First-in-Human for Advanced Therapy Medicinal Products

Tânia Sofia Casaca Formigo[1,*]

Thesis to obtain the Master of Science Degree in Biomedical Engineering – November 2018 Supervisors: Prof. Dr. Beatriz Lima[2,3] and Prof. Dr. Margarida Diogo[1,4]

[1]Instituto Superior Técnico, University of Lisbon, Portugal; [2]Faculty of Pharmacy, University of Lisbon; [3]Research Institute for Medicines – University of Lisbon; [4]Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon

*Email: taniaformigo@tecnico.ulisboa.pt

ABSTRACT: Remarkable efforts have been made in the development of novel medicinal products, allowing to shift from a "one size fits all" healthcare approach to a personalized medicine. Advanced therapy medicinal products (ATMPs), based on cells, tissues or genes, offer innovative solutions to unmet medical needs and life-threatening conditions, representing a groundbreaking pharmaceutical field that shows great value but face some complex challenges. Due to ATMPs complexity and unique characteristics, regulatory framework must be tailored on a case-by-case basis, to efficiently evaluate these medicinal products for further market entrance. The present master thesis aims to discuss preclinical requirements, mandatory for conducting first-in-human studies of ATMPs, with a focus on cell-based medicinal products and using NVivo software for a more efficient analysis. Firstly, scientific guidelines amending non-clinical studies for small molecules, biotechnology pharmaceuticals, cell-based and gene therapy medicinal products were compared. Secondly, six different ATMPs case studies were analysed, assessing similarities and dissimilarities in preclinical development programs, as well as risk-based approach influence for considered non-clinical evaluations. Results showed that although ATMPs have specific characteristics, some non-clinical studies must be performed independently of the medicinal product's features, on the other hand, certain should only be conducted if outcomes are considered relevant for ATMPs evaluation. Furthermore, it was possible to conclude and discuss common preclinical studies' goals between different ATMPs, as well as relevance of standard preclinical evaluations when considering these innovative medicinal products. Moreover, importance and benefits of risk identification and appraisal, during non-clinical evaluations, were also discussed for selected case studies.

KEYWORDS: Advanced Therapy Medicinal Products, Cell-Based Therapy, Gene Therapy, Preclinical, First-in-Human, Non-clinical, Risk-Based Approach, Marketing Authorization Application

1. INTRODUCTION

The insertion of a new pharmaceutical in the market is frequently an arduous procedure which requires tight regulation to assure safety and efficacy to the targeted population. The whole development process entails different steps, namely research and development, the preclinical, clinical and post-authorization phases. These stages must be tailored according to the pharmaceutical being developed and must comply with different regulatory documents [1]. Over the past decades, a great investment and progress has been seen when it comes to
advanced therapy medicinal products (ATMPs), which include cells, tissues or genes in their constitution and aim to deliver a more personalized solution to unmet medical needs. However, such novel treatments are, in consequence of their patient-specific target and structure, more complex and heterogeneous than standard pharmaceuticals and must follow specific directives to identify safety, tolerability and efficacy [2]. Since 2008 regulation have been effectively adopted for these pharmaceuticals, namely Regulation (EC) No 1394/2007[3], aiming to help in product's market insertion.

ATMPs diversity led to the elaboration of different regulatory documents, from scientific guidelines to reflection papers, that must be considered when developing an ATMP. The effort to create such diverse regulatory documents amending ATMPs is an evidence of the improvements that have been made in trying to increase the number of available ATMPs, which pose

great value to targeted populations [4]. However due to the required case-by-case development approach, ATMPs still face several hazards and the number of available ones remains quite low [2].

Due to the need for unique regulatory documents, considering ATMPs characteristics and complexity, since 2008 regulation have been effectively adopted for these pharmaceuticals, namely Regulation (EC) No 1394/2007 [3], aiming to help in product market insertion and in complex development program supporting market introduction [5]. However, there is still no scientific guideline particularly providing recommendations on requirements of preclinical studies for further first-in-human studies, considering all ATMPs categories.

The major potential impact that ATMPs show to the healthcare community served as the core motivation for this study. After some literature review within the topic, it was clear that appropriate preclinical development steps were of key importance for these pharmaceuticals' market entrance, allowing to minimize clinical trials' risks and achieve a safer product evaluation process when starting clinical studies with first administrations in humans. These two aspects combined together led to the conduction of a comparative analysis with regards to preclinical steps, mandatory for first-in-human studies of ATMPs, with a particular focus on cell-based medicinal products.

To conduct a significant investigation, this study was divided in two major phases, allowing to acquire relevant information to conclude on different topics of the present thesis. Firstly, different scientific guidelines, with regards to preclinical development steps, were compared, relating product characteristics with recommended preclinical studies. Secondly, different ATMPs' European Public Assessment Reports (EPARs), particularly somatic cell therapies and tissue engineered products' EPARs, were analyzed, preclinical studies were compared and discussed according to particular products' features, such as mode of action or route of administration.

Moreover, this thesis was developed using a step-wise approach, allowing to use information obtained from the previous steps as basis for the following ones. To simplify several documents' analyses and data organization, the software NVivo was used.

