk of insertional mutagenesis and vector mobilization.

Dysregulated expression of the transgene of interest may induce undesired effects to the cells or to the host and this is a very important point that should be evaluated in vitro and in vivo. Toxicology studies should capture any potential toxic effect and they should be designed to detect the persistence of the expression of the therapeutic gene. A potential immune response to the transgene product should also be taken into account when designing toxicology studies.

Insertional mutagenesis resulting in oncogenesis (e.g. leukaemia/lymphoma) is a theoretical safety concern that needs to be carefully evaluated as stressed in the updated guideline, in accordance with the *Reflection paper on management of clinical risks deriving from insertional mutagenesis*.[15] Product-specific safety concerns of insertional mutagenesis and clonal expansion leading to tumorigenicity after engraftment of transduced cells have to be assessed by genomic integration profile analysis as part of toxicology and biodistribution studies. It is important to remind developers that, in order to reduce the risk of insertional mutagenesis, any possible strategy should be taken into account (i.e. self-inactivating vector configuration). The risk of vector mobilization, where vectors spread or 'mobilize' from transduced cells and infect additional cells within or outside the initial host, is a theoretical concern that should be evaluated depending on the vector chosen.

3.3 | Specific products: immune cells (such as CAR-Ts), induced pluripotent stem cell-derived products, genome edited cells

The revised guideline introduces recommendations regarding newly developed products, including CAR-T cells, T cell receptor (TCR)-modified T cells, iPSC-based products and products derived by genome editing. This reflects the increase in clinical experience with these therapies and allows for consideration of new tools for genetic modification of cells. Since experience with these products has been limited so far, these recommendations are points for consideration rather than prescriptive guidance.

As CAR- and TCR-modified T cells are products with an immunological mechanism of action, an appropriate animal model or a good in silico or in vitro alternative approach is needed to address their activity and safety. Nonclinical development should mainly focus on target antigen expression analysis to evaluate potential on-target/off-tumour activity and specificity assessments to rule out off-target activity. Such nonclinical safety assessment strategies require a tailored approach based on both the nature of the product and the intended application.[16]

Cell-based products derived from iPSCs hold great potential for novel therapeutic approaches to regenerate impaired tissues, but they also present a risk of insertional mutagenicity and oncogenicity associated with the possible use of integrating viral vectors and intrinsic risk of teratoma formation. The new guideline stresses the importance of

designing nonclinical in vivo and in vitro studies that could evaluate any untoward effects possibly due to the iPSCs.[17]

In support of the development of cell-based products derived by genome editing, the new guideline underlines the importance of selecting a relevant animal model for toxicity studies that could predict potential off-target toxicities and potential immunogenicity against the modified cells. Appropriate in vitro analyses are recommended in case of absence of a relevant in vivo model.

Toxicity tests still rely on animal models, even though transgene toxicity is highly species-specific. The guideline suggests designing in vivo tests in surrogate animal models, either in animals containing xenograft human tissues or a host-specific transgene. The encouragement to invest in alternative in vitro and in silico models in accordance with the 3Rs principles is welcomed by the academic developers. This is not only important for animal wellbeing, but from a cost-effectiveness and safety perspective it is also wise to research alternatives. However, the development and validation of 3Rs approaches can be challenging, costly and complex, and it requires collaboration and coordination among various stakeholders such as academia, regulators, validation bodies and ethical committees. The regulatory acceptance of the 3Rs testing approaches may vary depending on the type, purpose and scope of the testing. The *EMA Guideline on the principles of regulatory acceptance of 3Rs testing approaches*[18] describes the mechanisms by which an alternative novel method could be considered for use in a regulatory context. With the aim to help the developers, EMA has many initiatives ongoing to promote the alternatives to animal testing; in 2021 EMA launched a 3Rs-specific initiative in its Innovation Task Force (ITF) to encourage dialogue on new nonclinical methods.

4 | CLINICAL CONSIDERATIONS

The section regarding clinical aspects has been updated based on development and experience gained since the publication of the original guideline. In this relatively young and rapidly evolving field, severe, life-threatening and fatal toxicities have been observed in early clinical trials, requiring consideration of the specificities of GM cells when progressing these complex products from the nonclinical stage to first-in-human trials[19] and further trials supporting the MAA. While at present precedent cases for some product classes, like medicinal products containing genome edited GM cells, are still limited, there is sufficient regulatory experience with the assessment of MAAs of other product classes like genetically modified CD34+ cells and CAR-T cells.

