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Advanced Non-animal Models in Biomedical Research

Immunogenicity testing for advanced therapy medicinal products

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This collaborative study was coordinated by Laura Gribaldo on behalf of the JRC's EU Reference Laboratory for alternatives to animal testing (*EURL ECVAM*).

The collection of non-animal models described in this report is publicly available from the *JRC Data Catalogue*.

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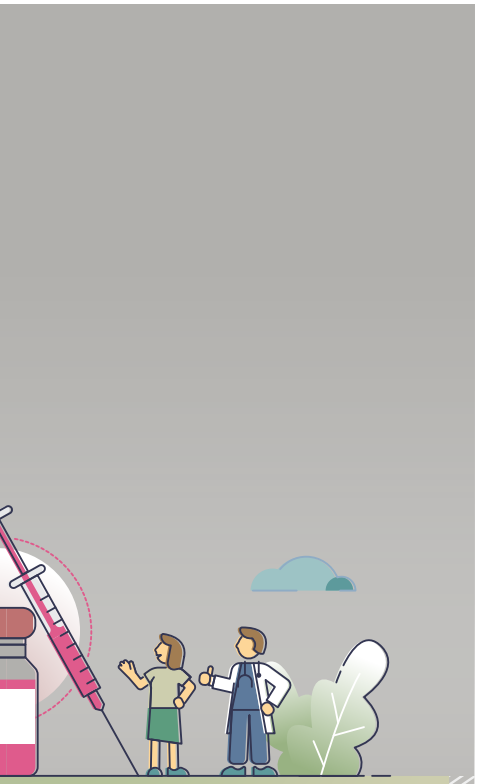


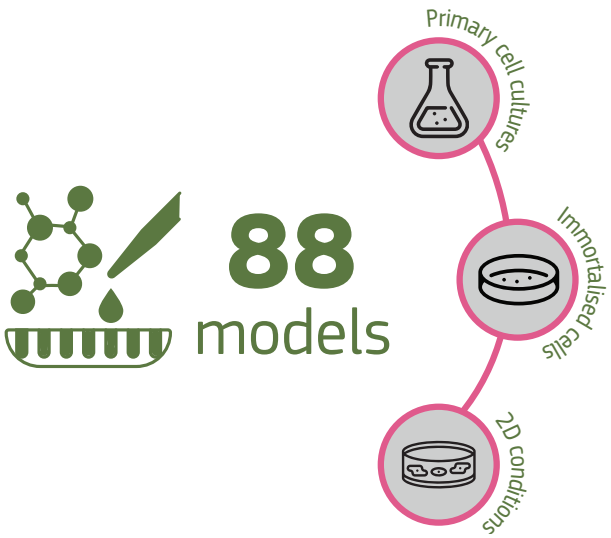
Abstract

Advanced therapy medicinal products (ATMPs) are a fast-growing class of innovative biological products. They include somatic cell therapy, gene therapy, tissue-engineering and their combinations, and they hold the key for new treatments for rare diseases and the establishment of personalised medicine.

Nevertheless, several hurdles are currently hampering the faster development and wider clinical use of ATMPs, one of which is safety and their immunogenic potential. Testing for immunogenicity of cell-based, gene-based or tissue-engineered products in animals has uncovered the intricate limitations of *in vivo* models. There is, therefore, a growing need for innovative non-animal methods to better mimic patient immune responses to ATMPs.

The JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) conducted a study to review the state-of-the art of advanced non-animal models used in immunogenicity testing for ATMPs. The majority of the 88 models that were identified were based on *in vitro* techniques with a limited amount of *in silico* methods, and most of these focused on the investigation of cell therapy products. Furthermore, most of the identified methods used classical 2D primary cell cultures in a low throughput format, typically yielding limited amounts of measurement information.

This review highlights a pressing need for the development and acceptance of more sophisticated and innovative non-animal approaches in this field which will pave the way for the broader use of ATMPs in the clinic.



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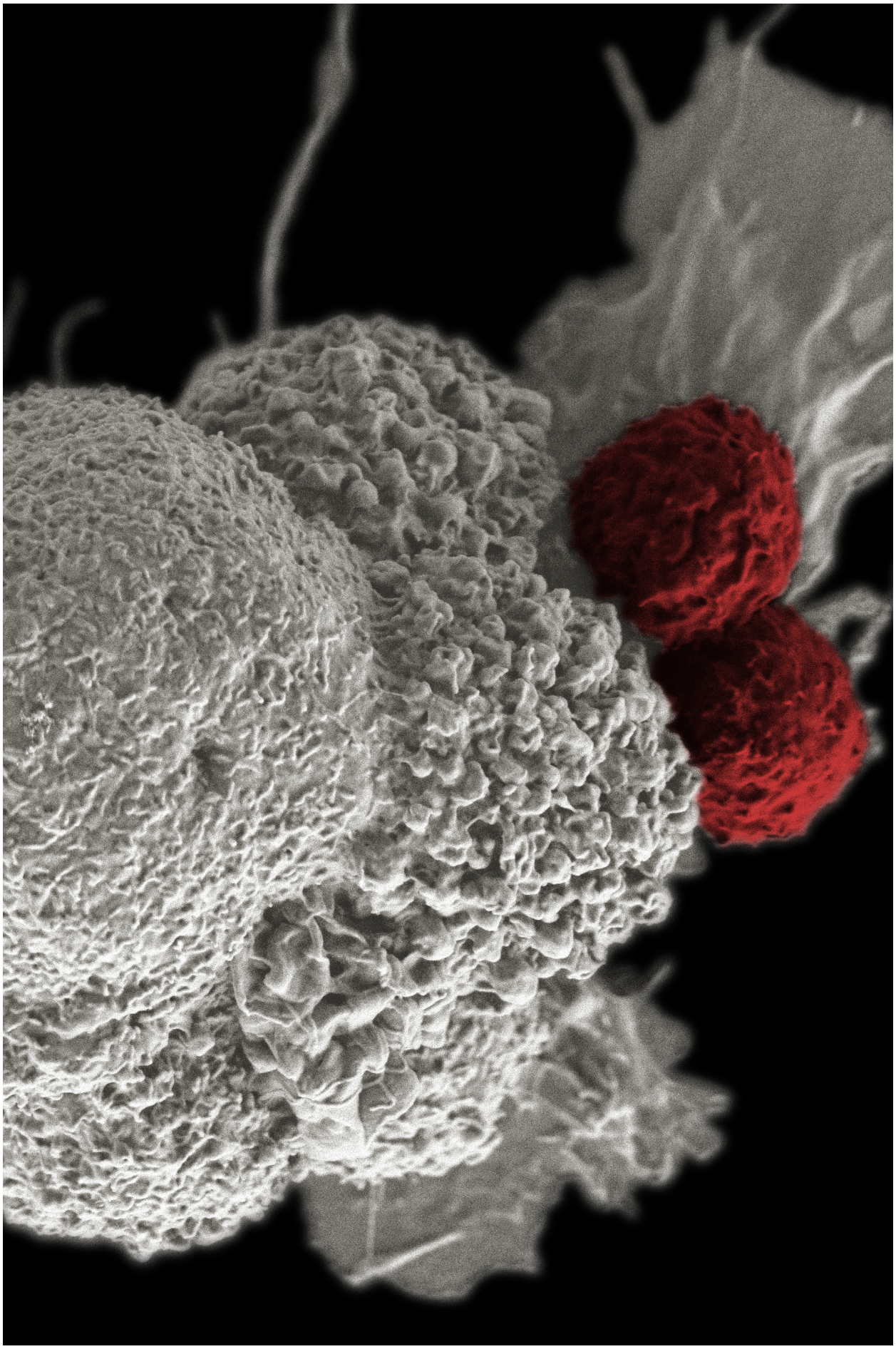


1 Introduction

The steady progress made in recent years in biotechnology research has led to the emergence of advanced therapy medicinal products (ATMPs). ATMPs are innovative therapies with distinct characteristics and include gene therapy products, somatic cell therapy products, tissue-engineered products and combined products[1]. These are expected to reshape our therapeutic approach towards several diseases, confer important health benefits and also substantially impact the world economy. The global regenerative medicine market was expected to grow at a compound annual growth rate of 23.6% from 2016 to 2021 and reach 39 billion USD[2].

Up until early 2020, a total of 14 ATMPs were granted marketing authorisation by the European Commission, following positive evaluations by the European Medicines Agency (EMA). Nevertheless ~1/3 of them were later withdrawn from the market. It is evident that several hurdles are currently preventing the wider clinical use of ATMPs. These include commercial viability (e.g. limited target groups in rare diseases), insurance reimbursement issues (mostly due to lack of evidence proving the effectiveness of an ATMP versus a non-ATMP for a given pathological condition), insufficient standardisation in manufacturing, inadequate training of prescribers (to consider ATMPs as potential choices for their patients), limited efficacy and drug development, complicated administration issues, as well as safety issues (including mutagenicity and immunogenicity).

The promising future of this inherently complex group of medicinal products will only come to fruition if the whole range of stakeholders join forces and overcome the major remaining bottlenecks like immunogenicity. Immunogenicity aspects are a critical point to consider for safety and efficacy assessment of an ATMP, particularly cell-based products. Cell-based therapies fall into two broad classes, i) those derived from the patient's own cells (autologous) and ii) those derived from a donor's cells (allogeneic) (Buzhor et al. 2014; Heslop et al. 2015; Schroeder 2014; Zhang et al. 2015). Autologous cell-therapy has the advantage of being immunologically matched to the patient but, being a one-to-one treatment, is bespoke in nature. As such, both the economic cost of these interventions and the delay on patient treatment can be very high, potentially limiting their uptake in financially constrained healthcare systems. With the latter, allogeneic treatments offer the opportunity of providing a one-to-many ("off-the-shelf") treatment and, if scalable, may be more efficient in addressing the needs of large patient populations. However, in order to meet these needs, allogeneic treatments will need to "escape" immune rejection. In general, the immunological challenges faced by allogeneic products due to potential donor-host incompatibilities, discourage their development and this leads to their application currently lagging behind that of autologous products (Parmiani et al. 2011).