2. METHODOLOGY

In order to acquire all the relevant information and obtain the essential regulatory documents to perform a significant analysis, different research questions were established, for example: 1) *"What is the mandatory information to be available before first-in-human studies?"*; 2) *"Are there any differences in preclinical data package for different pharmaceuticals?"*; 3) *"How risk-based approach influenced each ATMP non-clinical evaluation?"*. Bearing in mind the proposed questions, some key words and expressions were used to identify
relevant regulatory documents available in EMA website [6], particularly: *Advanced Therapy Medicinal Products, Cell-Based Therapy, Gene Therapy, Preclinical, First-in-Human, Non-clinical, Risk-Based Approach, Marketing Authorization Application*. Besides research in EMA website by key word, also the sections *Human Regulatory* > *Research and Development* > *Scientific Guidelines* > *Non-Clinical* > *Non-Clinical Development and Human Regulatory* > *Advanced Therapies* > *Scientific Guidelines* were consulted, to assure that all relevant scientific guidelines, commission directives and reflection papers, as well as some presentations, were considered for further analysis.

Since a large number of documents had to be considered, assessment of all information available became a demanding task. To facilitate readings and information synthesis, the software NVivo, from QSR International, was used. NVivo is a qualitative data analysis software, that allows data storage, categorization, organization and management, offering information research tools such as queries and comparison diagrams [7].

Characterization of preclinical studies for different pharmaceuticals

One can anticipate relevant differences between different types of pharmaceuticals such as their origin or manufacturing processes, which leads to significant dissimilarities during the whole development procedure and consequently the preclinical stage [8]. This analysis allowed to assess why some studies are relevant for a particular type of medicinal product and others are not considered significant to be apply.

Bearing in mind these product-specific characteristics, four scientific guidelines were studied and compared, assessing differences and similarities between them. For standard drugs, particularly small molecules, *ICHM3R2 – Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals* [9] was considered, which gives a broad guidance to preclinical drug development
process, particularly before first drug's administration in humans. When it comes to biotechnology-derived
pharmaceuticals, *ICHS6- Preclinical safety evaluation of biotechnology-derived pharmaceuticals* [10] was studied. With regard to advanced therapy medicinal products two documents were analyzed, namely *Guideline on Human Cell-based Medicinal Products* [11] and the *Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products* [12], both containing relevant information for the pharmaceutical' developers with regard to preclinical data package for marketing approval.

This analysis was performed using Nvivo software to easily compare and gather conclusions on this topic.

The essential data package for standard medicinal products was included in a mind map created with the software and represented in figure 1. This map was used for further analysis of case studies' EPARs, with nodes been automatically generated from the mind map and allowing to perform a classification and analysis of each preclinical study required for the different pharmaceuticals' categories considered. This methodology allowed a more efficient comparison between the four guidelines, as well as a clearer perspective of what are the similarities and disparities in preclinical requirements for different pharmaceuticals, considering each product category's characteristics.

Definition of relevant case studies

Additionally, it was considered relevant to compare different European public assessment reports (EPARs) in order to have more specific examples of preclinical study designs and how the pharmaceuticals' characteristics influence the correspondent non-clinical data package. In the most desired scenario the relevant case studies chosen would still be available on the market. Besides, it was required that these products showed some common features and also some dissimilarities to allow a meaningful comparison between preclinical studies performed. Accomplish these two requirements proved to be not feasible due to the very low number of ATMPs available. Thus, it was necessary to consider some medicinal products that have already been withdrawn form market. However, it is relevant to stand out that none of the relevant cases chosen were withdrawn due to safety reasons, making them still relevant to be used as case studies.

After identification of all marketing authorizations for ATMPs obtained until the end of 2017, the pharmacotherapeutic group, the ATMPs sub-category and the therapeutic indication were compared to assess the relevant case studies for further assessment.

After identification of the relevant ATMPs to conduct a significant comparison of preclinical studies, the information present in each medicinal product's EPAR was summarized and analyzed, using NVivo as a research tool to help identify the key points to gather meaningful conclusions and perform a more efficient analysis.

Comparison of Chondrocyte-based medicinal products

After comparison of selected EPARs, ChondroCelect, Maci and Spherox were classified as chondrocyte-based medicinal products, due to their similar expected therapeutic outcome, mode of action and method of administration. With NVivo software, using a similar methodological process to the one discussed in previous section the case studies comparison was performed.

Classification of ATMPs and evaluation of risk-based approach influence

ATMPs considered as relevant were classified according to their method of administration and possible risks inherent to their used. Further from this classification, also the risk-based approach was kept in mind during the discussion conducted for the categorization attributed to each ATMP, evaluating the influence of this methodology implementation in each ATMP's development process.

Overview of common characteristics of preclinical studies considered

After the previously mentioned analyses conducted, it was considered relevant to perform an overview of major common preclinical features identified, independently of the ATMPs' characteristics. Besides the insights acquired from the comparative study, also the *Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products* [13] was considered. Although ATMPs are not in the scope of this document, common points were identified when it comes to relevant studies required and so it was considered relevant to compare them with the outcomes of the case-studies analyses performed.