The updated guideline builds on this experience and addresses distinctive features that are expected to be considered in the clinical development of medicinal products containing GM cells with the goal to generate robust efficacy and safety data. These specific features include: the complexity of products, product characteristics and manufacturing considerations; limitations due to the difficult extrapolation from animal data; uncertainty about frequency and duration of side effects, immunogenicity and malignant transformation; the need for long-term efficacy and safety follow-up; and the collection and administration procedures.

The guideline emphasizes the need for good predictions of the clinical effect, which could be due to the gene product, the transduced cells or both, and this information can be important to establish the posology.

Several factors must be taken into consideration to select a starting dose. As first-in-human trials are mostly performed in specific patient populations, as opposed to healthy volunteers, the starting dose should be safe and have a measurable pharmacologic effect. Guidance is provided on how to derive the starting dose when data relevant for calculations of *no observed adverse effect level* (NOAEL) or *minimum effective doses* (MEDs), as for other types of investigational medicinal products (IMPs), are limited. Clinical considerations based on the specificity of a patient's disease (in the case of CD34+ GM cells) are also important factors when selecting the starting dose.

The subsequent sections in the guideline recapitulate important factors to design a clinical trial with GM-containing products. It is fundamental to establish efficacy and assess the pharmacodynamic activity of the medicinal products containing GM cells, such as the cell engraftment and duration of the therapeutic effect. Conventional pharmacokinetics studies are not relevant for this kind of product, but it is important to assess the cellular kinetics, biodistribution and persistence of GM cells and the level of transgene product in target and non-target tissue. Clinical trials should be designed case-by-case to assess all those important aspects and the choice should be discussed and justified. An immunogenicity assessment is typically included in the development with the intent to understand the potential immune response to the modified cells and/or the transgene product. Of particular importance is that the clinical trial should be able to assess the duration of the therapeutic effect and the detection of short- and medium-term adverse events that can be associated with the administration of the product. To study long-term efficacy and safety, the guideline recommends a 15-year follow-up for patients treated with these products.

4.1 | Clinical development of CAR-T cells

In its Annex, the revised guideline addresses specific aspects regarding the clinical development of CAR-T cells. Since the approval of the first CD19-targeting CAR-T cell products in the EU in 2018, research and development activities in this area of adoptive immunotherapies have further increased. Not only have CAR-T cells emerged as new treatment modalities for patients with non-Hodgkin lymphoma, but there has also been considerable progress with the exploration of new CAR-T cell targets in malignant and non-malignant conditions, in addition to off-the-shelf allogeneic CAR-T cell developments.

While the considerations outlined in the guideline build on regulatory and scientific experience in haematological malignancies, they are equally relevant and applicable for other indications and treatment areas.

Characterizing in vivo cellular kinetics, CAR-T cell persistence in blood and target tissues, defining dose-limiting toxicities, assessing pharmacodynamic activities and identifying the recommended dose for later stage clinical trials are the main objectives of exploratory studies.

The efficacy section outlines requirements for the design of confirmatory trials. Recommendations are provided for the different scenarios, patient populations in late line *vs.* earlier lines of treatment, and the relevance of standard-of-care treatment options for the preferred randomized controlled trial (RCT) design and conditions where non-parallel controlled single arm designs might be acceptable.

Experience with approved CAR-T cell products and incremental knowledge gained in clinical trials, be it single arm or randomized controlled trials, as well as from observational studies, is used to inform new CAR-T cell developments but is also raising new questions. Whether the characterization of the target population for target antigen expression is required or not is a question that has evolved from the totality of data generated with CD19-targeting CAR-T cells. While not specifically addressed in the guideline, the principle of applying a case-by-case approach for this and other design elements of the clinical development is here again emphasized.

The safety section refers to the CAR-T cell specific toxicities and provides guidance for the generation of a robust data package that allows risk detection and monitoring of the commercial product under real-life conditions. Acute toxicities like cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome (ICANS) are linked to pharmacologic properties of CAR-T cells. The increased clinical experience with authorized CAR-T cell products has resulted in the development of consensus grading and treatment recommendations[20] by learned societies. This harmonization effort is appreciated and the use and application of the consensus criteria in the clinical development of CAR-T cell products is highly recommended.

As pharmacovigilance requirements are not specific for CAR-T cells, they are addressed in the general chapter.