As stated in the original 2008 guidelines and confirmed in the recent 2018 update[3], the EMA stressed that unwanted immunogenicity and its consequences - including anaphylaxis, graft versus host disease, graft rejection, neutralizing

1 Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) Nº726/2004

2 *www.marketsandmarkets.com/PressReleases/regenerative-medicine.as*

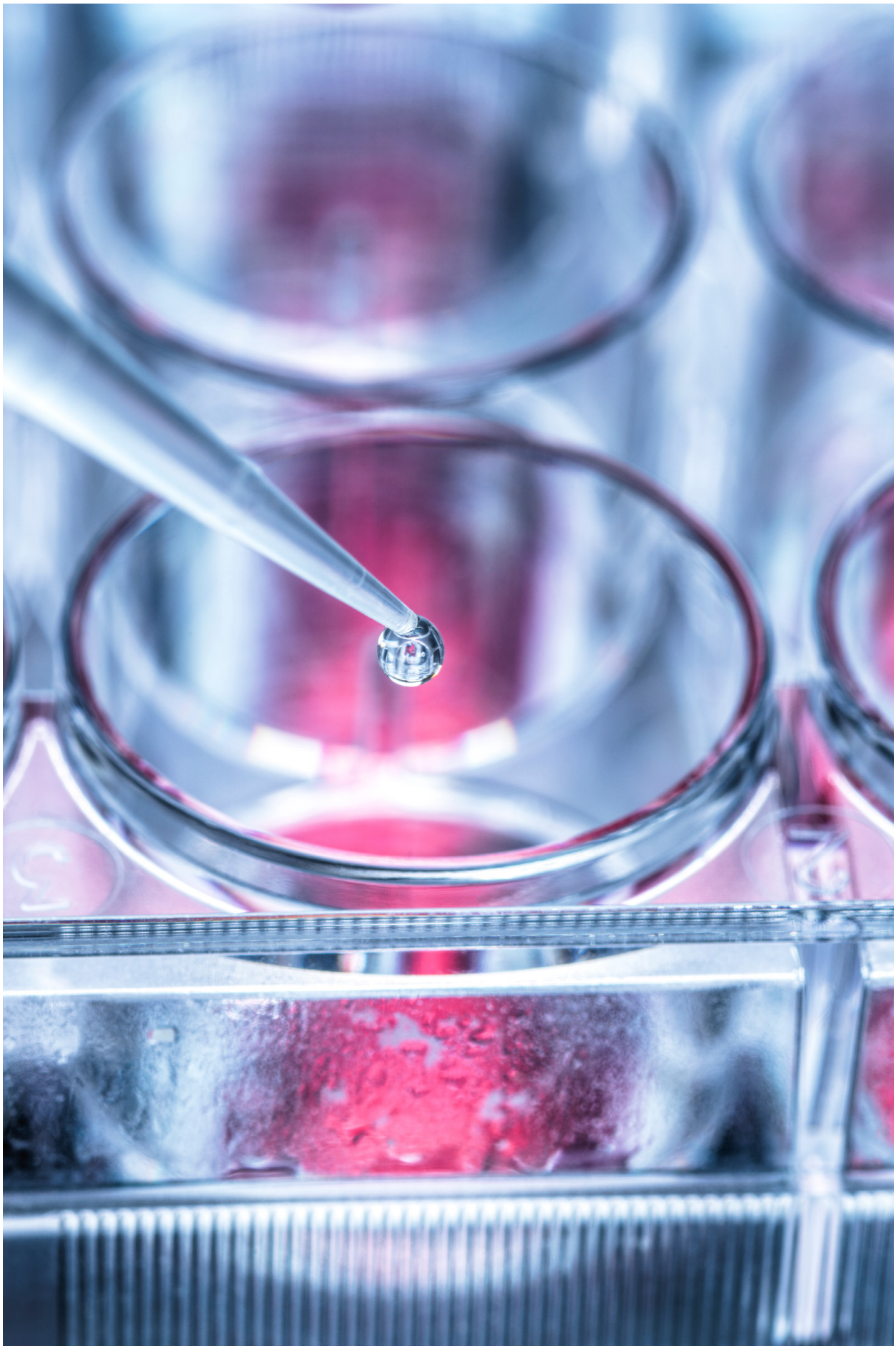
3 EMA Guideline on safety and efficacy follow-up and risk management of Advanced Therapy Medicinal Products EMEA/149995/2008: *www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-safety-efficacy-follow-risk-management-advanced-therapy-medicinal-products-revision_en.pdf*

antibodies, hypersensitivity reactions, immune deficiencies, cytokine release syndrome, inflammation, etc - need to be carefully considered in the context of ATMP marketing authorisation evaluation (for both safety and efficacy). As a consequence, the development of allogeneic products will require a strong focus on addressing the immune response. Whether embryonic or induced pluripotent stem cell-derived allogeneic products for wide scale use will require HLA-matching remains to be determined (Aron Badin et al. 2019). This may depend in some degree on whether it is necessary for allogeneic cells to participate in tissue regeneration processes and remain within the repaired tissue, or if their short-term trophic effects might be more effective. Another factor will be the site of cell transplantation, given that certain tissues or organs, such as the eye or parts of the central nervous system, are more immune-privileged than others. Lastly, strategies might be engaged to encapsulate the donor cells to hide them from the host immune systems in scenarios where the cells provide a paracrine effect (Perin and Silva 2011).

Testing the potential immune effect of a cell-based product in animals exposes many limitations of this type of *in vivo* model. In fact, two different outcomes must be monitored: the behavior at the site of injection (i.e. de-differentiation or unwanted differentiation), and behavior at distant sites, after the migration to unwanted tissues. Moreover, given that cells react in a species-specific manner, nothing might happen upon injection into animals when there is no relevant interaction with animal tissue. However, the animal's immune system will finally recognise human cells as foreign and react in order to eliminate them. This immunogenicity can therefore lead to artificial immunotoxic effects that would not occur or would occur to a lesser extent in patients in an autologous setting (Ciccarese et al. 2016; June 2007). Conversely, the rapid elimination of the cells may mask potential adverse events that would occur at a later stage in patients. Immunodeficient animals or the use of homologous models (e.g. the use of mouse adult stem cells in mice to mimic the cell-based medicinal product to be used in humans) are not satisfactory alternatives. Thus, it becomes evident that animal models may not be the optimal choice in providing reliable data to allow extrapolation of findings to humans (from pre-clinical to clinical).

The EU Directive 2010/63/EU on the protection of animals used for scientific purposes is a primary piece of legislation to ensure and improve the welfare of laboratory animals and to promote the more rigorous application of the 3Rs. To this end, the advancement of alternative (non-animal) methods and the replacement – where possible – of animal methods with non-animal ones, are of paramount importance. Non-animal (mostly *in vitro* and – to a lesser extend - *in silico*) approaches have been developed in the recent years as exceptional immunogenicity assessment assays for predicting the potential relative immunogenicity of antibody-based biotherapeutics. The generation of such *in vitro* assays specifically engineered for ATMPs (Strong, Neumeister, and Levi 2017; Tsilimigras et al. 2017; Zhang et al. 2017) have the potential to positively impact their development and bring more of them to the market and the clinic.

Here we present the results of the systematic literature review of scientific peer-reviewed publications using non-animal methods to test ATMP immunogenicity. The systematic search was performed in *PubMed*, *Scopus* and *Web of Science* databases considering publications from January 2014 to March 2019 in English language. The literature analysis was performed on the data extracted from 88 key articles.



2 Methodology

The review strategy that was used retrieved 15,157 candidate abstracts. After a selection based on titles and abstracts, 222 scientific articles were retrieved for the full-text analysis.

The full-text analysis resulted in the selection of 88 articles, from which all the identified data were extracted and analysed.

2.1 Selection criteria

The systematic search strategy considered any scientific article describing or dealing with *in vitro* human models, or methods, or assays, or test systems in the field of immunogenicity testing for ATMP, based on the dynamic classification shown in Annex-Table 1 as inclusion criteria.

In addition, scientific articles describing or dealing with any *in silico* model, such as algorithms, or mathematical / computational models, or simulations it was considered as inclusion criteria any were also included.

The following initial set of flagged search terms was determined as inclusion search terms, for the publications retrieval, based on title/abstract analysis:

model OR assay* OR "test* system*" OR "in vitro" OR "ex vivo" OR in-vitro OR ex-vivo OR organoid* OR spheroid* OR 3D OR coculture OR co-culture OR microfluidic* OR microphys* OR biops* OR explant* OR "cell culture" OR "stem cell*" OR stem-cell* OR "primary culture" OR simulation* OR algorithm* OR mathematic* OR computation* OR chip*

The proposed search strategy considered the exclusion criteria listed in Annex-Table 2 and the following initial set of flagged search terms were determined as exclusion search terms for the publications retrieval based on title/abstract analysis:

"mouse model" OR murine OR mice OR rat OR rats OR "Controlled Study" OR "Priority Journal" OR "Major Clinical Study" OR "Animal Experiment" OR "Animal Model" OR "Animal Tissue" OR "Prognosis" OR "Follow Up" OR "Follow-Up" OR "Retrospective Stud" OR "Prospective Study" OR "Case Control Study" OR "case stud*" OR "case-stud*" OR "Nude Mouse" OR "Psychology" OR review OR "Case Report" OR questionnaire* OR "Diagnostic Imaging" OR "Mammography" OR cross-sectional OR survey* OR "Meta-Analysis" OR "meta-analysis" OR hiv OR infection* OR aids OR hepatitis OR influenza OR "clinical trial*" OR xenotransplant* OR xenograft* OR papilloma* OR gvhd OR "qualitative study" OR workshop OR sympos* OR "conference* proceeding*" OR cohort OR descent OR ancestr* OR participant* OR population OR gwas OR "genome wide analysis" OR "methyl* analys*" OR polymorphism**

2.2 Information sources

To perform the systematic literature search, it was agreed to focus on human-based models published in the last five years (January 2014 up to March 2019). In order to generate the most inclusive datasets, multidisciplinary citation databases and indexing services (*Web of Science* and *Scopus*) and the specific biomedical sciences citation database, *PubMed*, were used.

