

Regulatory Issues in Developing Advanced Therapy Medicinal Products with Stem Cells in Europe

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11.1 Introduction

This chapter discusses European requirements for development of Advanced Therapy Medicinal Products (ATMP) based on stem cells, describing the framework for clinical trials and for marketing authorization, as well as the critical issues and challenges for developing stem cell-based ATMP.

The recent advances of biotechnologies have opened new promising perspectives for the development of ATMP, including stem cell therapy and regenerative medicine, which may potentially have a great beneficial impact on many human diseases, including among others cancer, degenerative and genetic diseases.

ATMP are based on the cutting-edge progress of biomedical research and on the use of novel and sophisticated technologies progressively aiming at patient-tailored interventions.

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ATMP comprise a variety of novel therapeutic strategies, including Cell Therapy, Gene Therapy and Tissue Engineered Medicinal Products (CTMP, GTMP, TEP, respectively).

In Europe, as in most countries in the world, ATMP are considered as medicines, therefore they are covered by the pharmaceutical legislation.

11.2 European Regulatory Frame for ATMP

ATMP are covered by the European legal frame for medicinal products through the EU Regulation 1394/2007 [1]. The current EU definition of GTMP and of CTMP is contained in the new Annex 1 that has been recently issued amending Directive 2001/83/EC [2], while the definition of TEP as well as of combined ATMP is contained in the EU Regulation 1394/2007.

GTMP are defined as those biological medicinal products that contain or consist of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; in addition, their therapeutic, prophylactic or diagnostic effects relate directly to the recombinant nucleic acid sequence they contain, or to the product of genetic expression of that sequence. Therefore, medicinal products such as viral or non viral vectors, plasmid or bacterial vectors, recombinant oncolytic viruses, genetically modified cells, cancer immunotherapeutics (so called “cancer vaccines”) are considered GTMP if they fulfil the current definition. It should be noted that, according to new Annex 1, vaccines for infectious diseases are no longer considered GTMP [2].

Somatic CTMP are defined as those biological medicinal products that: i) contain or consist of cells or tissues that have been subject to substantial manipulation so that their biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, or ii) contain or consist of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor, and iii) are presented as having properties for, or are used in or administered to human beings with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues [2].

TEP are defined as those biological medicinal products that contain or consist of engineered cells or tissues; and are presented as having properties for, or are used in or administered to humans with a view of regenerating, repairing or replacing a human tissue. Cells/tissues shall be considered ‘engineered’ if they have been subject to substantial manipulation or are not

intended to be used for the same essential function(s) in the recipient and the donor [3].

Medicinal products for stem cell therapy and regenerative medicine are considered as CTMP or TEP, whereas if genetically modified they are considered as GTMP.

ATMP may also contain a medical device (MD), such as e.g, biomaterials, scaffolds, matrices. In this case, EU Regulation 1394/2007 stipulates that the combination of ATMP and MD is defined as combined ATMP and that it is handled completely under the pharmaceutical legislation. Therefore, while MD directives are applicable to the MD before it is put in the combination, the whole combined ATMP shall be subject to EMA evaluation. Any type of MD that is present in the combination shall meet the MD essential requirements; the dossier for market authorization application of the combined ATMP shall include description of MD physical characteristics, performance and design methods, as well as evidence of conformity and, where available, results of assessment by a Notified Body [4].

Through EU Regulation 1394/2007, ATMP are within the frame of EU Regulation 726/2004 according to which no medicinal product can be marketed in Europe without an approval. For biological medicines, EU- wide market authorization is granted or denied by the European Commission through the centralised procedure taking place at the European Medicine Agency (EMA) [5]. In addition to those Regulations, the main EU regulatory framework for ATMP includes also Directive 2001/20/EC regulating clinical trials [6].

In the EU Regulation 1394/2007 a specific Committee for Advanced Therapy (CAT) is created. CAT covers the scientific areas relevant to ATMP, including gene therapy, cell therapy, tissue engineering, MD, biotechnology, surgery, pharmacovigilance, risk management, ethics.

CAT is responsible for ATMP evaluation in the centralised procedure, but EMA final opinion to the European Commission is always given by the Committee for Human Medicinal Products (CHMP).

Two new procedures specific for CAT have been established in the European Regulation 1394/2007: ATMP classification [7] and certification of quality and non-clinical data [8]; the latter is available for SME only.

European Regulation 1394/2007 also contain two very important legal requirements for ATMP: i) long term follow-up of safety and efficacy, in order to build up knowledge on ATMP long term effects [9], and ii) an active traceability system enabling to track the ATMP used to the patient who received it, and vice-versa, for 30 years after ATMP expiry date [10].

Development of medicines includes basically two steps: preclinical and clinical development. The first part includes designing the substance/molecule that represents the medicine, proving its activity and testing its safety in a preclinical model, while the latter part includes translating those results into human subjects by means of clinical trials.