Academic developers commented that the types of clinical trials in the academic setting differ from industry-initiated trials. More first-in-human studies with ATMPs are initiated in academia, and more often orphan indications and/or treatments for (ultra-)rare diseases are pursued.[21,22] On top of the issues related to manufacturing and control, as addressed previously, these types of trials are more challenging because of the longer recruitment duration and risk of underpowering if fewer patients are eligible than anticipated. Additionally, for such long-running trials, the standard of care to which the ATMP under investigation is compared might have changed over time, which could then require a further extension of an already lengthy trial (with the new standard of care) or even new trial. Cell-based immune therapies are now being developed targeting many more antigens and it is to be expected that CAR-T and TCR-T therapy will get a more prominent role in curative treatment rather than exclusively being used as a last-line therapy. There is ample experience with CAR-T and TCR-T cell trials, and for both products the safety profile is very well defined as are the production pipeline and risks. The use of real-world evidence data from approved therapies could be discussed with the regulator. Eventually, CAR-T and TCR-T combination therapies will be explored: personalized therapies tailoring CAR-T and TCR-Ts in different combinations to target a mix of tumour antigens to increase clinical benefit. Approval of such combination therapies under the current clinical trial setup would require studying the individual therapy *and* all the possible combinations. With a growing library of CAR-T and TCR-T receptors becoming available, testing all possible combinations is not feasible and it will therefore not be possible to generate a fully comprehensive data package for a fully individualized approach. Instead, safety profiles can be extrapolated from available real-world evidence and combined with the nonclinical data of a newly made combination, an RBA capturing all potential risks and uncertainties, and a clinical protocol designed to consider these uncertainties (severity of side effects, more careful dose escalation, total dose not exceeding the evaluated 'safe' dose for the single dose therapies, and informed consent).

The clinical part of the GM cell guideline is intentionally kept less descriptive than other parts in the guideline, especially to allow flexibility in the clinical development of ATMPs. It is the responsibility of the developer of the ATMP to conduct clinical trials to determine adequately the efficacy and safety profile of the new product. It is important to seek the advice of regulatory authorities, especially if less conventional clinical development strategies are to be applied: both national authorities and EMA are open to such a dialogue, for example, via the scientific advice procedure.

5 | POST-AUTHORIZATION CONSIDERATIONS AND THE USE OF REAL-WORLD EVIDENCE

The recommendation in this guideline is for a 15-year long-term patient follow-up. This is based on data from patients developing leukaemia caused by insertional oncogenesis of integrating vectors and other safety considerations. The reported cases of leukaemia were linked to the treatment with CD34+ GM cells, while cases of a causal relationship between CAR-T cell treatment and insertional oncogenesis have been limited to a few rare cases in the context of specific transduction methods, unrelated to commonly used integrating vector types. This aspect may soon be adapted to the particular risk associated with specific therapies. For example, the increasing knowledge base of CAR-T cell treatments is expected to provide the necessary data to reconsider the current request for 15-year, long-term patient follow-up. The duration of follow-up will have to be balanced against the safety signal of secondary T cell malignancies.[23]

The use of data from real-world evidence (RWE) to support regulatory decision-making is being considered by the regulators globally and is gaining in relevance.[24] Several initiatives are driving the harmonization, collection and use of RWE for regulatory decision-making in general.[25] For advanced therapies, the need to lower uncertainty, frequently without the traditional tools of blinded RCTs, makes RWE a potentially key tool. All approved ATMPs in the EU have post-authorization requirements that involve RWE or data from registries.[26]

Academic research has a double involvement on this aspect: on the one hand, the early identification of relevant data sources, including registries, will help to drive and prepare the ground for future data collection of academic's ATMPs, and, on the other hand, many registries and other relevant data sets (including hospital data) are in the hands of non-profit institutions.

The traditional setup of clinical trials is not suitable to prove efficacy and safety in 'N-of-1' or 'N-of-very-few' studies.[27] This poses a risk that these ultra-orphan indication trials are prematurely terminated or not initiated because obtaining market approval is not possible. Alternative methods need to be investigated to encourage the development of these therapies, like the use of RWE, historical controls and data extrapolated from other studies.

Improvements to simplify and reduce data entry would be necessary and appreciated for current and future registries.

These perspective from the academic developers on the use of registries and RWE are not restricted to academic research and academic clinical trials. Most of the ATMPs authorized and under development are for orphan indications, so the concerns expressed above also apply to industry developers. The GM cell guideline does not address in detail these aspects, but as this is a new field, the EMA is developing additional guidance, such as the *Guideline on registry-based studies*[28] to assist developers of novel medicines.