Furthermore, grey literature sources of information were monitored to retrieve news and/or highlights on non-animal methods in the field (Annex-Table 3).

2.3 Systematic search

We finally retrieved 88 full-texts from where the data were extracted and analysed. However, in order to conclude on the selected full-texts, we applied two strategies in five phases, as illustrated in Figure 1.

Using a bottom-up strategy, we initially retrieved a total number of 14,585 scientific peer-reviewed journal articles by searching in the information sources with search terms, assigning a score to each publication and ranking them according to the presence or the absence of specific terms (strategy A). In parallel, we used a top-down strategy with more stringent search terms for inclusion and exclusion and retrieved 572 articles (strategy B). By summing up the two results, we obtained a total of 15,157 peer-reviewed scientific articles.

After a selection based on titles and abstracts, we finally sorted out 222 publications for full text review that eventually resulted in the inclusion of 88 articles.

2.4 Method summary

The data from the scientific articles were extracted based on the following method-summary format including the fields that are reported in Annex-Table 4.

The resulting collection of advanced non-animal models is publicly available from the EURL ECVAM collection in the JRC Data Catalogue[4].

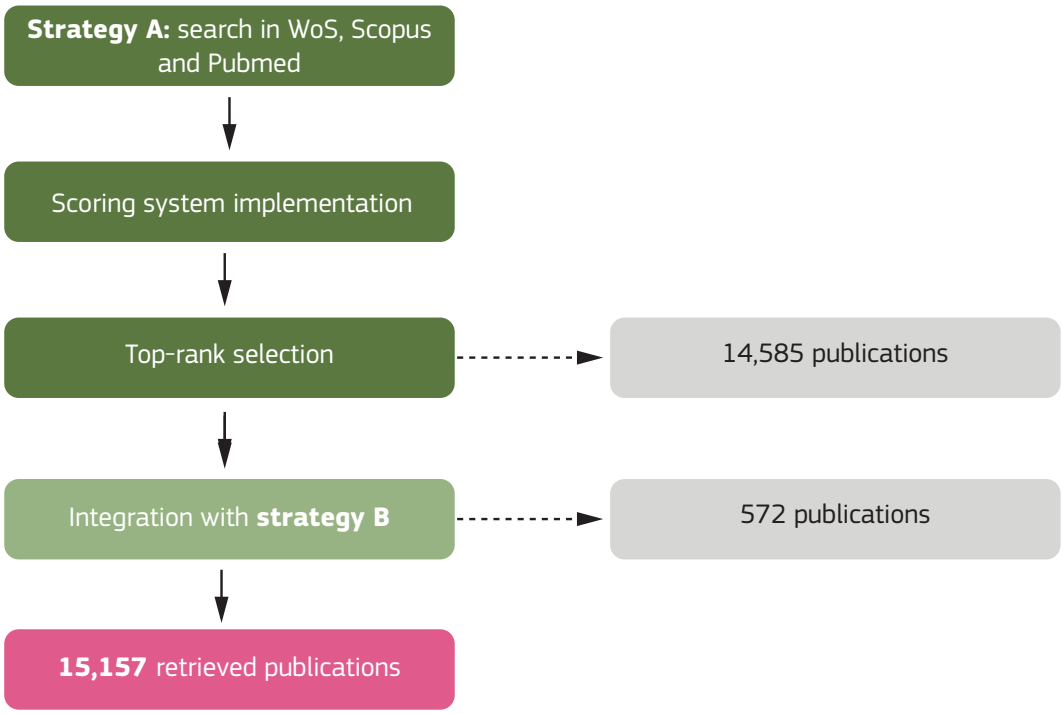
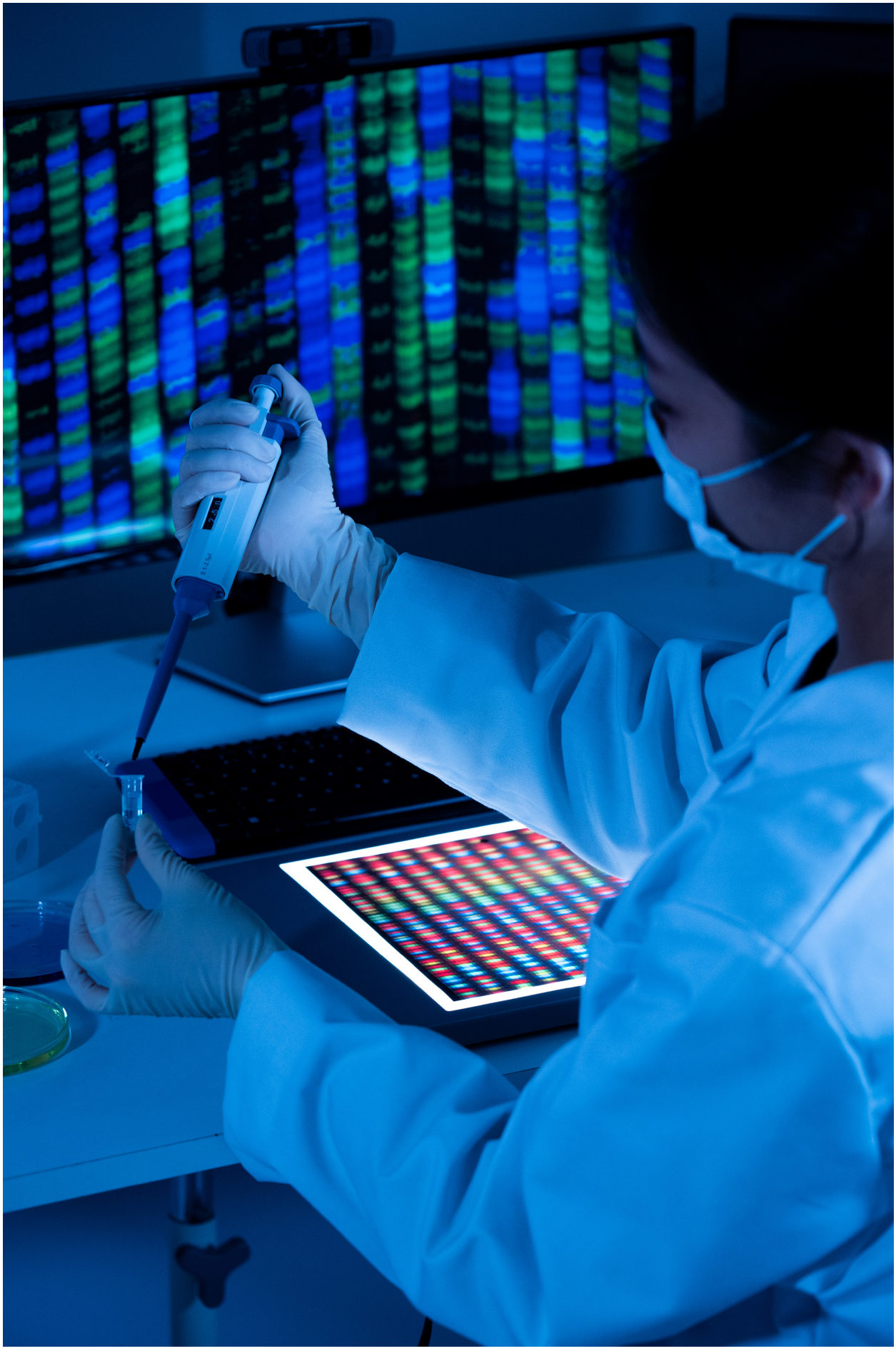


Figure 1: Selection process of the abstracts.

4 https://europa.eu/!DwGdTG



3 Results and discussions

3.1 Advanced non-animal models by ATMP class

ATMPs refer to gene, somatic cell and tissue engineered therapeutic strategies for human disease conditions, hence we studied the use of non-animal models for each type of strategy. The analysis of the 88 peer-reviewed scientific articles retrieved showed that, from 2014 to early 2019, the human models were mainly employed for testing the immunogenicity of cell therapies (73 articles; Figure 2).

We found 6 articles (one in 2014, three in 2015, one in 2017 and one in 2018) describing the use of human-based models for immunogenicity testing for gene therapies. On the other hand, 6 articles (one in 2014, one in 2015 and four in 2017) described the immunogenicity testing for *ex vivo* gene therapies employing non-animal models, and 3 articles (two in 2016 and one in 2018) focused on testing tissue engineering strategies. From 2014 to 2018, the median number of retrieved publications was 17, being lower in 2016 and higher in 2017, with 15 and 21 publications respectively.

Through the analysis of the scientific articles, we identified five applications of non-animal models (see Figure 3). In 66 publications the models were employed to assess immunogenicity during the advanced therapy development and 13 were also used to study the mechanism of action (MoA). In 5 articles, human-based models were used to qualify the ATMP, mostly mesenchymal stem cells (MSCs). In the remaining 4 articles, authors presented the theoretical and experimental development of their models and the possible use in immunogenicity evaluation of ATMPs.

