European Commission

Advanced Non-animal Models in Biomedical Research

Immunogenicity testing for advanced therapy medicinal products

Executive Summary

Joint Research Centre

EUR 30334/4 EN

This Executive Summary describes a study conducted by the JRC's EU Reference Laboratory for alternatives to animal testing (*EURL ECVAM*) to identify current and emerging non-animal models and methods being used for biomedical research related to immunogenicity testing for advanced therapy medicinal products.

The resulting collection of non-animal models are analysed in a JRC Technical report (Canals, J. M., Romania, P., Belio-Mairal P., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Rossi, F., Straccia, M., Daskalopoulos, E. P., and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research – Immunogenicity testing for advanced therapy medicinal products*, EUR 30334/4 EN, Publications Office of the European Union, Luxembourg, 2022, ISBN 978-92-76-49091-3, doi:10.2760/7190, JRC126997) and publicly available from the *JRC Data Catalogue*.

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JRC126997

EUR 30334/4 EN

PDF	ISBN 978-92-76-49093-7	ISSN 1831-9424	doi:10.2760/158699
Print	ISBN 978-92-76-49094-4	ISSN 1018-5593	doi:10.2760/904354

Luxembourg: Publications Office of the European Union, 2022

Design and layout: Debora Valsesia and Adelaide Dura.

How to cite this report: Gribaldo, L., Dura, A., Daskalopoulos E. P., Straccia, M. and Whelan, M., *Advanced Non-animal Models in Biomedical Research – Immunogenicity testing for advanced therapy medicinal products – Executive Summary*, EUR 30334/4 EN, Publications Office of the European Union, Luxembourg, 2022, ISBN 978-92-76-49093-7, doi:10.2760/158699, JRC126997.

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Advanced therapy medicinal products (ATMPs) are innovative therapies expected to reshape our approach towards several pathologies, particularly those that do not respond adequately to conventional treatments, like cancer, neurodegenerative and cardiovascular diseases.

ATMPs are based on gene therapy, somatic-cell therapy, engineered tissues and combinations of those[1] (see Box 1).

Therapies that use biological material from a human donor can cause an unintended and potentially serious reaction of the immune system in a recipient. Thus, immunogenicity testing is an important step in the development of these therapies since it aims to predict an adverse immune response of a patient prior to receiving a particular treatment (see Box 2).

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) No 726/2004.

Europe is a world leader in ATMPs

The European Commission has granted a considerable number of marketing authorisations for ATMPs since 2009, following positive scientific evaluations by the European Medicines Agency and its relevant Committees. This indicates the value of these therapies and supports the considerable investments being made in this field in Europe, particularly in research and development.

However, some ATMPs have been withdrawn from the market because of several obstacles that prevent wider clinical use. These include small target groups (e.g. rare diseases) limiting return on investment, insurance reimbursement issues, as well as poor efficacy and safety due to unwanted immunogenicity.

Moreover, fundamental biological differences between the immune system of different species frequently limit the human applicability of immunogenicity testing results carried

Box 1. **Classes of advanced therapy medicinal products (ATMPs)**

Advanced therapy medicinal products (ATMPs) can be classified into three main categories:

- **gene therapy medicinal products (GTMPs)**: possess an active component containing or consisting of a recombinant nucleic acid (gene). The insertion of the recombinant gene into the body may be used to confer a therapeutic (e.g. to treat a range of pathologies, including genetic disorders, cancer and other chronic diseases), prophylactic or diagnostic effect.

- **somatic-cell therapy medicinal products (sCTMPs)**: manipulated cells (other than germ cells) that are not intended to be used for the same essential function in the body. These products can be administered to humans and confer a pharmacological, immunological or metabolic action, with the aim to treat, prevent or diagnose a disease.

- **tissue-engineered products (TEPs)**: engineered cells or tissues (of human or animal origin) that can be used for repairing, regenerating or replacing human tissue.