6 | DISCUSSION

The requirements in the guidelines are the expectations from the regulators for developers pursuing an MAA. They will, in principle, guide the clinical development of any product, but this does not imply that all these requirements are expected to be in place for the initial (exploratory) clinical trials. A separate *Guideline on the quality, non-clinical and clinical requirements for investigational ATMPs in clinical trials* is currently in development, and this will provide guidance, especially for early clinical development stages.[10]

The authorization of a medicinal product containing GM cells, as for all other medicines, will require that a positive risk–benefit balance is demonstrated. This requires data showing that the product is consistently manufactured and of adequate quality, and that the safety and efficacy profile is favourable. The RBA allows for a lot of flexibility in the system that regulators have been using to good effect, especially for low-prevalence, life-threatening or seriously debilitating diseases.[29]

It is relevant to mention three specific challenges. First, medicinal products containing GM cells are complex products and therefore quality requirements will have to address both the cellular and the recombinant nucleic acid components. Small changes to the manufacturing process can result in changes to the quality profile with potential effects on the safety and efficacy of the product and might put into question some of the results of the nonclinical and clinical studies. It is therefore highly recommended to complete the product characterization and process development as early as possible, ideally before the start of the clinical development. Major manufacturing changes during or after the confirmatory (pivotal) clinical trial are discouraged, as this might result in the raising of major concerns during the marketing authorization procedure.

Secondly, in the EU, GM cells are classified as genetically modified organisms (GMOs) and therefore information specific to Directive 2001/18/EC on deliberate release into the environment of GMOs needs to be provided. For clinical trials with genetically modified cells, the requirements from this Directive have been adapted based on the specific characteristics of these products: a Common Application Form (CAF) and corresponding Good Practice Document have been developed for human cells that are modified by means of retro/lentiviral or adeno-associated viral vectors, including genome edited cells.[30] The adaptive requirements can also be applied to human cells genetically modified without the use of viral vectors. The CAF aims to facilitate the approval of clinical trials with medicinal products containing GM cells in the EU and will also form the basis of the additional information that needs to be included in the MAAs of medicinal products containing or consisting of GMOs. More information can be found in the *EMA pre-submission guidance* (question 3.4.3).[31]

Lastly, the most appropriate design of a clinical study is to randomize patients against a placebo, comparator product or standard of care. However, for ATMPs, including medicinal products containing GM cells, such RCTs are seldomly performed for feasibility reasons, ethical concerns or lack of a comparator or standard of care (e.g. for ultra-rare indications or late-stage diseases). However, RCTs will remain the gold standard, also for ATMPs. As mentioned in Annex I to the revised guideline, the design of the confirmatory study for CAR-T cell products should follow a randomized controlled design (against a standard treatment), unless a scientifically sound justification can be provided for a non-parallel controlled single arm design. However, many authorized ATMPs are based on adaptive, small, open-label, uncontrolled and single-arm pivotal trials.[29] When an MAA is based solely on single-arm clinical trials, it is important to contextualize these clinical data with the help of real-world or registry data (e.g. on the natural course of the disease or on non-pharmaceutical treatments such as surgery, bone marrow transplantations).

An additional consideration, not addressed in the guideline, is patient access. Patient access to ATMPs after marketing authorization has been granted can only be guaranteed if a therapy is evaluated as cost-effective by the national health technology assessment bodies and becomes eligible for reimbursement. Cost reduction is therefore an important topic, even more for academic developers. Reducing unnecessary and uninformative testing can help reduce manufacturing costs and prevent 'minimum testing requirement inflation', whereby an extensive (and expensive) set of quality tests are performed because they have gained the reputation of being a 'necessity' and are therefore considered as the new minimum testing requirement, even if not all of the tests in such a package are relevant to the critical quality attributes of the product. Flexibility in designing the testing strategy to cover the uniqueness of the medicinal product containing GM cells, as opposed to a 'one-size-fits-all' approach, is the best way to ensure the correct tests are performed and safety and quality are

guaranteed. The academic viewpoint is fully understood by the regulators and acknowledged in the guideline which allows the RBA principles to be taken as the basis for setting the appropriate quality controls, and to guide the nonclinical and clinical development.

To conclude, product development of advanced therapies should be based on a risk-based approach where the developer can define the development programme based on the specificities of the product, taking into account known and theoretical risk factors and prior knowledge from similar products. For all aspects of development, including elements that are not or are not extensively addressed in the available guidelines, or deviations from the guideline requirements, developers can always seek scientific advice on their quality, nonclinical and clinical development programmes and on the post-authorization evidence generation strategy.[32]

DISCLAIMER

The views expressed in this article are the personal views of the author(s) and may not be understood or quoted as being made on behalf of or reflecting the position of the European Medicines Agency or one of its committees or working parties.

AUTHOR CONTRIBUTIONS

Patrick Celis and Giada Farinelli contributed equally to this article. All authors were involved in the conception and design of the article, review of the literature and the drafting and final approval of the manuscript.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

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