AMBITION STATEMENT
STEP-BY-STEP PLAN FOR THE IMMUNOLOGICAL RESEARCH FIELD IN THE NETHERLANDS

Table of Contents

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Preface

This document is the ambition statement “Step-by-Step Plan for Immunological Research in the Netherlands.”

The importance of the immune system in health and disease is highlighted by the fact that more than 25% of all candidate drugs currently in clinical trials target the immune system. This includes agents aimed at enhancing and/or training the immune response (such as in vaccination and cancer), as well as agents that suppress immune activity (such as in allergy and autoimmune diseases). The development and application of many new agents depend on excellent fundamental and applied immunological research.

Immunological research encompasses the fundamental and applied science of complex processes at the molecular, cellular, tissue, and organismal levels. Many of these processes have been and are modeled using animal models. The field of immunology is rapidly evolving, leading to increasing recognition of the limitations of animal models and the realization that they are not always the most suitable approach to answer scientific questions. New technological and medical advances have led to the development of innovative human models, which have the potential to replace certain animal models. These innovative animal-free methods, or so-called ‘new approach methodologies (NAMs),’ allow us to study specific immunological processes relevant to human diseases and infections in detail without the use of animal experiments.

Together with the Dutch Society for Immunology (NVvI), efforts have been made in recent years to develop a roadmap to accelerate the transition to greater use of animal-free models. The possibilities and barriers for research with minimal use of laboratory animals have also been mapped.

The ambition of the NVvI is to optimally acquire knowledge of the immune system that contributes to better health, prevention, and therapy. Therefore, the association aims to maintain and further strengthen the leading position of Dutch immunology, both in human and veterinary fields. At the core of this ambition is the scientifically optimal choice of the best models to answer relevant research questions. We continually make rational and strategic use of combinations of (disease) models with and without laboratory animals. The NVvI board supports solid scientific initiatives within the framework of the 3Rs (refinement, reduction, replacement) for both human test models and animal models that are currently indispensable for certain questions. For complex immunological processes, where interactions between organs and the whole organism are essential, animal models will remain necessary for the time being. Moreover, the development and use of innovative animal-free models can reduce the use of laboratory animals. These models have the potential to replace certain animal models because they may better reflect human processes.

It is crucial that immunologists have access to the best possible approaches and technologies to create the greatest scientific value for humans and animals. The starting point is always the scientific question and the most suitable research model, with uniform European legislation providing an adequate ethical framework. The purpose of this ambition statement is to keep the discussion about the choice of the most appropriate model for answering scientific questions current and to continue engaging with researchers on this topic. The advantages and limitations of animal-free and animal models have been analyzed, and goals have been set for both the near and long term. Finally, the main barriers to the implementation of animal-free models have been identified, and ways to address these barriers are discussed. To this end, we have formulated six recommendations for the development, improvement, and implementation of animal-free models. The aim is to advance science while simultaneously reducing scientific dependence on animals.

Creation of this Document

Developing a step-by-step plan for the use of laboratory animals and animal-free innovations within immunology requires great care, due to the complex ethical considerations and the sensitivity of the societal debate. To arrive at a broadly supported step-by-step plan within the immunological research field, we undertook a series of initiatives in consultation with the National Committee for Animal Testing Policy (NCad) from December 2023 to March 2026, including a sounding board group, input from Young NVvI, and a consultation open to all 1,200 NVvI members. The latter was both written and oral during a feedback session on December 12, 2024, at the annual meeting. In addition, the draft version of this document was made available to all NVvI members for review via the NVvI website.

The Ambition Statement for Immunological Research was inspired by the comprehensive Vision for Cardiovascular Animal (Free) Innovation and the underlying consensus article by Van der Velden et al. (Cardiovascular Research, 2022 https://doi.org/10.1093/cvr/cvab370).

Summary of the Ambition Statement

1. **Start the dialogue from a shared, broadly supported ambition: excellent science with much less, refined use, or no use of laboratory animals**
Promote an integrated and question-driven use of human studies, animal-free, and refined animal models, where the choice of research model is primarily determined by scientific quality, societal relevance, and translation to humans or target animals. Further (interdisciplinary) collaboration should be encouraged, as different fields are needed to develop, optimize, and validate innovative animal-free technology.