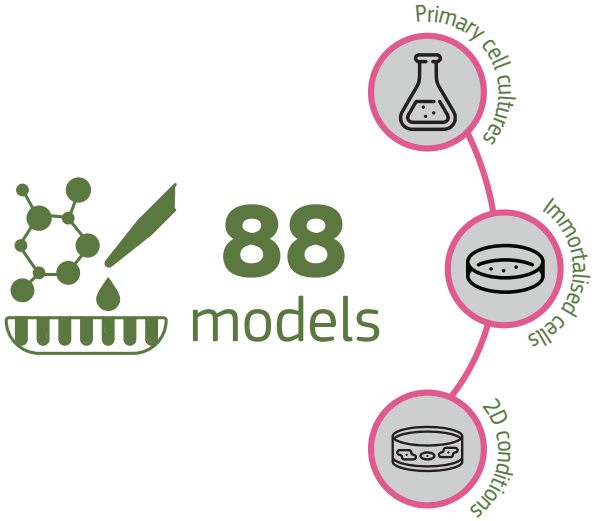
Furthermore, in Europe a fourth category exists, referring to products that consist of an ATMP combined with one or more medical devices ("**combined ATMPs**") as an integral part of the final product. An example of this class is cells that are incorporated into a biodegradable matrix or scaffold.

out using animal models. This can result in poor translation of pre-clinical findings into subsequent clinical phases, for example in deciding on the right dose that is both safe and efficacious for the patient.

The JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) therefore carried out a study to review the state-of-the-art of advanced non-animal models in use for immunogenicity testing in ATMPs.

Non-animal models and their application

The EURL ECVAM study analysed the scientific literature published from January 2014 to March 2019 and selected 88 peer-reviewed articles that used advanced non-animal models for testing immunogenicity of ATMPs.

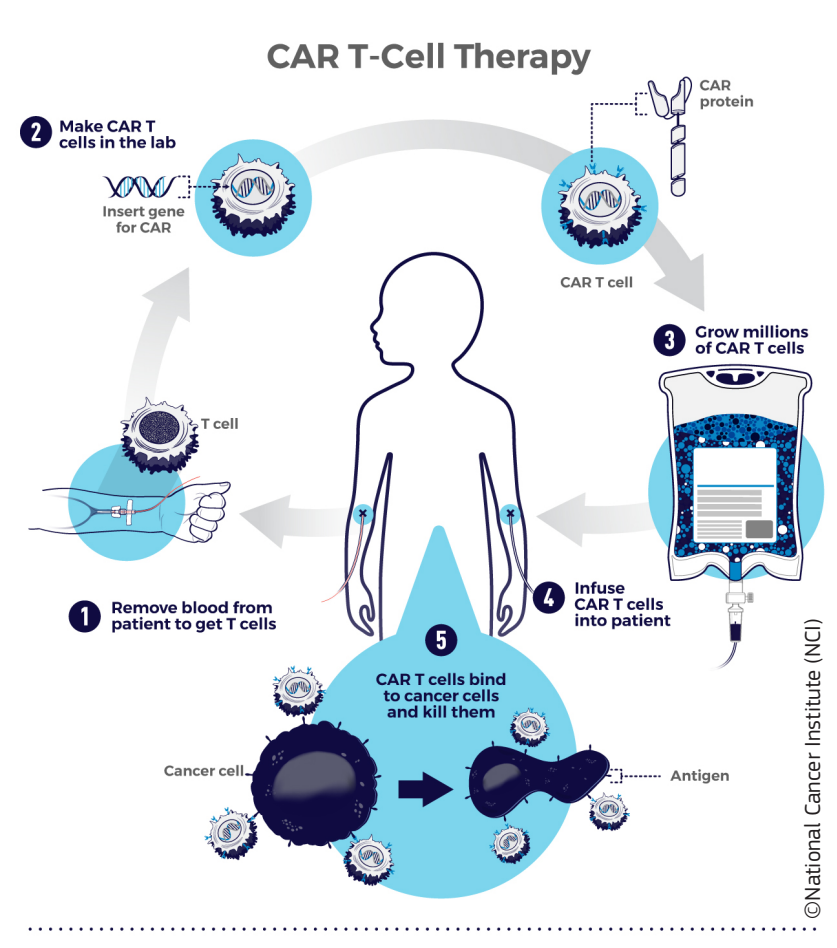


Box 2. Somatic-cell therapy and immunogenicity aspects

Cell-based products fall under two classes: autologous cells from patients themselves and allogeneic cells originating from another human donor. **Autologous cell therapy** has the advantage of low immunological reaction against the final therapy, however, being a one-to-one treatment, it has practical limitations and involves higher costs for national health systems. **Allogeneic cell therapy** can be broadly applied to a segment of well-defined patients and, importantly, could represent "off-the-shelf" therapies. However, immunogenicity aspects are a key limiting factor to the broad application of allogeneic ATMPs.