3. ATMPs and Drug Development Process

Advanced Therapy Medicinal Products (ATMPs) is a ground-breaking pharmaceutical field aiming to restore, correct or modify physiological functions, through a metabolic, pharmacological or immunological pathway [14]. This emerging biomedical area comprises different subtypes, all of them either based on tissues, cells or genes [15]. Somatic cell therapies use cells and tissues which have a different function, biological features or structural properties in a recipient, according to their intended clinical use, in comparison to its essential function in the donor, who can be the same person. With the manipulation that these cells or tissues undergo it is possible to cure, prevent or diagnose diseases, through an immunological, pharmacological or metabolic process [16]. Other ATMP subtype is the gene therapy medicinal product, in which the use of recombinant genes in the human body leads to a therapeutic, prophylactic or diagnostic effect [17]. Finally, a tissue-engineered product entails cells or tissues that have been engineered in a way that allow them to repair, replace or regenerate human tissue, without identical essential purpose in the recipient and in the donor [18]. Due to these therapies' biotechnologic background, each new ATMP developed is very specific and distinct from other products that might be already available [19]. Thus, quality, preclinical and clinical data required for pharmaceutical's evaluation is highly product-specific, which also requires a very expertise personnel

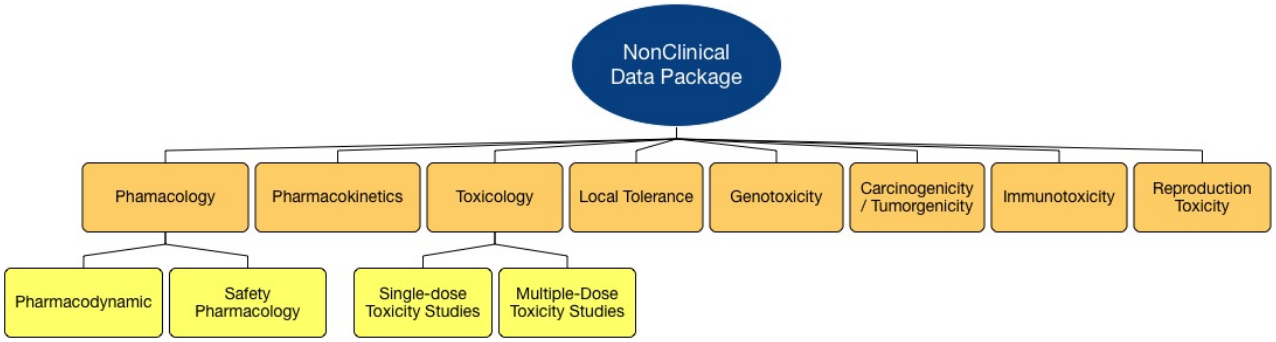

Figure 1 - Mind map with essential data package for standard medicinal products, during non-clinical studies (obtained using NVivo software)

responsible for the evaluation of product development, determining a positive or negative opinion towards it [3].

During the last few decades the regulatory framework for drugs entering the market has seen an enormous transformation, with the harmonization of the regulatory approval process being increasingly required [4]. With a focus on ATMPs, a centralized procedure is mandatory. With this approach, a single document is submitted, a Marketing Authorisation Application (MAA), that once granted allows the accessibility to the therapy in different countries within the European Union, providing a free movement of authorized medicinal products [5]. The entity responsible for drug's market entrance approval is the European Medicines Agency (EMA), together with its scientific committees, particularly CAT, the Committee for
Advanced Therapies, established in 2009, due to the increasing necessity of a specialized body for the evaluation of ATMPs submissions. This board is responsible for the assessment and draft opinion regarding submitted MAAs, that are afterwards discussed within the Committee for Human Medicinal Products (CHMP) to gather a conclusive opinion [20].

The complexity of ATMPs therapeutic field leads to the necessity of precise legal definitions. Thus, to cover all ATMPs' characteristics and suppress hazards to human's safety, some directives and regulations must be respected. Particularly, part IV of Annex I of Directive 2001/83/EC [21] is related to ATMPs field, as amended by Directive 2009/120/EC [16], documenting requirements for non-clinical, clinical and quality data. After 2007, Regulation 1394/2007 allowed to place under the same regulatory context the three different ATMPs subtypes, named previously, as well as provide a certification system for this scientific field.

When considering ATMPs, the regulatory framework differs from usual medicines available in the market. ATMPs' development considers the risk-based approach as a legislative background, to assure minimization of risks to which population might be exposed. With this method, the developers are capable of define and support the extent of quality, non-clinical and clinical data that has to be included in the MAA submitted to appraisal, keeping in mind a risk profiling method, in which risks and risk factors are identified during product's development [22].

Risk-based Approach

Considering ATMPs complexity a new methodology is required, to improve medicinal product's quality in an efficient way, reducing development expenses and eliminating unexpected jeopardies. In an attempt to identify and reduce risks which may provoke worse outcomes, since some years ago, a risk-based approach has been stated has the emerging methodology to assure products' quality [23].