2. **Make optimal use of existing knowledge and expertise in the development of disease models**
Ensure a broad and sustainably accessible infrastructure for human cells, tissues, and research data. Sharing knowledge can accelerate and broaden the deployment or further development of new animal-free immunological human test models and refined animal models. Open Science plays a crucial role here, alongside optimal access to and proper reuse of human research data. Position the NVvI as a coordinating and connecting factor for knowledge sharing, collaboration, and agenda-setting for animal-free and animal-friendly innovations at national and international levels.

3. **Ensure good communication and involve all stakeholders**
Facilitate clear and consistent communication to researchers, policymakers, patients, and society about the possibilities and limitations of different research models, to promote support, trust, and realistic expectations.

4. **Continue to make financial resources available for the development and validation of animal-free test models and for initiatives aimed at reduction and refinement of animal models**
Develop and secure structural funding instruments that support the development, validation, and implementation of animal-free models as well as the reduction and refinement of animal models, including funding for parallel and validation studies.

5. **Integrate the development of animal-free models into legislation, regulations, and policy**
Encourage validation and standardization of animal-free research models and promote early alignment with regulatory authorities and end users to accelerate implementation and acceptance. Good cooperation between researchers, regulatory authorities, and government is essential.

6. **Facilitate education in the field of animal-free research models and reduction and refinement of animal models**
Embed knowledge about innovative research models, model selection, and translation structurally in higher education and post-academic training, to ensure future-proof animal-free research and reduction/refinement of animal research, while maintaining quality.

Introduction

To gain insight into the mechanisms underlying the health of humans and animals (both in the wild and in production animals), immunology as an international discipline has a rich diversity of both animal models and animal-free models. Immunology is an extremely dynamic field, and many scientific breakthroughs have had a direct and indirect impact on the health of humans and animals (as evidenced by the awarding of many Nobel and Lasker prizes). Animal models have often played an important role in these breakthroughs. For example, they were essential in the initial development of cancer immunotherapy and mRNA vaccines for COVID-19.

The immune system not only plays a key role in defense but also in physiological development and processes such as tissue repair, metabolism, temperature regulation, and behavior in animals and humans. The immune system forms a mobile unit of diverse mechanisms that provide protection and repair in every conceivable anatomical compartment. Due to this complexity, the immune system presents a challenge to reliably and integrally model with immunological test models entirely outside living intact organisms. In this context, there is much attention within immunology for the development and application of new measurement methods and technology for research with fewer or no laboratory animals. Further development and validation of animal-free models will reduce the use of laboratory animals.

The use of animals as laboratory animals and the conduct of experiments with these animals is strictly regulated in the Netherlands and the EU. For keeping animals for laboratory research, there is more legislation and regulation than for keeping animals for any other purpose. Strict requirements are imposed on the qualifications of the personnel caring for the animals, the care system in which the animals are housed, and the experiments conducted with the animals. EU regulations stipulate that animals may only be used for research when there is a compelling scientific justification; when the expected benefits outweigh the harm to the animals; and when the goals cannot be achieved with animal-free, alternative methods (see EU Directive 2010/63/EU, amended in 2019, and the Dutch Animal Experiments Act, WoD 2014, based on EU regulations). If it can be estimated that the expected outcome can also be achieved without animal experiments, it is prohibited to conduct an animal experiment for this purpose.

Dutch immunological research holds a leading position worldwide. It focuses mainly on fundamental and translational scientific research and to a lesser extent on (legally required) safety research. The essential combination of in vivo research in laboratory animals and in vitro research in animal-free models has demonstrably led to new drugs and diagnostics for both humans and animals. Within immunology, there is a strong intrinsic motivation to use the most suitable research models needed to answer the scientific questions at hand. These can be animal models, animal-free models, or a combination thereof. As a result, immunological researchers contribute to the innovation of methods that reduce the number of laboratory animals and to animal-free methods. Immunological research uses a variety of innovative technologies. Examples include advanced flow cytometry of immune cells in blood and organs, new imaging techniques, and genome-wide analyses at the level of individual cells (single-cell sequencing and spatial transcriptomics). In addition, innovative cell and tissue culture technology, including organoids and organ-on-a-chip models, is used, enabling animal-free research. Depending on the technique, different terms are used, such as NAMs, human test models, in silico models, and in vitro models. These terms each refer to specific aspects of these research methods. For readability, we use ‘animal-free methods’ as an umbrella term in this vision.