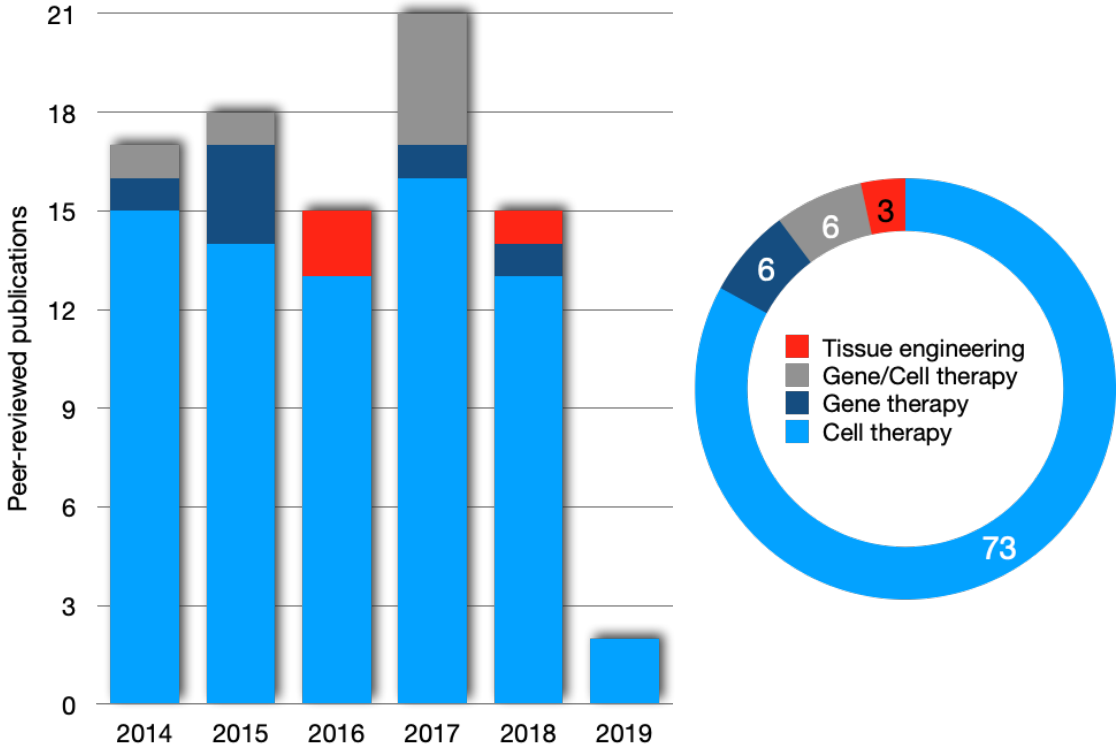


Figure 2: Distribution of the selected scientific studies by ATMP type in the period under consideration.

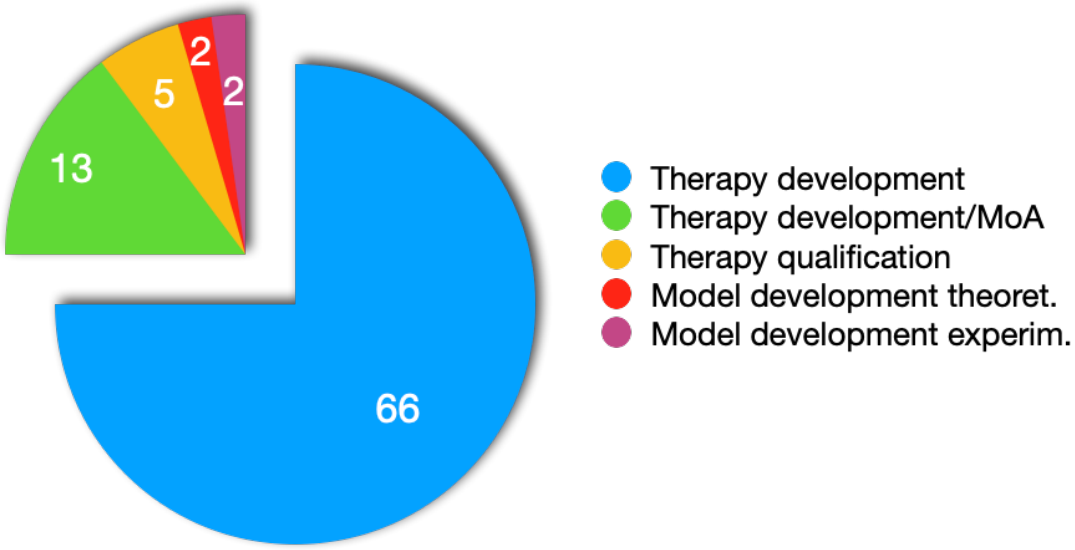


Figure 3: Study distribution by the use of non-animal models for ATMP immunogenicity testing.

3.2 Classification of models by disease feature

The intra-species transplantation of cells, tissues and organs from a donor to a recipient with non-identical genetics is commonly characterised by an immunological response by the recipient immune system. In ATMP development, this is a major challenge for their broad applications and the market release. In fact, 20 articles described models employed to test immunogenicity in allotransplantation paradigms for ATMP applications (Figure 4). In addition, 16 articles described the use of models for testing the immunogenicity of MSCs, which can be applied to several clinical paradigms from reducing inflammation to promote regeneration.

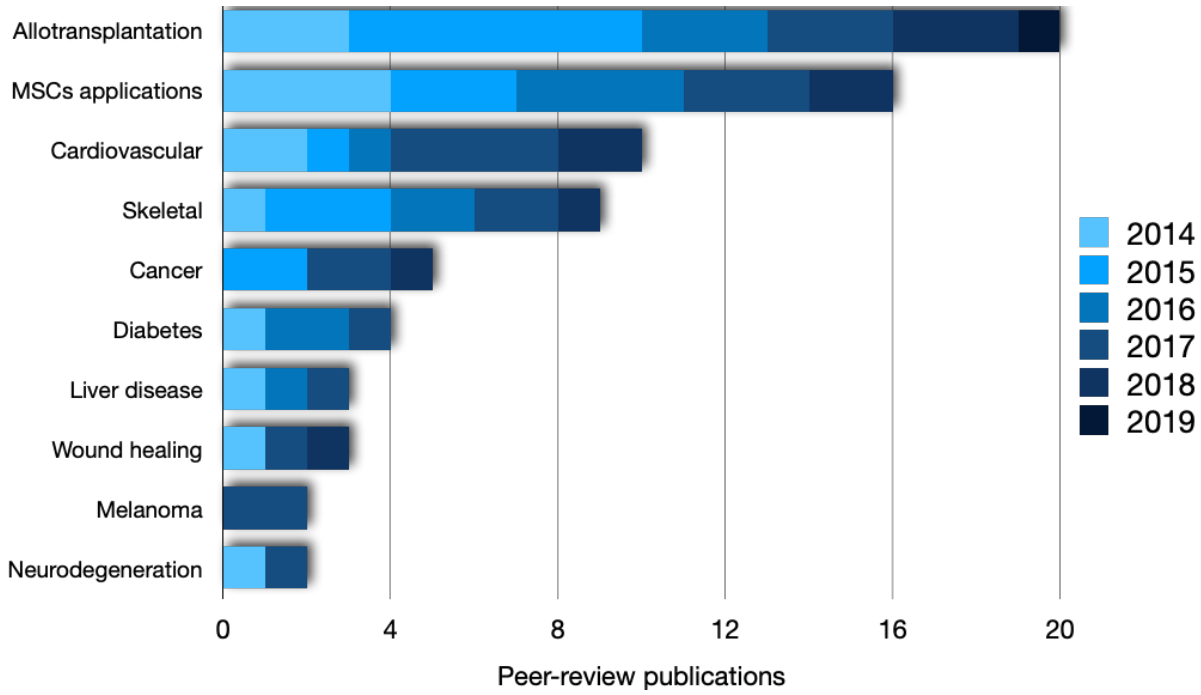


Figure 4: Distribution of the scientific studies by disease area of ATMP application during the period of study. Only disease areas with two or more than two articles are shown.

Immunogenicity testing for ATMP applications in cardiovascular diseases was another major area of study with 10 articles, followed by skeletal disorders (9), cancer (5) and diabetes (4) (Figure 4). Three articles described the use of models for testing the immunogenicity of ATMPs targeting liver diseases and three other focused on wound healing. Melanoma, inflammatory bowel disease and neurodegenerative diseases were other ATMP applications for testing the immune response. The remaining 12 articles described the use of non-animal models for testing immunogenicity of ATMPs targeting different diseases[5].

Analysing the specific aim for the use of advanced human-based models in the immunogenicity tests for ATMP, the modulation of immune response (29 articles) and immunogenic properties (24 articles) were the main features of study (Figure 5). The number of articles reporting studies on the modulation of immune response by these models was constant between 2014 and 2018, except for 2016, when only one publication was retrieved (Figure 5).

The molecular profiling of immune cells, alloreactivity studies, immune response mechanisms and regeneration strategies were the main focus of the immunogenicity tests studied in 25 scientific articles, using non-animal models as pre-clinical tools for ATMP testing. The remaining 10 publications for ATMP immunogenicity testing dealt with immunotherapies, strategies to diminish the immunogenicity and 3D modeling (Figure 5).

3.3 Type and source of cell-based models

Cell-based models were the most commonly used *in vitro* methods to test ATMP immunogenicity, being reported in 85 of the retrieved publications with a median of 17 articles per year between 2014 and 2018 (Figure 6).