The risk-based methodology is centered around the idea of inducing regulatory flexibility, by continuously monitoring risks and risk factors, with an early identification of hazards, that allows to control and minimize them in the risk management context. Moreover, with this method it is possible to specify the extent of non-clinical, clinical and quality data needed to be included in the MAA.

The methodology is focused on risks defined, by EMA, as an adverse effect of concern to the patient and third parties and which is related to drug's clinical use. The quantitative and/or qualitative product's characteristics that contribute to a specific risk is defined as a risk factor. From these two notions, the principle of risk profiling arises, with a characterization of each risk that is related to the product and not the overall product's risk. The risk profiling idea comprises different steps allowing to integrate all data available to design a profile to each hazard identified [22].

4. Preclinical Data Package supporting FIH

Prior to the conduction of clinical studies, it is mandatory to acquire a data package that allows to assess if it is reasonably safe to proceed to human administrations. This preclinical information acquired, with the use of adequate *in vitro* and *in vivo* models, serve as basis for the next development steps. Taking into consideration the specific characteristics that each pharmaceutical can show, particular requirements will apply, making this process product specific [22]. The ultimate goal of non-clinical procedures is to acquire the greatest amount of information possible, with

regards to drug's features, proof-of-principle and possible adverse effects once administered in humans, allowing to obtain information for human risk prediction. This is accomplished through the combination of *in vivo* and *in vitro* studies, which allows to conduct a more accurate extrapolation to human expected findings [10].
From the analysis performed with the software NVivo, aiming to compare scientific guidelines already named in section *2. Methodology - Characterization of preclinical studies for different pharmaceuticals*, it was possible to reveal some patterns in preclinical data package which is required for first-in-human administrations, even though these scientific guidelines are intended to be considered for pharmaceuticals of diverse categories. From the analysis of the results acquired it is possible to conclude that independently of the sort of pharmaceutical under development it is necessary to consider the same non-clinical data package and afterwards discussion on the relevance of each study must be conducted.
Firstly, when looking at pharmacodynamic studies, it was possible to comprehend that the main goals of such studies are equal, namely, proof-of-principle and mode of action evaluation. One important topic to stand out is the absence of specific tests recommended to assess pharmacodynamics in the regulatory documents analysed. This is justified by the diversity in pharmaceuticals' characteristics, that conducts to a diversity in PD tests to be performed. Therefore, not a single battery of tests is adequate for the different pharmaceuticals, even within the same category, such as small molecules. Thus, just the goal of data acquisition is relevant to be included in the scientific guidelines. When it comes to safety pharmacology studies, their goals are common and concerns the evaluation of effects on major physiological studies.

For pharmacokinetic studies significant differences were encountered. When considering small molecules, it is important to assess ADME, namely absorption, distribution, metabolism and excretion. On the other hand, for biopharmaceuticals and ATMPs conventional studies are not relevant. For biopharmaceuticals metabolic pathways are well comprehended, since degradation of small peptides and amino acids are expected to be seen, thus biodistribution, clearance and disposition must be assessed. When it comes to cell-based products, pharmacokinetic studies must allow to evaluate biodistribution, cells growth, viability, migration, differentiation and on the other hand, assess distribution, persistence, clearance, transcription of administered nucleic acid and germline transmission risk for gene therapies.

Considering toxicology, for small molecules and biopharmaceuticals it is relevant to perform single and repeated dose toxicity studies. On the other hand, for ATMPs single dose studies must have longer post-administration observation periods due to ATMPs expected long-term effects and multiple dose studies are not mandatory and must only be conducted if the ATMPs' characteristics and mode of administration justify such studies.

Local tolerance assessment must be performed according to the product under evaluation.

When it comes to genotoxicity, small molecules evaluation must include a gene mutation assay and for gene therapies it is relevant to assess possible genomic modifications, insertional mutagenesis and locals of genomic integration. Such studies are not relevant for cell therapies and biopharmaceuticals sine they are not expected to interact with the genetic material.

Considering carcinogenicity studies, small molecules must include these studies if the product is intended to be used for long periods of time. Cell-based medicinal products' studies must evaluate tumorigenic potential, due to possibility of neoplastic alterations induction, justified by the fact that these products show migration, differentiation and proliferation capacity.

Immunotoxicity studies are considered relevant to predict human inflammatory response and must be considered relevant for the different pharmaceuticals' categories considered, whenever the medicinal product's
expected mode of action or characteristics might affect immune system and lead to adverse outcomes.

Thus, from the evaluation of the four scientific guidelines considered, it was possible to acquire some insights of the different studies required for each medicinal product category, namely small molecules, biopharmaceuticals, cell therapies and gene-therapies, and discuss differences, as well as discuss similarities encountered, considering products' characteristics.