On the other hand, for many immunological research questions about health and disease, there is currently no suitable alternative to laboratory animals. This is understandable because an immune response or immunological disorder involves many different cell types and almost always a large number of organs. Modeling the complexity of many compartments and migrating immune cells is a very challenging task. Animal-free models often lack the

complexity needed to model disease processes. However, new developments such as multi-organ-on-a-chip and more complex organoids offer new possibilities, as does the development of more sensitive techniques for 'live' measurements in patients. Many successful research groups in immunology combine animal models with human test models. This combination demonstrably leads to improved treatments, more effective vaccinations, and new drugs including immunotherapy for cancer, allergy, and autoimmunity.

Although the focus of the Step-by-Step Plan for the Immunological Research Field is on human immunology, there is also important immunological research aimed at animal health. This includes not only (agricultural) domestic animals but also, for example, wild birds, as avian flu affects both domesticated poultry and wild birds, and now multiple animal species. The COVID-19 pandemic has strongly emphasized the joint importance of animal and human health (OneHealth concept), with the risk of pathogens that can jump from animals to humans (zoonosis). In such cases, animal models in combination with animal-free models are important to promote the health of both humans and animals. To improve animal health, the target animal itself is often used as a model.

For high-quality science with less use of laboratory animals, it is very important to continue developing new immunological test models that are more representative and predictive for the patient than an animal model. Given the exceptional complexity of the immune system and the involvement of many different cell types and organs described above, we face significant challenges, and effective test models that do not use laboratory animals will not be feasible for every disease in the near future. The NVvI aims to make significant progress in the next five years in both the development of animal-free immunological test methods and in reducing and refining animal models.

Ambition Statement for the Immunological Research Field

1. Start the dialogue from a shared, broadly supported ambition: excellent science with much less, refined use, or no use of laboratory animals

Ambition. The pursuit of innovation in immunological research is an ambition supported by many parties. There is ongoing attention to both the limitations and ethical implications of using laboratory animals, and a growing need to find alternatives. The use of animals in scientific research is a complex and often emotionally charged topic. Striking a balance between ethical considerations and scientific progress is extremely important and is therefore central in the EU directive and the Dutch Animal Experiments Act. There are currently no animal-free test models that capture the full complexity of immune responses and make the use of animals obsolete. At present, it is not possible to realistically predict when this will be the case. At the same time, we emphasize that animal models also have shortcomings and do not always model human biology well. The NVvI's principle is to conduct top-quality immunological research for the benefit of human and animal health with as few or no laboratory animals as possible. It is important to stress that the aim to reduce or phase out animal research should never compromise our ability to gain meaningful insights into the development and treatment of infectious diseases, allergy, autoimmune diseases, chronic inflammation, and cancer. In short: "Excellent science with, where possible, less, refined, or no use of laboratory animals."

Validation. It is important to emphasize that animal experiments are often essential for validating the safety and effectiveness of animal-free test models. Therefore, it is important to allow for nuance in the dialogue and to work towards an integrated approach that considers all aspects of animal use and 'animal-wise' innovation. 'Animal-wise' innovation includes both animal-free research and research with justified reduced and refined use of animals. To realize a joint step-by-step plan from the Dutch immunological research field towards reduction and eventual phasing out of animal research, investment in innovative technologies and research methods is required. This means sufficient time and resources must be invested in both the development of animal-free test models—including in vitro research (culture models, organoids, organ-on-a-chip, etc.), computer modeling, and clinical studies in volunteers—as well as in innovative animal models, such as integrating natural human commensals and pathogens into animal models. The immunological research community aims to phase out animal use in the long term, without loss of research quality and utility. Validating animal-free test models is a crucial step that must be strongly emphasized.

In this context, it is essential to support studies that focus on understanding the translational gaps between animal models and the human disease being modeled. It is essential to stimulate validation studies that compare results from animal experiments with animal-free models. Only with such systematic comparisons can it be determined which method is most suitable, including for regulatory applications. These validation studies are necessary for the transition to animal-free test models.

A key driver here would be specific funding for parallel studies (see the NCad Parallel Studies Report). This can help identify barriers, determine feasibility, and support the transition for researchers using animal methods. At the same time, investments are needed in high-quality, welfare-oriented animal facilities that can facilitate refinement of animal research and thus contribute to improved translation.