T cells were the most frequently used cellular models, among immune cell types used to test immunogenicity (56 articles). Although

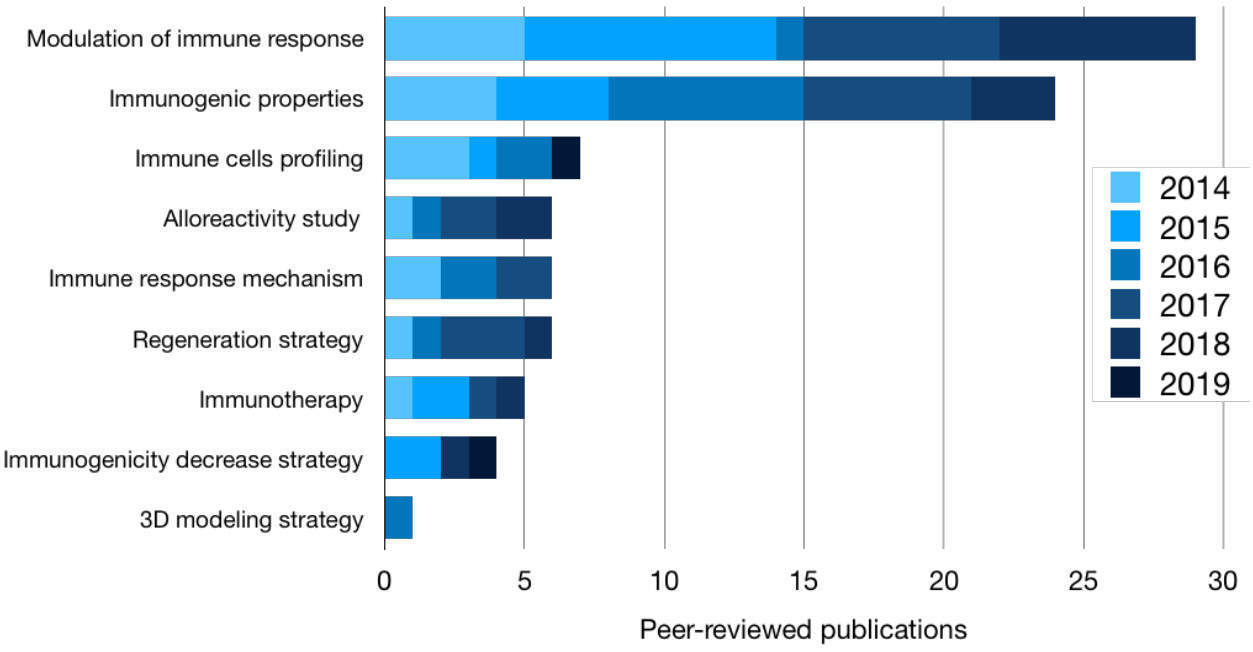


Figure 5: Distribution of the scientific studies by the specific aim of use in the ATMP immunogenicity test.

5 AIDS, alpha-1-antitrypsin deficiency, cartilage disease, cholangiocarcinoma, Ebola, eye diseases, immune disorders, immune oncology, multiple sclerosis, psoriasis, skin diseases, trauma.

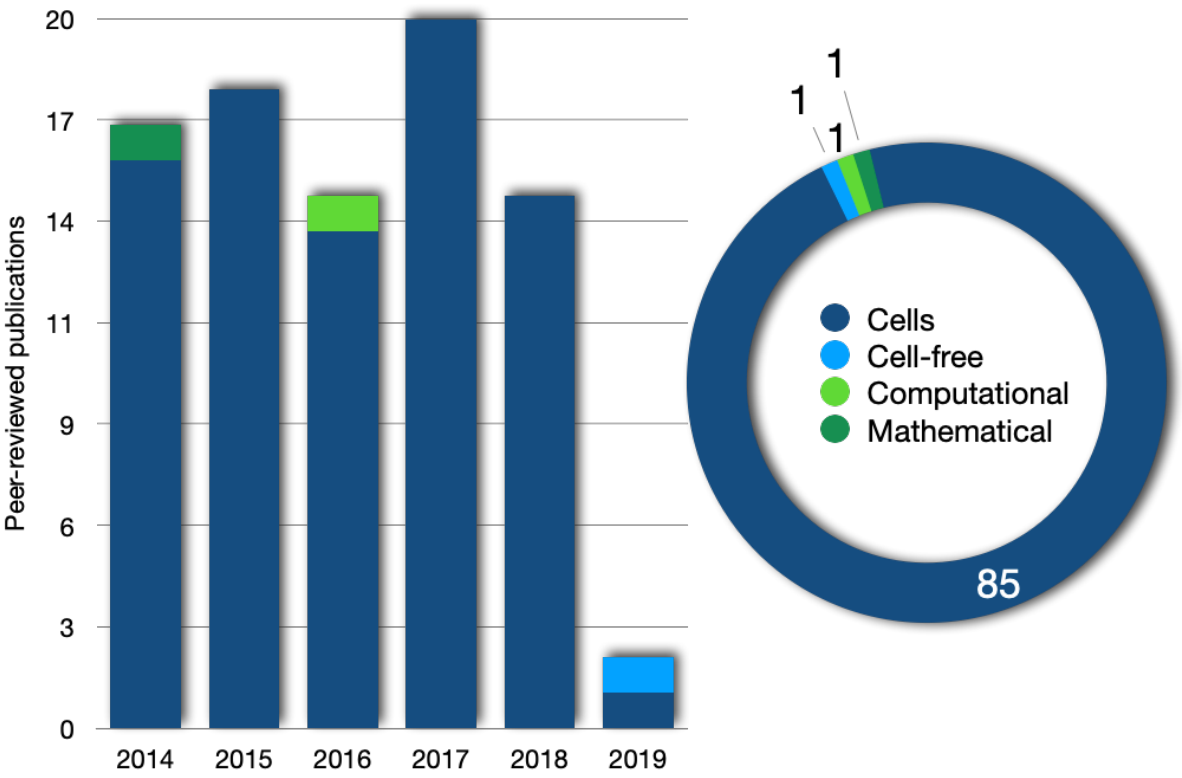


Figure 6: Scientific studies distribution by in vitro (cells and cell-free) and *in silico* (computational and mathematical*) human-based models.

*The classification for mathematical and computational models is based on what was reported in the original articles by the authors. We assumed they were following the definitions previously reported in the literature (Fisher and Henzinger 2007; Hunt et al. 2008).

immune cells were mostly used to test immunogenicity of the cell therapies products, in six publications they were used to assess gene therapies, using RNAs or viral vectors. Two articles reported *in silico* models to study alloreactivity and immune response in allotransplantation by simulating T cells and hematopoietic stem cells.

Seventy-seven articles employed primary cell culture to procure the immune cells (Figure 7), whereas five articles used immortalised cell lines (Figure 7). One article described multiple models employing both immortalised and primary cell cultures to test lentiviral gene therapy in an AIDS study (Wolstein et al. 2014) (Figure 7). Of note, Seet et al. (Seet et al. 2017) described a stem cell-based model to produce T cells from artificial thymic organoids, which can be a model of interest to provide T cells for ATMP immunogenicity tests.

3.4 Cell culture type and number of dimensions of cell-based models

ATMP testing for immunogenicity commonly requires an immune cell population that acts as responder to a stimulus. In our analysis the majority of tested ATMPs were cell therapies, consequently the immune cells tested were co-cultured with the cell therapy candidate in 82 publications (Figure 8). On the other hand, the three studies testing immunogenicity for gene therapies employed single immune cell cultures, such as A549, THP-1 macrophages, peripheral blood mononuclear cells (PBMCs), T lymphocytes and hematopoietic SCs (Figure 8).

Cells used in advanced human models were cultivated in bidimensional (2D) conditions in 76 articles (Figure 9). During the 2015-2018 period, there was a 15% average increase in

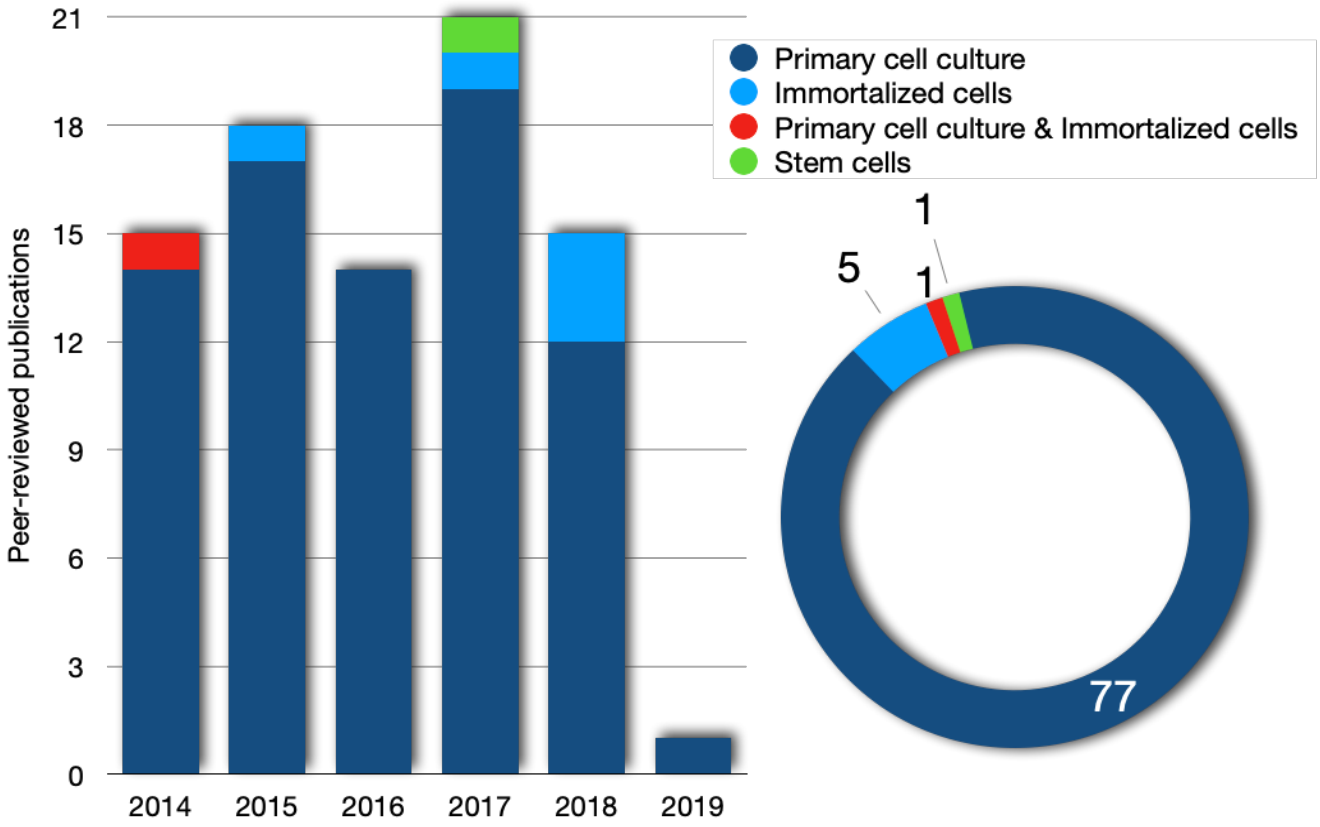


Figure 7: Scientific studies distribution by class of cells used to assess immunogenicity of ATMPs in pre-clinical phase.