Cell therapy spans multiple therapeutic areas, such as regenerative medicine, immunotherapy[1], and cancer therapy. Among the different types of immunotherapy, one of the most innovative is known as adoptive cell therapy, in which T cells (a type of immune cell) are given to a patient to help the body fight diseases, such as cancer. Recently, one kind of adoptive cell therapy utilising **chimeric antigen receptor T (CAR T) cells** has been proven to be a very effective treatment for patients with some forms of leukaemia.

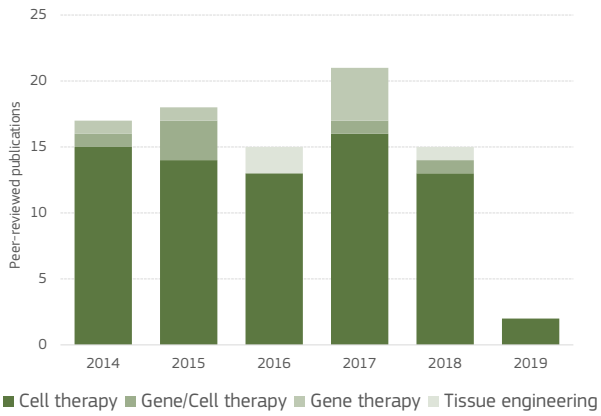
The methods involve taking cells from a patient's blood and adding the gene for the CAR receptor, which binds to a certain protein on the patient's cancer cells. Large numbers of the CAR T cells are grown in the laboratory and given to the patient by infusion. However, producing large amount of autologous T cells is complicated, requires a lot of time and can result in manufacturing failures, making the treatment inaccessible to some patients.



Here the promise of what are called 'off-the-shelf' therapies might offer a solution: immediately available allogeneic products produced from healthy donors and stored until they are needed, meaning that patients should not have to wait for the treatment. Nevertheless, the potential of allogeneic cell products to produce unwanted immunogenic reactions is a major barrier to their safe and efficacious use in the clinic.

1 Gribaldo, L., Dura, A., Straccia, M. and Whelan, M., *Advanced Non-animal Models in Biomedical Research – Immuno-oncology – Executive Summary*, EUR 30334/3 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-39989-6, doi:*10.2760/82873*, JRC125256.

The majority of them have been used to test cell therapy products (Figure 1).



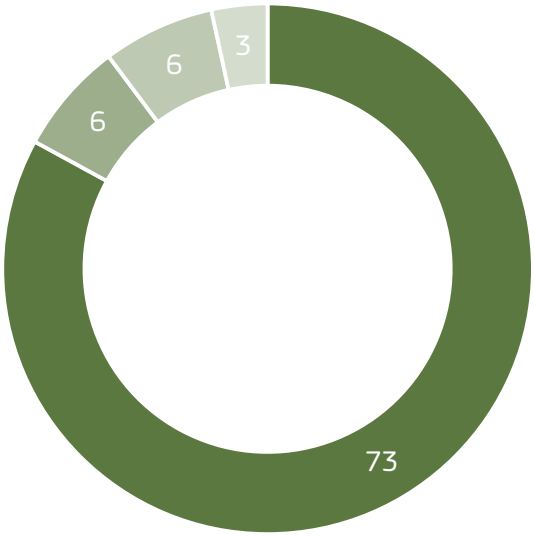


Figure 1: Use of advanced non-animal models for testing immunogenicity of different classes of ATMPs.

A great number of non-animal approaches used to test the immunogenicity of ATMPs were based on *in vitro* methods that mainly use cells and tissues cultured in the laboratory (Figure 2).

In contrast, it was found that computational and mathematical modelling (*in silico* methods) were less commonly used (Figure 2).