Collaboration. Some research areas within immunology will be able to make the transition faster than others. This depends on urgency, transition requirements, and available expertise. One of the goals should therefore be to increase expertise and make it available for innovative models to every researcher. Examples include the iPSC hotel and organoid centers that have

been or are being established at many universities. A key condition for increasing the chance of success is sharing technologies and knowledge.

More broadly, institutions should have, in addition to an animal facility, a facility for animal-free research, making animal-free technologies optimally available and shareable. Such a facility can also serve to further develop animal-free technology—so that they can replace less suitable animal models—and to start parallel and validation studies. Close connection with the animal facility, with support from the local Animal Welfare Body (IvD)/animal experiments committee, is necessary to ensure optimal choice of high-quality models. Such a facility can also be used for training purposes. Further interdisciplinary collaboration should be encouraged, as different fields are needed to develop, optimize, and validate innovative animal-free technology. The national consortium Human Organ and Disease Model Technologies (hDMT) offers immunologists the opportunity to collaborate interdisciplinarily with cell biologists, (tissue) engineers, and industry to further develop models and support implementation.

2. Make optimal use of existing knowledge and expertise in the development of disease models

In the context of the transition to animal-wise innovation for immunological research, it is important to have a clear overview of currently available animal models and animal-free models, in order to inventory the associated expertise. Because immunology plays an important role in various research areas, it is important to involve other research fields as well.

Human models. Within Dutch immunology, many initiatives are underway regarding the use and development of research models that do not use animals for human and animal health. The Netherlands holds an internationally leading position in this area. Appendix Figure 1 provides an overview of various test models as applied in immunology. There are many different models based on immune cell lines that allow for genetic manipulation and robust analyses. Innovative techniques enable genetic manipulation and long-term culture of primary human immune cells, making the use of primary cells in immunological models more attractive and feasible. It is therefore important to facilitate access to human primary immune cells from blood and tissues for every researcher via open access biobanks (for tissues removed during plastic surgery) and centralized facilities (blood banks, tissue centers). In addition, the reagents used in human test models should not be forgotten, as they often contain animal components or are produced using animal systems. The use of animal-derived products, such as cell culture reagents (e.g., fetal calf serum, growth factors, enzymes, Matrigel, and antibodies), should be minimized. Suitable non-animal alternatives are often available. It is understandable that researchers are hesitant to switch to other models: additional tests and validation experiments are needed, alternatives are not always suitable, and costs are often higher. Therefore, institutional efforts and specific funding opportunities are necessary.

Animal models. For many immunological diseases (such as infection, inflammation, allergy, and cancer) in humans and animals, there is currently no suitable alternative to animal models. However, it is recommended to focus on refinement of animal research. Recent developments show that, for example, refinement through semi-naturalistic housing and enrichment leads to a better (healthier) animal model, resulting in research outcomes with increased translation from animal to human. Also, the use of humanized mouse models and training the mouse immune system with gut microbiota from wild mice greatly increases comparability with humans. In addition, replacement can be pursued through innovations within animal models, such as the use of invertebrate species, like the fruit fly (*Drosophila*) and the worm (*C. elegans*).

Knowledge sharing. By exchanging knowledge between research groups working with animals and groups developing animal-free models, new immunological and human test

models, as well as refined animal models, can be deployed or further developed more quickly. NWO programs such as “More Knowledge with Fewer Animals” are good initiatives, enabling, for example, the funding of systematic literature reviews of existing literature describing animal experimental research. This leads to the consolidation of knowledge about animal models and suitable animal-free immunological test methods, which can then be more widely shared through publication.

Applying Open Science as broadly as possible is extremely important. Important resources include the Dutch consortium Human Disease Models and Technologies (www.hdmt.technology), the 3Rs Centre Utrecht (Utrecht University) (www.uu.nl/organisatie/3rs-centre), and the NC3Rs (UK) (www.nc3rs.org.uk) with extensive examples and databases. Innovative analytical techniques and methods provide an abundance of patient data (genes, proteins, metabolism). Access to and reuse of patient data enables other researchers to use these data for their own research. Regulations such as FAIR Science are already in place, but further efforts are needed to facilitate access to and proper use of these data. Initiatives to facilitate access to information about animal-free models should be encouraged. Access to such information should be easy for researchers, Animal Welfare Bodies (IvD), and animal experiments committees (DEC). This can be improved by more intensive contacts between animal-free initiatives, researchers, the IvDs within universities, the DECs, and the Central Committee on Animal Experiments (CCD). For example, a DEC member could be included in the management team of animal-free initiatives. In this way, awareness and transition are optimally facilitated.