5. Case Studies Analysis

Bearing in mind this thesis's goal and methodology, the case studies selected belong to somatic cell therapies (sCTMP) and tissue engineered products (TEP) categories and are identified in table 1, as well as their therapeutic indication. Six case studies were selected: Chondrocelect, from Tigenix, is classified as a TEP, formed by autologous chondrocytes expanded *ex vivo* and delivered as a suspension to treat symptomatic cartilage defects; Maci, from Genzyme, is also a TEP consisting of patient's own chondrocytes applied to a collagen matrix which is held in place by a fibrin glue and intended to have an effect on cartilage defects; Spherox from CO.DON, classified as a TEP is also intended to repair cartilage defects, consisting of spheroids containing human autologous chondrocytes associated to a self-synthesized matrix; Alofisel, from Tigenix, classified as an sCTMP is intended to have a therapeutic action on complex perianal fistulas in Chron's disease patients, consisting of expanded human allogeneic adipose stem cells delivered in a single suspension; Heparesc, from Cytonet GmbH&CoKG, classified as a sCTMP, intends to show a therapeutic action on urea cycle disorders, consisting

Table 1 - Selected Case Studies

ATMP name	ATMP classification	Therapeutic indication
ChondroCelect	TEP	Cartilage Defects
Maci	TEP	Cartilage Defects
Provenge	sCTMP	Prostatic Neoplasms
Holoclar	TEP	Corneal diseases
Zalmoxis	sCTMP	Graft vs Host Disease
Spherox	TEP	Cartilage Defects
Alofisel	sCTMP	Rectal Fistula
Heparesc	sCTMP	Urea Cycle Disorders

of human heterologous liver cells, intravenously infused through an intraportal catheter, in six daily doses; Zalmoxis, from MolMed, also classified as a sCTMP, intends to be used as an adjunctive therapy after hematopoietic stem cell transplantation, supporting immune reconstitution. It consists of T cells that have been genetically modified to contain a suicide gene which is activated, by antiviral drugs, if the patient shows some signs of adverse reaction after the transplant, allowing to selectively eliminate dividing cells, the alloreactive T cells.

Considering the similarities and differences, possible to encounter after analyzing these products' assessment reports, it was considered relevant to perform separate studies, already named in section 2 and discussed next.

5.1 Comparison of chondrocyte-based medicinal products

Bearing in mind the homologous section in *2. Methodology*, Chondrocelect, Maci and Spherox were considered to perform a comparison analysis between ATMPs' development processes.

Thus, from the methodology depicted in methodology section, and through the use of Nvivo software, it was possible to obtain a matrix, presented as table 2, illustrating the relation between different medicinal products' assessment reports and studies categories, identified in figure 1. The referred matrix shows the relevance and consequent implementation of each study category for each medicinal product compared, with number 1 showing that the study was conducted and number 0 ascertaining the absence of it. From this matrix analysis, it was possible to understand some similarities and differences for chondrocyte-based medicinal products. When it comes to repeated-dose toxicity, genotoxicity and reproduction toxicity, no applicant performed such studies, considering them not relevant, bearing in mind the products' features and mode of action. On the other hand, some studies were conducted in all three medicinal products' non-clinical processes, namely primary pharmacodynamics, pharmacokinetics and local tolerance.

Pharmacodynamic studies were conducted for all three products to assess proof-of-principle and demonstrate some evidence of proposed mode of action. Thus, all ATMPs were evaluated when it comes to the repair tissue formation capacity, *de novo* tissue properties, medicinal product integration in the host and degree of defect filling, the regeneration of the defect site and formation of new and adequate tissue, allowing to assess proof-of-principle. Furthermore, it is relevant to state the conduction of biomechanical studies for Maci and Spherox, relevant due to intended functional repair and allowing to evaluate tissue properties essential to restore knees' normal function. Only for Maci, secondary pharmacodynamics, assessing outcomes not correlated to the intended one, and safety pharmacology were evaluated due to presence of a collagen matrix from porcine origin within this TEP which might lead to some undesired outcomes.

When it comes to pharmacokinetic studies, standard ADME studies were considered not relevant for all three ATMPs evaluated during this stage. Bearing in mind chondrocyte-based medicinal products' characteristics, biodistribution was evaluated, particularly assessing cell migration capacity from implantation site, which must be reduced since these products are intended to show local outcomes.

Within toxicology studies, single-dose studies were conducted for all three considered ATMPs, with adequate post-administration evaluation periods to assess later outcomes. On the other hand, repeated dose toxicity studies were not performed for any of the considered tissue engineered products. These decisions can be justified based on products' mode of action and intended single administration, as well as expected long lasting effects, for all of them. Besides, as durations are considered relevant to assess long-term effects, no need for repeated dose studies was considered.

Tumorigenicity studies, were conducted for all three chondrocyte-based medicinal products and only for Maci this study category did not had dedicated studies. No further studies were required for Maci due to absence of mutagenic potential, evaluated during genotoxicity studies conducted. Tumorigenicity evaluation is of major relevance for these ATMPs since it is essential to evaluate cells' migration and proliferation capacity, which might lead to relevant tumorigenic potential.

Finally, local tolerance studies, is one of the categories that had major relevance for these medicinal products' non-clinical development processes being performed for all ATMPs considered within chondrocyte-based category, with a particular relevance for Maci. Such studies allowed to evaluate any inflammatory outcomes that might occur due to medicinal product administration.