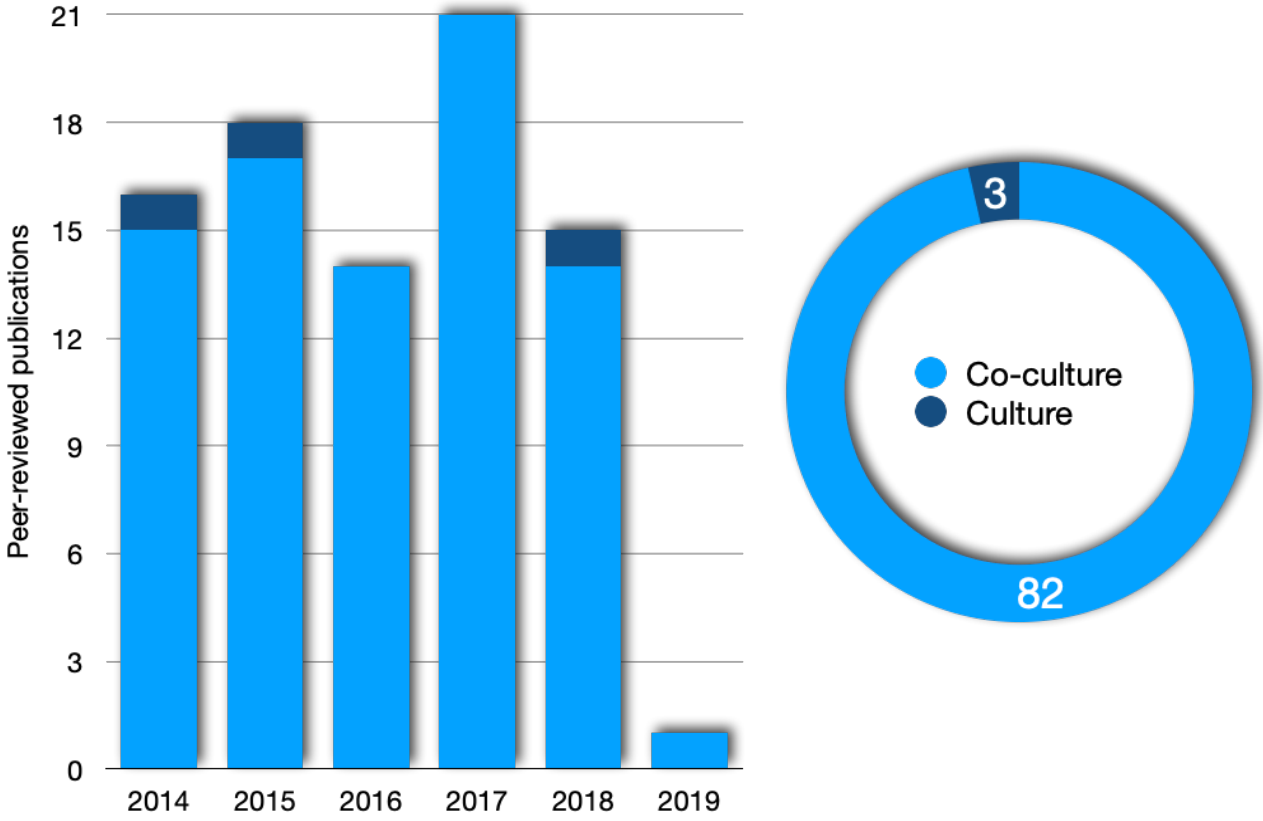


Figure 8: Scientific studies distribution by type of cell culture.

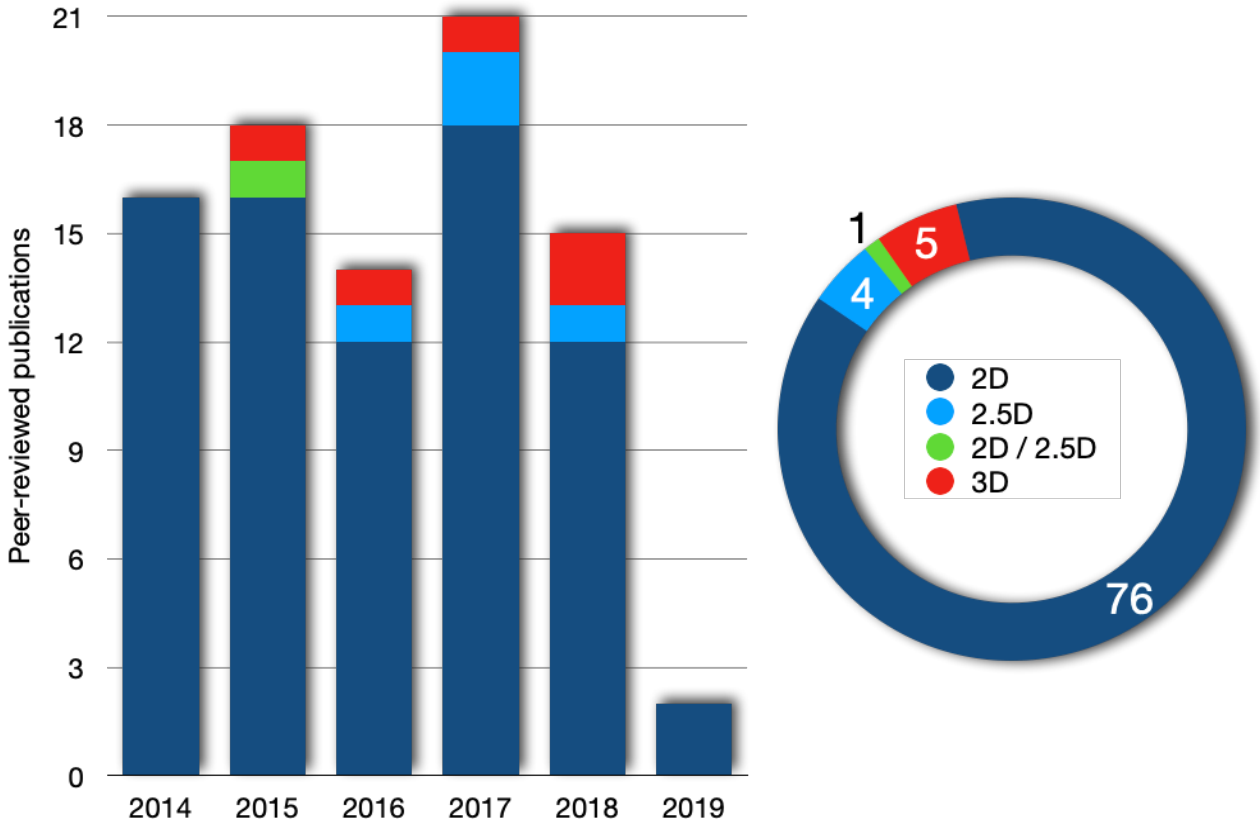


Figure 9: Distribution of articles by cell culture dimension.

the employment of models with more than two dimensions (2D/2.5D: n=1; 2.5D: n=5; 3D: n=5). The 3D cultures were used in three publications for testing scaffolds as tissue engineering solutions scaffolds. Cardiac spheroids immunogenicity was tested by co-culture with T cells (Mattapally et al. 2018) and organoids were used to mimic thymus formation for T cell production (Seet et al. 2017).

We also analysed the throughput and the content described in the articles and 76 studies employed the models in a low-content and low-throughput manner (see table below). We identified only one *in silico* study with a mathematical model with high-throughput and high-content application.

		THROUGHPUT		
		Low	Medium	High
CONTENT	High	0	0	1
	Medium	1	3	3
	Low	76	1	3

3.5 Status of identified non-animal models

The vast majority of the studies, namely 84 articles, employed human-based models for immunogenicity testing for ATMPs that are commonly used in research, as well as in pre-clinical applications (Figure 10).

Meanwhile, only four studies were describing proof-of-concept use of new models to study the immunogenic potential of ATMPs (Figure 10).

In 72 articles, the models had direct relevance for the study features of the ATMP immunogenicity test aimed by the authors (Figure 11). On the other hand, we considered supportive their use for the immunogenicity testing for ATMPs in 16 scientific articles based on the authors' hypothesis (Figure 11).

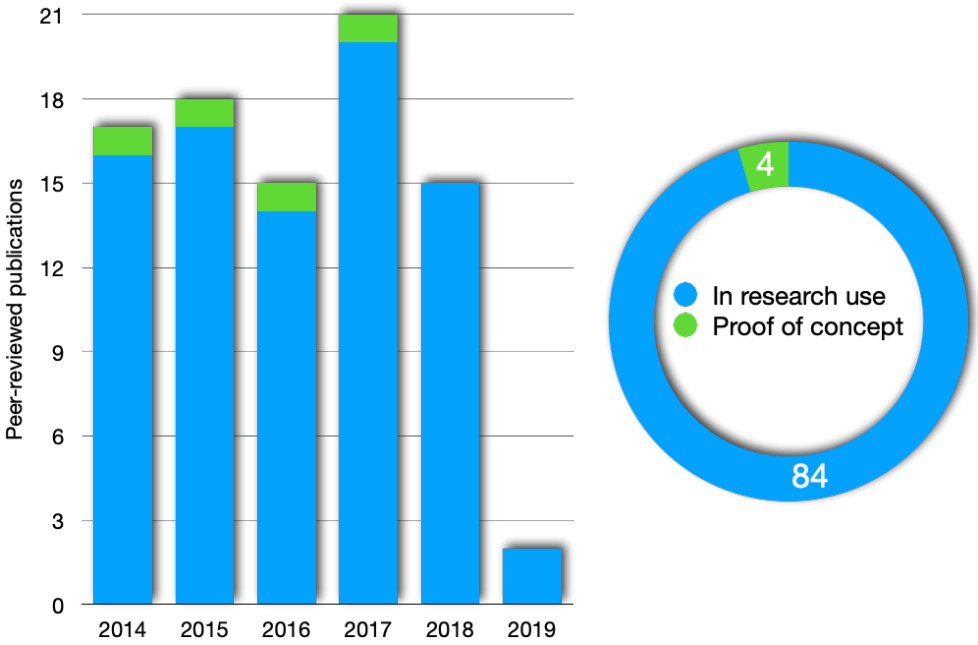


Figure 10: Number of scientific articles classified for status.