More progress needed

Notwithstanding the high investments in this field, the use of non-animal models is still rather uncommon to test the immunogenicity of

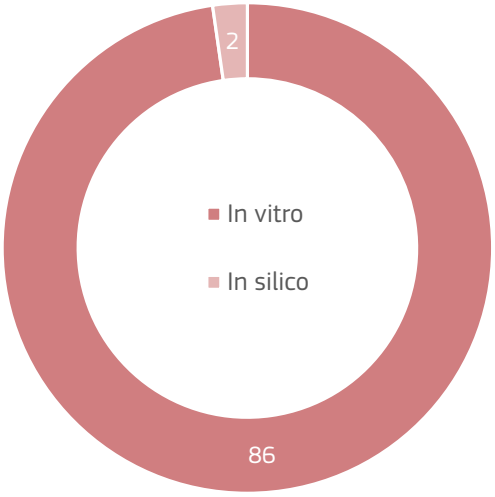


Figure 2: Types of advanced non-animal models used for testing the immunogenicity of ATMPs.

ATMPs, and the publication trend has remained rather constant over the observed period.

This study highlighted that there is certainly room for improvement in several areas. These include: achieving a more reliable and scalable supply of immune cells, commonly based on voluntary donors; increasing the number of samples that can be tested in an immunogenicity experiment; and implementing more advanced measurement technologies such as 'omics to generate more information from a test.

More innovative approaches could also be pursued through the incorporation of new technologies for high-throughput and high-content analysis. In addition, *in silico* models of the immune system should be definitely exploited further.

The knowledge base

This study has produced a unique highly-curated knowledge base that contains a detailed description of 88 non-animal models in use for immunogenicity in ATMPs. It is freely available to download from the EURL ECVAM collection in the JRC Data Catalogue[2], together

2 *https://europa.eu/!DwGdTG*

with a JRC Technical report[3] that describes the review methodology and presents the main findings (see Box 3).

This unique knowledge base can serve the needs of multiple stakeholders:

- **researchers** can identify models and methods that can be adapted and applied to tackle their own research questions;
- **educators** can provide the latest information on the state-of-the-art to their students;
- **funding bodies** can consider trends, identify impactful research avenues and target promising areas for investment;
- **project evaluation committees** can ensure that project proposers have properly considered the use of non-animal models and methods in their research proposals;
- **National Contact Points** and **National Committees**[4] can ensure proper knowledge sharing on non-animal methods within Member State networks and organisations involved in biomedical research using animals.

Findings of this study can also inform policy, in relation to the protection of animals used for scientific purposes, setting of research priorities to progress the development and uptake of non-animal methods, and the promotion of innovative human-relevant

3 Canals, J. M., Romania, P., Belio-Mairal P., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Rossi, F., Straccia, M., Daskalopoulos, E. P., and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research – Immunogenicity testing for advanced therapy medicinal products*, EUR 30334/4 EN, Publications Office of the European Union, Luxembourg, 2022, ISBN 978-92-76-49091-3, doi:*10.2760/7190*, JRC126997.

4 As referred to in Directive 2010/63/EU for the protection of animals used for scientific purposes.

Box 3. Knowledge base of advanced non-animal models

This study is a part of a series that EURL ECVAM is carrying out to review available and emerging non-animal models being used for research in seven disease areas. Details on the published studies are available on the *EURL ECVAM website*.

In this study approximately 15,000 peer-reviewed publications on immunogenicity testing for advanced therapy medicinal products (ATMPs) were initially retrieved and screened for representative papers describing innovative and promising advanced non-animal models.

An important outcome of this study is a highly-curated knowledge base containing detailed descriptions of 88 non-animal models being used for testing immunogenicity in ATMPs. It is easily downloadable as a spreadsheet file from the EURL ECVAM collection in the *JRC Data Catalogue*.

This knowledge base is complemented with a *Technical Report* that provides an in-depth analysis of the models identified and of the review methodology used.