Table 2 - Non-clinical studies performed for chondrocyte-based medicinal products

	A: ChondroCelect	B: MACI	C: Spherox
1: Primary Pharmacodynamics	1	1	1
2: Secondary Pharmacodynamics	0	1	0
3: Safety Pharmacology	0	1	0
4: Pharmacokinetics	1	1	1
5: Single-dose Toxicity Studies	1	1	1
6: Multiple Dose Toxicity Studies	0	0	0
7: Genotoxicity	0	0	0
8: Tumourigenesis - Carcinogenicity	1	0	1
9: Reproduction Toxicity	0	0	0
10: Local Tolerance	1	1	1

For Maci, several local tolerance studies were performed, due to the presence of a collagen membrane with porcine origin, which might lead to some inflammatory response. For that reason, most of these studies were conducted to assess local tolerance to this non-cellular component.

5.2 Classification of ATMPs according to their characteristics and risks

From the evaluation of ATMPs' characteristics, it was considered relevant to develop a new classification system, particularly based in medicinal products' mode of administration and risks that each ATMP pose. Firstly, to obtain the new classification system, medicinal products were compared according to their application site, which allowed to comprehend that some use a local administration and others a systemic one, such as an intravenous delivery. Moreover, it was also possible to understand that some ATMPs under study showed local risks and other presented systemic ones. Thus, the classification present afterwards was developed and each ATMP analyzed was included in a single category, discussing the risk-based approach influence for each ATMP's non-clinical studies.

ATMPs with a local application and posing local risks

Chondrocyte-based medicinal products, namely Chondrocelct, Spherox and Maci, were included in this category. Besides from their local administration sites, considering that these ATMPs are not expected to contact blood circulation, they are not expected to pose any risks away from implantation site, thus only posing local risks inherent to their use.

When looking at risk-based approach influence for these three ATMPs, it is important to stand out that Chondrocelect was authorized for market entrance in 2009 and only in 2013 the guideline supporting risk-based approach implementation was released. On the other hand, Maci came to the market soon after the named guideline was made available and Spherox received the European marketing authorization in 2015. Thus, differences in the way non-clinical studies were conducted are possible to find when comparing this three ATMPs, with Spherox and Maci showing greater evidence of this methodology implementation in contrast with Chondrocelect's non-clinical studies. Thus, it was possible to understood that the existence or absence of practical guidance for risk-based approach implementation influences the way non-clinical studies are conducted, with Chondrocelect not showing as great evidence of this approach implementation as the other two products do.

All medicinal products pose the risk of lack of efficacy, but since chondrocyte-based medicinal products are intended to restore knee's functional properties, efficacy is an important feature to be evaluated for these products, with specific functional studies being performed, such as testing of biomechanical properties like aggregate modulus. These studies were performed, as part of primary pharmacodynamics, for MACI and Spherox and only for ChrondroCelect such functional evaluation was not conducted. Besides these biomechanical properties evaluated for MACI and Spherox, all ATMPs underwent structural properties evaluation of *de novo* tissue formation, allowing to prevent lack of efficacy issues such as graft failure caused by delamination.

The other possible hazard identified was graft hypertrophy, due to inappropriate cell proliferation, which might lead to pain or joint swelling, not allowing to restore normal knee's functional properties, without symptoms, as desired. This risk was assessed with evaluation of tumor development, analyzing cell senescence and ectopic tissue formation, through biodistribution and tumorigenesis studies performed.

Furthermore, local inflammation was also identified as a possible adverse event, particularly due to medicinal products expected local effects and local implantation site. Thus, all three ATMPs considered for this category underwent local tolerance studies, assessing Graft versus Host Disease, inflammatory signs and tissue necrosis.

Conclusively, by following a risk-based approach, particularly for Maci and Spherox, it was possible to conduct only relevant studies considering medicinal products' characteristics, reducing the number of studies performed and the number of animal models used. One particular example of risk-based approach implementation for these medicinal products is the absence of repeated-dose toxicity studies, being justified by the single administration procedure used for chondrocyte-based products, which validate the omission of such evaluation.

ATMPs with a local application and posing systemic risks

Belonging to the category of ATMPs with a local administration approach and posing systemic risks is Alofisel. This medicinal product is administered through

a local intralesional injection in the anal fistula wall. Alofisel entered the market after guideline on risk-based approach was made available, thus it was possible to identify clear evidence of this approach implementation throughout Alofisel's assessment report, allowing to minimize the number of studies performed and optimizing the relevant data acquired.

The first risk identified was tumorigenicity, due to stem cell proliferation capacity, making it relevant to evaluate cell proliferation, senescence and telomerase activity, allowing to comprehend the risk of tumor formation.

Besides, migration was also identified as a relevant risk, since cells may show some migration capacity from injection site, due to their stem cell nature, making it relevant to assess distribution from local delivery location. Furthermore, since in Alofisel administration site there is a high number of capillaries and blood vessels, it is possible that the medicinal product enters the systemic circulation inadvertently, making it relevant to evaluate an intravenous route, studying systemic medicinal product distribution.