As for any other medicine, in Europe clinical trials with ATMP are covered by the Directive 2001/20/EC [6], that stipulates that for all medicines, clinical trial approval is the responsibility of Competent Authority in each European Member State (MS). Therefore clinical development takes place at national level. For any given clinical trial to be performed in a given MS, the EU Member State performs a separate evaluation and authorization procedure. This is also the case when a multinational trial is to be carried out, which may represent a difficult task if reviewers have divergent opinion in the different EU MS on the same clinical trial proposal. Procedures and initiatives have been put in place by EMA and national Competent Authorities to decrease the chance that such divergences occur and to facilitate an efficient translation of research discoveries into effective ATMP.

EMA and national Competent Authorities are also concerned that patients are offered SC-based medicinal products only under controlled conditions, such as e.g. in clinical trials or if market authorized. To ensure patients' safety, SC-based medicinal product development should comply with the highest standards, as for any investigational medicinal product, under the supervision of statutory regulatory bodies.

Thus a number of guidance documents (guidelines or reflection papers) describing quality, preclinical and clinical requirements for CPMP/TEP as well as for GTMP, have been produced by EMA to help applicants in developing their products. All guidance documents are available through the EMA website [11].

The main guidance documents for CTMP/TEP is the guideline on human cell-based medicinal products [12], while for GTMP it is the guideline on quality, preclinical and clinical aspects of gene transfer medicinal products [13].

Other guidance documents are available, covering aspects such as for example: risk based approach [14], chondrocyte-based CTMP [15], potency testing of cell-based immunotherapy medicinal products for the treatment of cancer [16], clinical aspects related to TEP [17], genetically modified cells [18], risk of germ-line transmission for GTMP [19], long term follow up for GTMP [20], environmental risk assessment for GTMP [21], non-clinical studies required before first clinical use of GTMP [22].

CAT has recently produced a reflection paper [23] that covers specifically stem cell (SC)-based ATMP. This reflection paper addresses all medicinal products that are presented for marketing authorization and that use any types of SC as starting material, regardless of SC differentiation status in the final product.

11.3 Stem Cell-Based ATMP

SC therapy holds the promise to treat degenerative diseases, cancer and repair of damaged tissues for which there are currently no or limited therapeutic options. SC-based ATMP in general can be classified as CTMP or TEP; if they are genetically modified, they are classified as GTMP. When they contain a MD, they are considered as combined ATMP.

SC-based ATMP can be obtained from adult stem cells or pluripotent stem cells, such as mesenchymal/stromal stem cells (MSC), hematopoietic stem cells (HSC), tissue-specific progenitor cells.

They can be also prepared from human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSC).

Although stem cells share the same principal characteristics of potential for self-renewal and differentiation, SC-based medicinal products do not constitute a homogeneous class. Instead, they represent a spectrum of different cell-based products for which there is a variable degree of scientific knowledge and clinical experience available. For example, while MSCs or HSCs have been more extensively used for therapeutic purposes, this is not the case for hESCs or iPSCs.

Despite their clinical potential, SC-based ATMP bear also potential risks, that require a thorough evaluation before clinical use. Different levels of risk can be associated with specific types of SC. For example, the risk profile associated with iPSCs is expected to be different from those of adult SC (e.g. MSCs or HSCs) for which a substantial amount of clinical experience has already been gained.

The risk profile of SC-based ATMP depends on many risk factors, such as for example the type of stem cells, their differentiation status and proliferation capacity, *in vitro* manipulation steps, the route of administration and the intended site for clinical effect, the irreversibility of treatment or on the other hand the risk of cell loss, the long-term survival of engrafted cells.

The risks so far identified in clinical experience or the potential risks (i.e. those observed in animal studies) include tumour formation, unwanted immune responses and the transmission of adventitious agents.

Cell plasticity and product differentiation might also affect results generated during development, therefore it is expected that nonclinical and clinical studies are performed with well defined and characterized product so that results can be better interpreted.

11.4 Quality Issues for Stem Cell-Based Product Development

The risk of transmitting adventitious agents, such as viruses or TSE agent, is common to all biological medicinal products.

Such risk is to be dealt with by building the safety of the final product from the safety of starting cells and raw materials. Therefore, screening of donors [24, 25] and of all reagents that are used in the production process [26] for presence of infectious agents is a mandatory activity.

Based on their characteristics of unlimited self-renewal and high proliferation rate, SC are often defined as cells able to form teratomas *in vivo*. Therefore, inherent with SC therapy is the risk of tumorigenicity. To control this risk, lineage-commitment before administration to the patient is a desirable characteristics of a SC product. To obtain the intended lineage-committed cell population, production process is critical.

Process factors (e.g. separation methods, growth factors, serum) as well as conditions and duration of *in vitro* culture can affect cell population composition and differentiation capacity *in vivo*, thus affecting also the mode of action. Therefore, impact of those factors should be carefully taken into consideration when planning and executing production process as well as quality controls. Critical manufacturing steps that are employed to reach required differentiation stage should be controlled with relevant markers to ensure the intended phenotype is maintained.

Since identity of the SC-based ATMP is defined by self renewal capacity (proliferation) and expression of specific markers, and the starting cell material (*i.e.* bone marrow, fat tissue, umbilical cord blood) is often a mixed cell populations, identity of the cell population as well as the heterogeneity profile of final product should be carefully defined and characterized. Several markers can be employed to establish identity, investigating cell type, lineage commitment, terminal differentiation and/or functionality. Whatever the test method chosen, the cell identity markers should be specific for the intended cell population and should be based on biological or molecular mechanism of therapy.