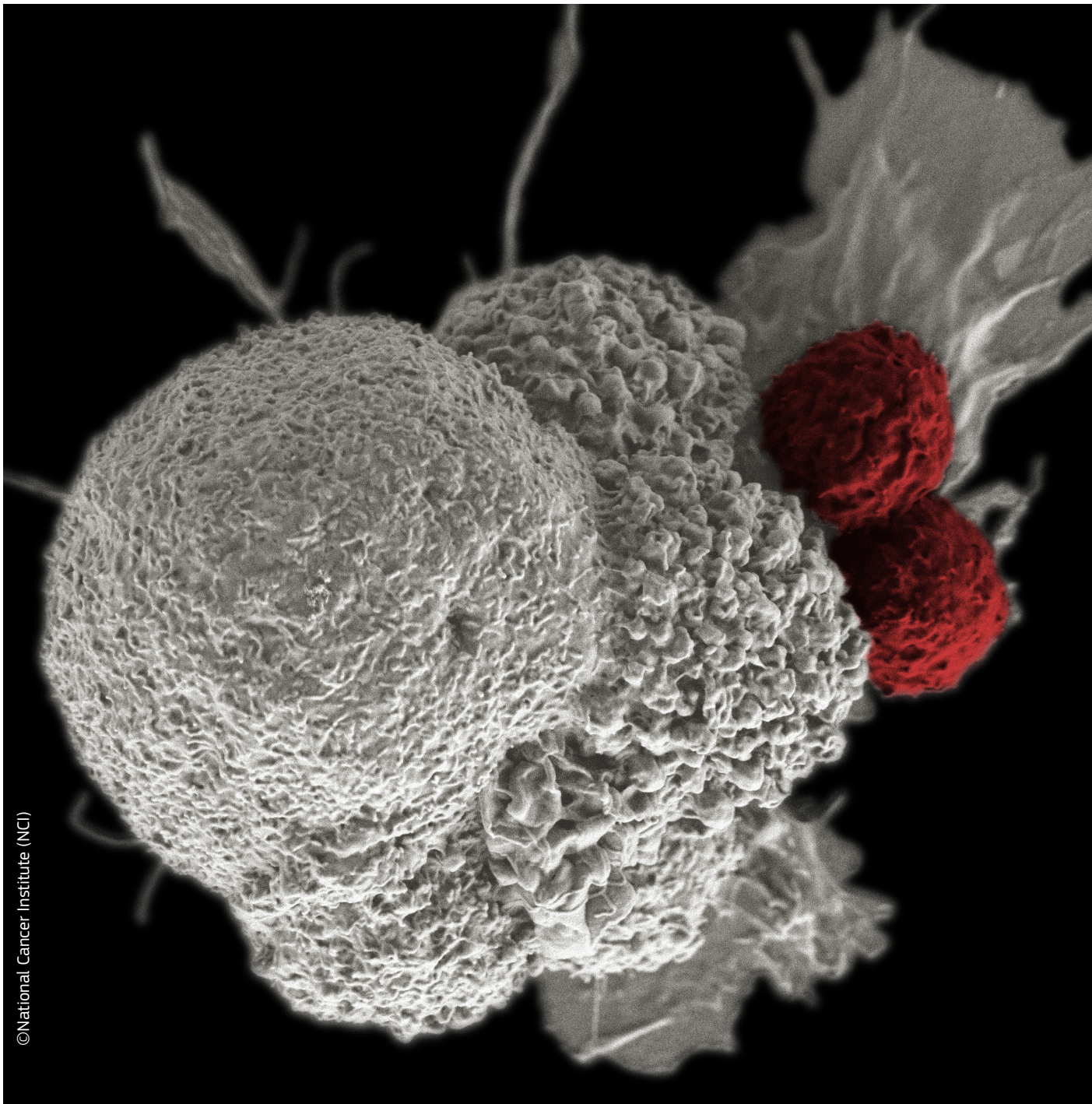
scientific approaches to support Europe's Beating Cancer Plan[5] and Cancer Mission[6].

Finally, this knowledge base can serve as a means to explore the strengths and limitations of both animal and non-animal models used in biomedical research, to stimulate a healthy scientific debate, to challenge mind-sets, and to pave the way for conducting better and more predictive science. Thus the knowledge base can act as a bridge across methods and disciplines in the biosciences field[7] to improve biomedical research quality and promote innovations for the ultimate benefit of patients and society.

5 *https://ec.europa.eu/health/system/files/2022-02/eu_cancer-plan_en_0.pdf*

6 *https://europa.eu/!wt73cr*

7 Carusi A., Whelan M. and Wittwehr C., *Bridging Across Methods in the Biosciences – BeAMS*, EUR 29852 EN, Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-76-11181-8, doi:*10.2760/190697*, JRC116305.



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KJ-NJ-30334-EN-N

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doi:10.2760/158699
ISBN 978-92-76-49093-7