Finally, immunotoxicity was also identified as a possible risk, considering Alofisel immunoregulatory expected effects, which might lead to some adverse events, caused by unwanted immune response. Thus this hazard was assessed during immunogenicity studies.

ATMPs with a systemic application and posing local risks

Within this category it is possible to find Heparesc. This medicinal product is administered using portal vein bloodstream, as a concentrated cell suspension, posing relevant risks inherent to its administration approach.

Considering Heparesc composition, intended mode of action and administration approach, it was clear to recognize that key unfavorable aspects regarding to its use are related to the administration approach, an intravenous delivery, particularly an intraportal cannulation, which might lead to possible complications such as portal vein thrombosis and pulmonary embolism. Thus, during biodistribution studies, sinusoidal uptake capacity was evaluated as well as hemodynamic alterations, allowing to evaluate possible local outcomes.

ATMPs with a systemic application and posing systemic risks

Zalmoxis was classified as belonging to this category, due to its particular characteristics, namely the genetic modification of T cells administered to the patient.

The first risk identified was the Graft versus Host Disease (GvHD), since in case the treatment fails immunotoxicity events may occur, leading to a Graft versus Host Disease scenario. This ATMP is constituted by T cells genetically modified to include a suicide gene which respond to ganciclovir, allowing to selectively kill T cells containing the suicide gene, when Graft versus Host Disease occurs and proliferation of such cells is verified. If T cells do not respond as expected to ganciclovir, they will continue to proliferate which can lead to serious adverse events and conduct to undesired administration of immunosuppressive drugs. Thus, functionality studies were performed during primary pharmacodynamic evaluations. These studies allowed to assess the response to ganciclovir that T cells, containing the suicide gene showed and in this way evaluate the efficacy of the suicide system used in Zalmoxis.

Moreover, also carcinogenicity was identified as a relevant hazard, due to this ATMP's possible oncogenic risk, related to the potential of insertional mutagenesis caused by vectors integration in the human genome. To assess such potential hazards insertional patterns, gene expression profile and clonality of cell populations were studied during *in vitro* carcinogenicity evaluation.

5.3 Overview of shared non-clinical testing properties for considered ATMPs

From the extended research performed to find meaningful regulatory documents, bearing in mind the scope of this thesis, it was not possible to find a guideline assessing requirements for FIH studies when considering ATMPs, since such regulatory document is still not available due to ATMPs relative novelty. However, the document *Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products* [13] was considered relevant to be analyzed in comparison with overall conclusions gathered throughout previously discussed study phases. Though recommendations present in this guideline are not within the scope of advanced therapy medicinal products, general considerations may be applied to ATMPs' development processes [24]. The previously referred guideline include non-clinical considerations regarding efficacy and safety concerns, particularly pharmacodynamics, pharmacokinetics and toxicology, which were found to be in common with all evaluated ATMPs' non-clinical development processes.

Overview of animal models used during non-clinical development program

Firstly, one important point to stand out when it comes to non-clinical evaluations is the relevance of the animal models used. Such models should allow to obtain significant outcomes, mimicking the intended therapeutic effects and possible adverse events and allowing to reflect human disease in all its characteristics. Thus, both small models, for example mice, and larger animal models, such as sheep or horse, must be considered to reflect as much as possible as well as extrapolate human expected

outcomes. One important example of the relevance of an adequate animal model's choice is obtained when looking at the animal used to evaluate chondrocyte-based medicinal products, for which functional studies were developed using larger animal models, due to higher similarities of anatomical and physiological characteristics, allowing to better predict human outcomes. On the other hand, for Alofisel and Zalmoxis, medicinal products of allogeneic origin, immunocompromised small animal models (i.e. mice and rats) were used allowing to evaluate possible therapeutic outcomes or adverse events and avoiding immediate immune recognition of human cells and their consequent rejection, due to xenogeneic medicinal product origin.

When it comes to Heparesc, a rabbit animal model and rabbit liver cells were used to assess possible therapeutic outcomes and adverse events. For this ATMP, it is important to stand out that despite the existence of a more relevant animal model mimicking one of the intended indications, rabbits and rabbit cells were used in an attempt to correlate non-clinical outcomes obtained with further clinical results. Thus, it was possible to generally support the concept of intraportal hepatocytes perfusion and indirectly evaluate proof-of-concept outcomes, although this animal model of disease lacks demonstration of human expected outcomes when it comes to efficacy of the ATMP.

Additionally, recommendations related to the use of *in vitro* models can be found in the considered guideline for FIH studies and they were possible to be observed in all considered ATMPs' non-clinical development processes, apart from Heparesc, for which only animal studies were performed. Such *in vitro* systems are crucial, particularly allowing to study human cell structures and avoid misinterpretations of possible human use consequences caused by animal models' outcomes.

Overview of pharmacodynamic studies performed

The aforementioned guideline addresses primary pharmacodynamic studies as intending to provide outcomes on product's mode of action and functional outcomes, a recommendation followed in every considered ATMP's non-clinical program evaluated. Besides, secondary pharmacodynamics and safety pharmacology studies must only be conducted on a case-by-case basis considering expected medicinal product outcomes, following a risk-based approach.