For pluripotent SC as well as somatic SC there is a risk of genomic instability in culture. Culture conditions (e.g. feeder cells and excipients) influence SC genomic stability. Therefore, to decrease such risk the presence of proliferative and pluripotent cells tolerated in the final product should be measured by means of e.g. cytogenetic analysis, telomerase activity, proliferative capacity, senescence, etc., and limited.

Biological activity (i.e. potency) of SC-based ATMP can be investigated by means of expression of relevant macromolecules, such as for example growth factors, enzymes, cytokines, and/or formation of extra cellular matrix cellular structures, and/or cell-cell interactions (e.g. immune activation/inhibition), and/or differentiation or self-renewing capacity or migration. *In vivo* functional assays may also be employed.

More probably, a combination of different assays may be needed to confirm the potency of a SC-based product. Whatever the assays chosen, they should be utilized both at quality and non-clinical level.

11.5 Non Clinical Issues for Stem Cell-Based Product Development

As compared to cell-based medicinal products that contain only differentiated cells, for SC-based medicinal product non-clinical evaluation may need to be more substantial.

Non clinical studies should evaluate different aspects including proof of concept, biodistribution and microenvironment (*niche*), ectopic tissue formation, *in vivo* differentiation, immune rejection and persistence, tumorigenicity. Potential inflammatory/immune response to SC product is also very important, since it underlies the risk of stem cell elimination and of long term failure of treatment.

Notwithstanding a thorough quality control program, a SC-based product may still contain cells in an undifferentiated proliferative state, that bear the potential for tumor formation.

Therefore during development appropriate tests should be carried out to minimize risk of transformation and tumor formation, in particular when using hESC or iPSC, that have a relatively higher potential risk than other types of SC.

Both tumorigenicity and chromosomal stability should be evaluated in the SC product before initial clinical use.

Traditionally, preclinical studies for testing safety of medicines are carried out in animal models.

Even though animal models reflecting the addressed disease would be ideal, in practice this can be prevented by several limitations. In fact, not only the relevant model strain may not be available, but also large animal models may be preferable for studying surgically implanted products, or for long-term evaluation of tissue regeneration and repair or in those situations where the animal size, organ physiology or immune system is relevant for the clinical effect (e.g. regeneration of tissue). Very likely, more than one animal species or strains might be needed. *In vitro* models may also provide additional and/or alternative ways to address some specific aspects.

Ideally, the human cell product should be used, thus requiring immune-suppressed animals. However, for studying aspects such as e.g. persistence or functionality, homologous animal models might provide the most relevant system, even though predictiveness of such a model may be limited because of the still limited knowledge of the similarity between animal and human SC differentiation processes.

11.6 Clinical Issues for Stem Cell-Based Product Development

Ideally, nonclinical evidence on the proof-of-concept and safety of the SC-based product is expected before administration to humans.

In practice, as discussed above, there may be cases where sufficient nonclinical data cannot be obtained. In such cases the evidence should be generated in clinical studies by including additional end points for efficacy and safety, for instance to address the effect of an altered microenvironment (e.g. by inflammation, ischemia).

Clinical studies should evaluate different aspects including proof-of-concept, mode of action, dose finding, biodistribution, persistence, ectopic presence of SC product, safety and long term efficacy.

The mode of action of a SC-based product may be directly dependent on the stem cell population, on molecules secreted by the cells or on their engraftment in the host tissue.

Studies to follow the cells during the clinical studies, i.e. to assess biodistribution of SC product, may be important, depending on the SC product risk profile and its mode/site of administration. Due to a number of circulating SC higher than in physiological condition, abnormal distribution may occur leading to ectopic engraftment in non-target tissues.

On the other hand, such studies may be very imposing on the patients and in practice are hampered by the lack of suitable non invasive tracers.

Developing and validating new non-invasive methods for biodistribution studies in humans as well as markers or tracers for tracking cells in clinical studies is a highly desirable activity for future development of the field.

A specific safety concern relates to the SC ability to form teratomas. If a tumor is observed in a treated patient, then an investigation (e.g. genetic analysis) on whether it is due to SC product or to endogenous causes should be carried out.

From the clinical efficacy point of view, it is necessary to set up appropriate structural and morphological endpoints. Those should enable to study tissue regeneration, repair or replacement both at short term and at long term. It should be considered that for SC-based products impairment of efficacy is also a safety issue.

11.7 Conclusion

Stem cell therapy may represent great hope for many diseases and degenerative conditions, but a thorough evaluation of quality as well as safety and efficacy characteristics of the SC- based medicinal product is critical during clinical development in order to obtain a safe and efficacious SC-based ATMP. Developers of SC-based products are strongly encouraged to engage in dialogue with the CAT/EMA as well as with national Competent Authorities at an early stage of this process.

The cooperation between all the actors in the development process is crucial to contribute in the continuous effort to protect the European patients, while helping in developing safer and more efficacious SC- based ATMP.

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