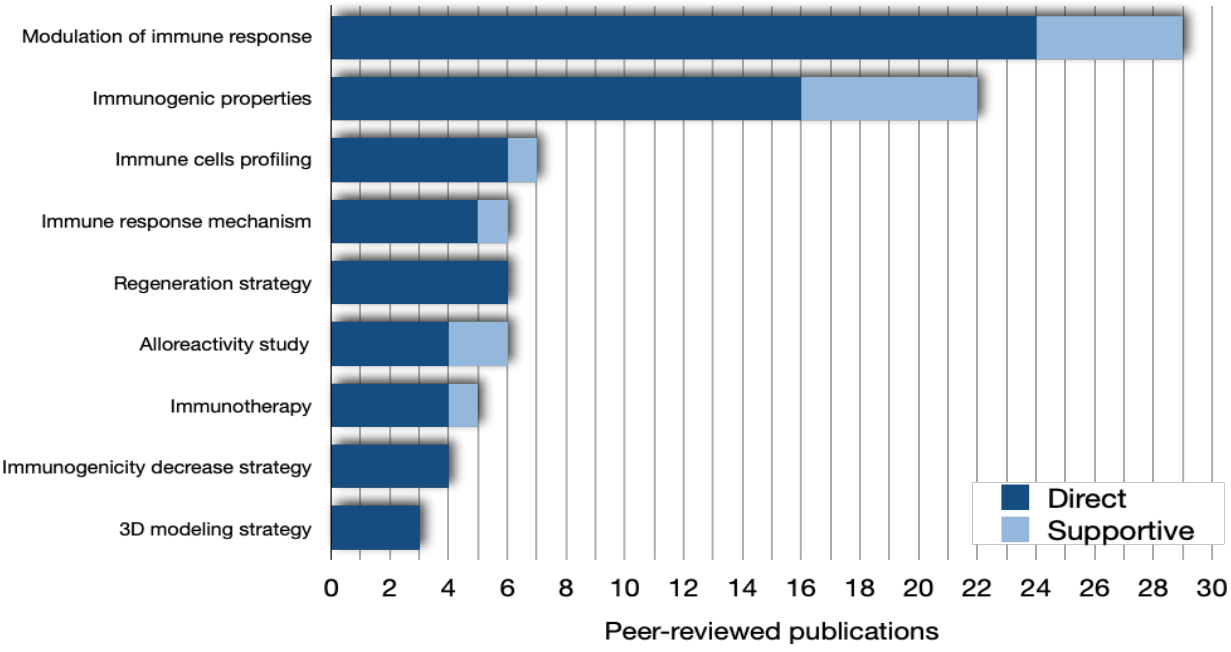
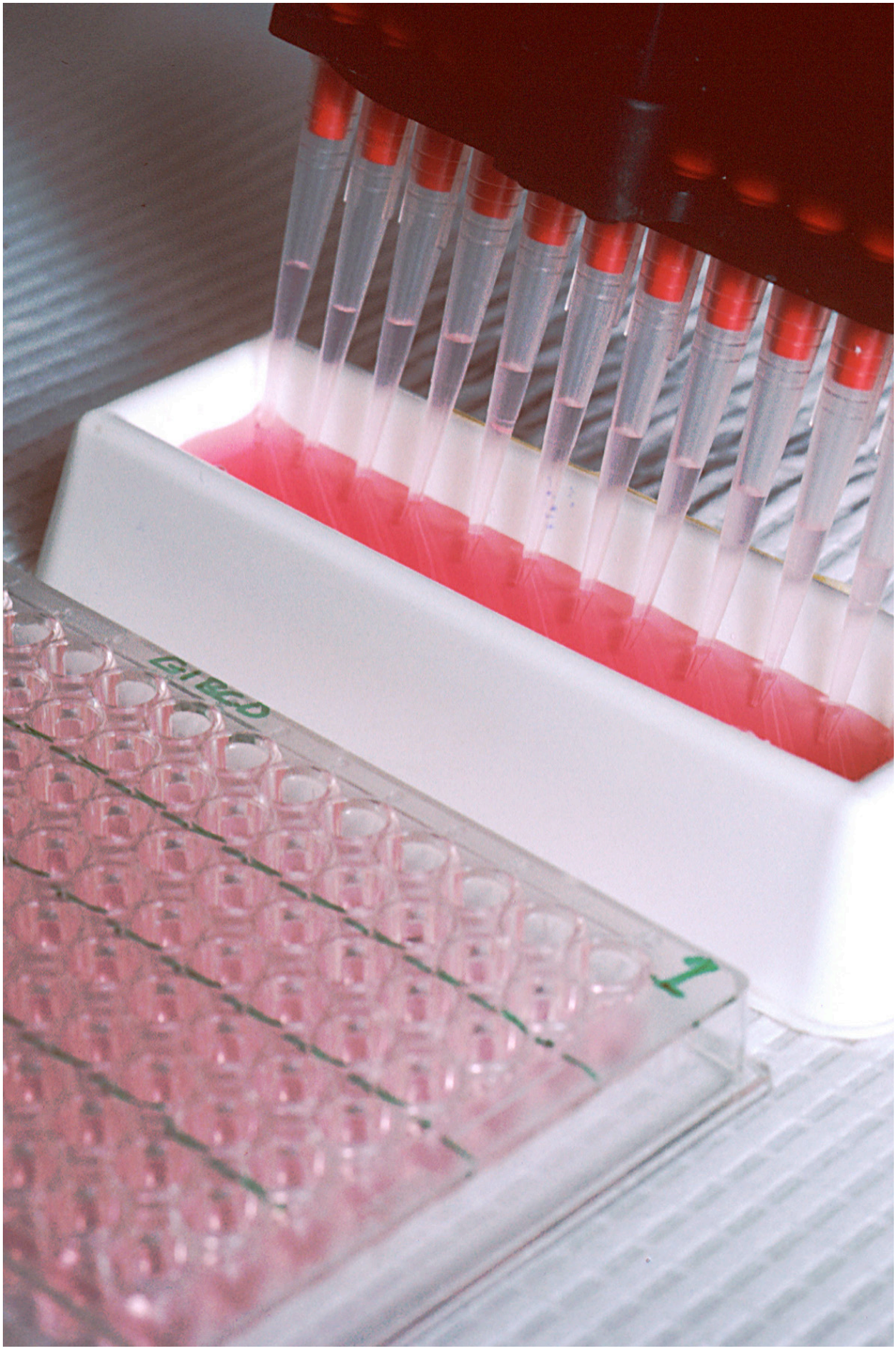


Figure 11: Distribution of the scientific studies by relevance of models' use for each specific aim of application.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4 Conclusions

The scientific development of cell and gene therapies started in the 20th century. The first attempt of cell therapy in humans was a human bone-marrow transplant to reconstitute the bone marrow of irradiated patients with acute leukaemia (Thomas et al. 1959). The first clinical study on gene therapy was reported in 1990 with a strategy involving immunotherapy in order to treat patients with metastatic melanoma (Rosenberg et al. 1990). Since then, the number of clinical trials registered in *ClinicalTrials.gov* for cell and gene therapies has kept increasing. Currently, there are 35,405 registered clinical trials for cell therapies and 4,295 registered for gene therapy[6].

ATMPs – as with all medicinal products intended for human use - are highly regulated[7], in order to assure the (efficacy and) safety of the treatment. Although the current European regulatory testing requirements for ATMPs are mostly based on the use of relevant animal models, there is a lot of potential for the implementation of advanced human-based models and especially *in vitro* models[8].

In particular, European regulation requires the test of immunogenicity for xenogeneic products, including the study of the host immune response triggered by xenogeneic cells bioactive products[9]. Of note, this regulation acknowledged that appropriate animal models reproducing the disease or condition of the patient with similar pathophysiology are not always available, especially when the host immune system is required to achieve the therapeutic result[10]. In these situations, non-animal models would be of paramount importance in providing the necessary safety data.

Our systematic review of human-based models for testing the immunogenicity of ATMPs, identified 88 articles using *in vitro* or *in silico* models to study the immune response to cell, gene or tissue engineering products.

Considering the huge amount of clinical trials involving ATMPs, we consider that the number of scientific articles employing these models was very limited (0.22%). In addition, many of the identified studies were also using animal models in their analysis. Lastly, most of the non-animal models were well-established methods, using primary cell cultures mostly based on isolating fresh immune cells from individuals or from frozen human tissues donation, underlying low innovation in this field of biomedical research. Only one article described a proof-of-concept development for a new method to procure T cells, whose first aim was to provide a source for T cell therapies and not to generate a human-based immunogenicity testing model.

The implementation of low and/or medium content and throughput analysis was another indicator of low innovation and research in the immunogenicity testing field for ATMPs.

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6 Search performed on the 31st of March 2020 in ClinicalTrials.gov. Search terms were: “Cell therapy”; “Gene therapy”.

7 *www.ema.europa.eu/en/human-regulatory/overview/advanced-therapies/legal-framework-advanced-therapies*

8 *www.ema.europa.eu/documents/scientific-guideline/reflection-paper-providing-overview-current-regulatory-testing-requirements-medicinal-products-human_en.pdf*

9 Regulatory provision for immunogenicity testing for ATMPs: EMEA/CHMP/410869/2006; EMA/CAT/571134/2009; and EMEA/CHMP/CPWP/83508/2009.