Overview of pharmacokinetic studies performed

As recommended in the mentioned guideline, all ATMPs performed pharmacokinetic evaluations, which allowed to support outcomes obtained in pharmacodynamic studies and further assess biodistribution. Within pharmacokinetic evaluation, for all ATMPs, standard pharmacokinetic studies (i.e. ADME) were not considered relevant, due to medicinal products cell-based nature and only biodistribution was considered appropriate to be assessed.

Overview of toxicology studies performed

Finally, as well as it occurred with pharmacodynamic and pharmacokinetic studies, also toxicology evaluation was conducted during non-clinical studies of all evaluated ATMPs. Within toxicology evaluation, main differences were encountered for repeated-dose studies, only performed for Heparesc, since this ATMP is intended to be administered using six daily doses, thus justifying the relevance of repeated-dose assessments. As the remaining ATMPs are only administered once, this evaluation was not performed.

6. Conclusions

Advanced therapy medicinal products have been exhibiting great impact in healthcare over the past several years, allowing to obtain therapeutic positive outcomes to so far unmet medical needs and life-threatening conditions. However, as time goes by, the number of ATMPs already available in the market remains quite low when compared to the positive impact this innovative pharmaceutical field has showed. This unpleasant reality is justified with the complex nature of these medicinal products and their patient-specific characteristics, making the market entrance an arduous procedure. Due to ATMPs relative novelty and complex nature there are still an unsatisfactory number of regulatory documents identifying the specific requirements for these medicinal products' market entrance approval. It is also important to stand out the absence of a single scientific guideline when it comes to requirements for first-in-human studies that includes gene therapy, cell-based and tissue engineered products. Such guideline could be analysed in conjunction with Regulation (EC) No 1394/2007 [3] and other regulatory documents concerning ATMPs, allowing broader guidance to be given and helping in non-clinical development process. Due to the absence of a scientific guideline amending FIH requirements for ATMPs it was considered relevant, firstly to compare guidelines assessing preclinical data package for different medicinal product categories. From this study phase, it was possible to comprehend that the core goal of preclinical studies was identical independently of the medicinal product under evaluation. All non-clinical studies must allow to acquire relevant data on safety and efficacy of the intended mechanism of action and mode of administration, allowing to acquire information on proof-of-principle and assess possible human adverse events from extrapolation of results obtained with the animal models used and *in vitro* studies performed. However, the way this data is acquired is product-specific. The best example of

diverse requirements for ATMPs and standard pharmaceutical products are found for pharmacokinetic evaluations. When it comes to small molecules, absorption, distribution, metabolism and excretion must be assessed, on the other hand, for cell-based medicinal products, cells migration and biodistribution must be considered. Thus, although these studies are distinct they allow to obtain information with regard to the same category, pharmacokinetics.

Further analysis performed, and divided in three major phases, intended to compare and evaluate non-clinical studies performed for ATMPs that showed some similar and distinct features, namely ChondroCelect, Maci, Spherox, Alofisel, Zalmoxis and Heparesc.

Firstly, chondrocyte-based medicinal products were compared. For instance, for all of these ATMPs, larger animal models were evaluated to better mimic human expected outcomes and perform relevant functional studies to assess successfulness of the evaluated medicinal product. Another relevant point was the absence of repeated-dose studies for chondrocyte-based medicinal products since all are intended to be administered once and to show long-term effects.

Secondly, bearing in mind the risk-based approach, it was possible to assess the most relevant studies conducted acording to the risks and risk factors identified for a medicinal product, considering its mode of administration and intended therapeutic effect. From this study, it was possible to observe that ATMPs entering the market after the guideline on risk-based approach for ATMPs was made available show greater impact of this methodology implementation in their non-clinical development programs, than ATMPs entering the market previous to this guideline release.

Finally, the third case studies phase, intended to gather general insights of non-clinical evaluation programs. Thus, it was possible to comprehend that the goals of all studies performed were common to all ATMPs assessed, as well as the animal models used, with particular focus on large animal models, used for all chondrocyte-based medicinal products, to perform relevant functional studies and mimic human anatomical and physiological characteristics. Besides it was possible to comprehend that some studies were only performed if the risks identified justified so such as local tolerance or repeated dose studies.

Conclusively, the comparative study conducted during this thesis development allowed to gather some conclusions on requirements for FIH studies, after the analyses of relevant scientific guidelines and the six different ATMPs preclinical evaluation processes. Further from the conclusions obtained, one major point stands out from the study conducted, the relevance of a dedicated scientific guideline on preclinical studies required to initiate FIH administrations, containing recommendations according to the risk-based approach and bearing in mind the complex and diverse nature of all medicinal products classified as ATMPs. Although this pharmaceutic field still remains very complex, we expect that during the next several years more regulatory documents will be made available, allowing to simplify ATMPs' development and further market entrance, increasing the number of patients using these innovative medicinal products.

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