10 *www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy_en.pdf*

Considering the overall findings of this systematic review of advanced models for immunogenicity testing for ATMPs, the main conclusions are the following:

1 There is a low but constant number of publications relating to the use of non-animal models to test immune response for cell therapy products.

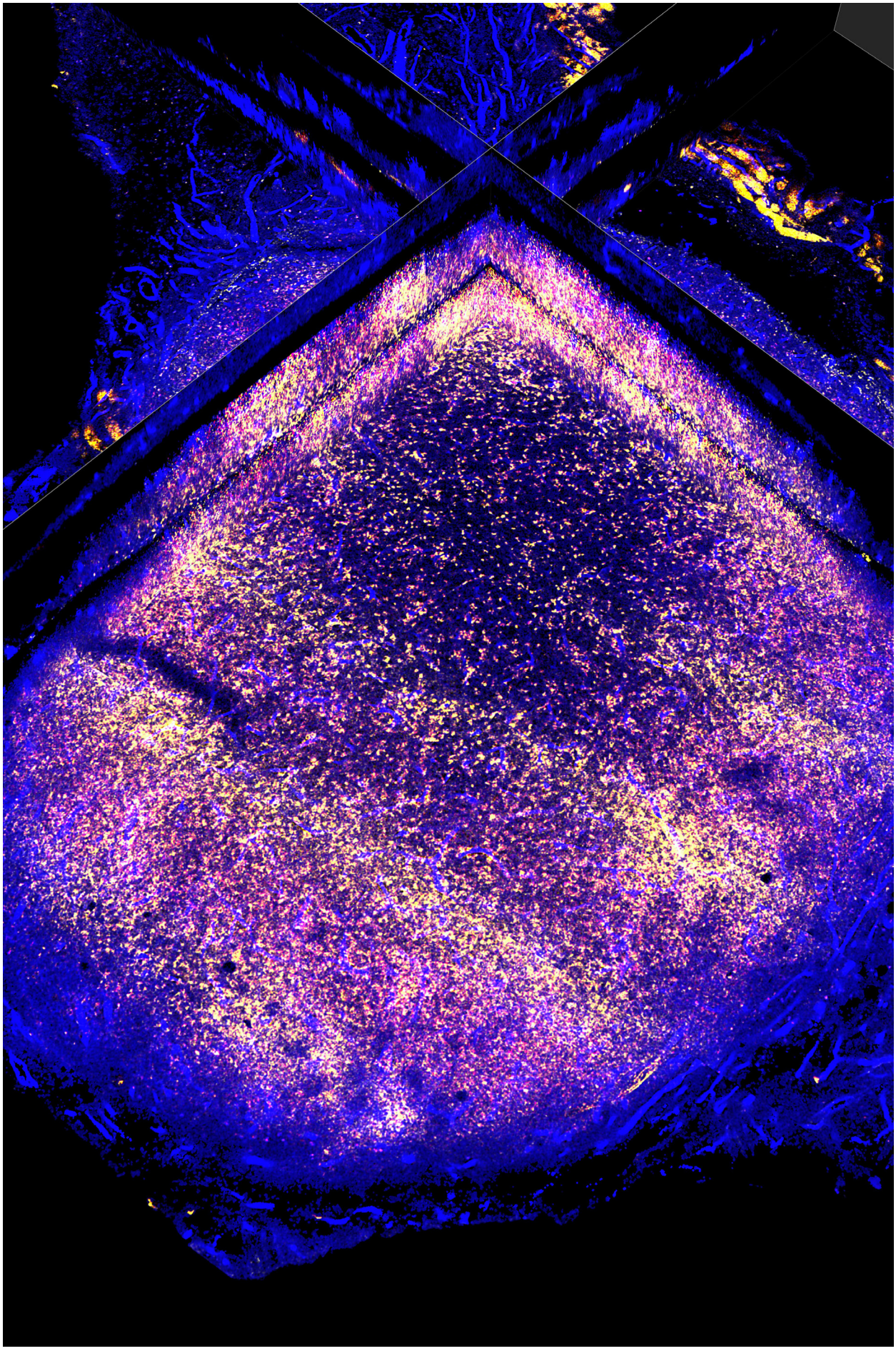
2 Allo-transplantation is the biomedical field mostly studied through human 2D primary culture T cells, with particular focus on immune response modulation in order to reduce allogeneic product rejection.

3 Models using gene therapy and tissue engineering therapy are under-represented in comparison to cell therapies that focus on immunogenicity of ATMPs.

4 The use of human-based models for the immunogenicity testing for ATMPs is offering a wide range of opportunities for innovation, from the development of new testing models to more high-throughput and high-content methods of analysis.

Although our study was originally focused on generating a repository of peer-reviewed scientific articles employing human-based models to test the immunogenicity of ATMPs, it also provided the opportunity to analyse the current situation in the field. Hence, some key actions can be proposed to promote the development, standardisation and utilisation of these models in the context of ATMPs:

- Study the regulatory and scientific challenges hampering a major expansion of the use of non-animal models in this field.
- Strengthen the development of support platforms with ATMP expertise, in order to further develop novel models for specific pre-clinical safety assessment applications, perhaps with targeted research funding.
- Define relevant non-animal models for answering specific scientific questions (i.e. which model for which pathology / disease state) and to push towards their standardisation.
- Bring test developers together with relevant stakeholders to better define needs and oppurtunities and accelerate development and application.



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6 Annex

Table 1: Inclusion criteria used to retrieve scientific articles from literature.

1. Cells cultures and/or co-cultures in 2D, 2.5D, 3D or Microphysiological Systems (MPS)
a. Primary cell cultures
b. Immortalised cell lines
c. Stem cells (SCs)
i. Pluripotent SCs
• Induced pluripotent SCs (iPSCs)
• Embryonic SCs (ESCs)
ii. Multipotent SCs
• Somatic SCs
• Fetal SCs
2. *Ex vivo* material
a. Biopsies
b. Organotypic cultures
i. Explants
ii. Whole organ or organ slice
3. Cell-free assays
Biochemical assays
4. Gene reporting assays

Table 2: Exclusion criteria used to retrieve scientific articles from literature.

1. The study does not deal with immunogenicity testing for ATMP (cell therapy, gene therapy and tissue engineering)
2. Secondary literature (review, meeting abstract, etc.)
3. Duplicate
4. No *in vitro* or *in silico* model or method
5. *In vivo* study
6. Test method not able to measure endpoints
7. The study does not focus on development/characterisation of a valuable alternative test method/model
8. No information on applications
9. The study does not provide mechanistic/pathophysiological or biological relevance
10. No biomedical research application
11. No valuable non-animal model or method
12. Non-English articles
13. Retracted publication
14. Published before 2014

Table 3: Specialised information sources on immunogenicity testing for ATMP applications used for literature searches.

Agencies	
U.S. Food and Drug Administration	*www.fda.gov*
La Agencia Española del Medicamento y Producto Sanitarios	*www.aemps.gob.es*
European Medicines Agency	*www.ema.europa.eu*
Societies	
European Society of Gene and Cell Therapy (ESGCT)	*https://www.esgct.eu/*
British Society for Gene and Cell Therapy (BSGCT)	*https://www.bsgct.org/*
International Society for Cellular Therapy (ISCT)	*https://www.celltherapysociety.org/*
International Society for Stem Cell Research (ISSCR)	*http://www.isscr.org/*
Groups	
European Immunogenicity Platform	*https://e-i-p.eu/*
Events	
Biologics & Biosimilars Congress	*http://www.global-engage.com/event/biologics-biosimilars-congress/*

Table 4: Agreed categories for data extraction.

Field	Definition	Drop-down option
Model number	Model of immunogenicity which is described in a paper	NA
Disease area	ATMP disease target	For example: Cardiovascular Liver disease Melanoma Cancer
Study/Disease feature	The disease feature studied by the model	For example: Immune response mechanism Immunotherapy Regeneration strategy
ATMP type	Type of advanced therapy	Cell therapy Gene therapy Gene / Cell therapy Tissue engineering
Category	The category of non-animal model assigned to the model	*In vitro* *In silico*
Type	More specifications of the model category	Cell-free Cells Computational Mathematical
Cells	If the model employs cells, this field specifies which kind of cells are used	Immortalised Primary cell culture Stem cells
ATMP input	ATMP to be tested	For example: iPSC CAR-T cells T cells
Cell culture type	If the model employs cells, this field specifies the type of cell culture	Cell free culture Co-culture NA
Cell culture dimensions	If the model employs cells, this field specifies the dimensions of the cell culture	2D 2.5D 3D
3D type	If the model uses 3D cell cultures, this field specifies the type of the 3D dimension	Scaffolds Spheroids Organoids
Model Cell Source 1 to 3	If the model employs cells, this field specifies the cell source	For example: A459 NKs T cells
Biological endpoints	List of potential biological endpoints used in a model system to describe the disease mechanism and/or study focus	For example: Activation marker Inflammation response, proliferation, apoptosis Macrophage activation T cell activation

Throughput	Regarding productivity/automatisation of the model	High Medium Low
Potential	Types of model application	Model development / experimental Model development / theoretical Therapy development Therapy development / Mechanism of Action Therapy qualification
Potential 2	Possible multiple model application in addressing disease features	Yes (The method/model has future potential for its immunogenicity applications). No (The method/model has no future potential for its immunogenicity applications). n/a (not specified)
Relevance	Biological relevance of the model for the disease feature in replacing animal models	Direct (The model is sufficient for the conclusions of the study). Supportive (The model is partially supporting the conclusions of the study).
Status	Model developmental stage	In research use Proof of concept
Content	Quantity of information retrieved	High Medium Low
Predictive	Whether the model was used with a predictivity purpose or not	Yes No n/a
DOI or link	Digital Object Identification number to retrieve the publication abstract. If not available, an alternative link is provided	NA
First author name	Name of the first author of the peer-reviewed article	NA
Year	Publication year from 2014 to 2019	2014 2015 2016 2017 2018 2019

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