Role of the NVvI. The pursuit of animal-wise innovation for immunological research is an ambitious goal that requires collaboration and awareness of the challenges and opportunities. The NVvI considers it one of its priorities to facilitate this dialogue and to work with all stakeholders towards a better future for both patients and laboratory animals. It is essential to position the NVvI as a coordinating and connecting factor for knowledge sharing, collaboration, and agenda-setting for animal-free and animal-friendly innovations at national and international levels. The NVvI can create and increase awareness of the possibilities of these animal-wise innovations, both among researchers and within training programs (see also recommendation 4 regarding education). During the annual NVvI Spring meetings (200-300 participants) or Winter meetings (500-600 participants), attention should be paid to this topic, for example through a workshop on innovative research models during the Winter meeting. But also on a European scale, as part of the European Federation of Immunological Societies, the NVvI should seize opportunities to advance the dialogue on animal-wise innovation. Finally, the NVvI also has a role in communication with societal organizations in the field of animal use and animal-free innovation (see below).

3. Ensure good communication and involve all stakeholders

Joint communication. Within the immunological research field, experimental knowledge is generally shared through scientific publications and meetings. However, this does not reach the general public or policymakers. To clarify why research is conducted with laboratory animals while simultaneously working on more refined animal models and alternative immunological test models without animals, clear and broad communication from the immunological research community is essential. This communication should target the general public, patients, and policymakers, and should provide a realistic picture of the possibilities and limitations of both animal models and animal-free test models. The guiding principle should be that top-quality immunological research is conducted for the benefit of human and animal health with as few or no laboratory animals as possible. To prevent polarization in the debate on animal use and to realize a shared ambition for animal-free innovation, an open and transparent dialogue is needed between scientists, physicians, industry, animal rights organizations, governments, policymakers, social organizations, and patient organizations. It is important that the NVvI remains in dialogue with organizations such as the Transition to

Animal-Free Innovation (TPI), Stichting Proefdiervrij, and the Ombion Centrum voor Proefdiervrije Biomedische Translatie, where its own positions can be presented in an open dialogue. Joint communication about what is and is not yet possible within immunological research can help reduce polarization. It is also important to involve patients and patient associations in this discussion.

Transparency agreement. We realize that there may be conservatism regarding animal-free innovation and that this requires attention. For the transition to the replacement of laboratory animals to actually take place, it is crucial how and by whom the message is communicated, both to researchers and to society. Creating a realistic picture of the possibilities for using animal-free methods, but also about the use of laboratory animals, is very important. One development in this area is the Transparency Agreement on Animal Experiments, which has been signed by many Dutch universities, academic hospitals, and knowledge institutions, including those conducting immunological research on humans and animals (Transparency Agreement on Animal Experiments - Stichting Informatie Dierproeven). The goal of this agreement is to provide society with optimal openness regarding animal experiments, the development of new immunological test methods without animals, what is being researched, and why this benefits the health of both humans and animals. Publishing nuanced examples of important developments in media accessible to the general public can play an important role in this.

Terminology "animal-free models." It is crucial that all stakeholders are included in the transition to replacing laboratory animals in immunological research. This means that all involved must trust that the discussion is not conducted selectively, but rather inclusively. Previous communication and discussion about reducing or replacing laboratory animals ("The Netherlands as world leader in animal-free innovation by 2025") has contributed to polarization in the debate on the use of laboratory animals and animal-free innovations, causing parties to oppose each other instead of joining forces to collaborate. To promote inclusivity and objectivity, it is important that terminology in the debate is chosen carefully. In discussions with stakeholders, the use of terms such as "transition" and "animal-free" can create the impression that the shift to animal-free methods is already a fait accompli, regardless of whether the right arguments are provided. Using terms like "immunological or human test models" instead of "animal-free models" emphasizes what we want to achieve, rather than what we want to exclude. Clear and careful communication with a well-defined common goal, as described above, is essential.

4. Continue to make financial resources available for the development and validation of animal-free test models and for initiatives aimed at reduction and refinement of animal models

Development of animal-free test models. Several promising animal-free models have been developed to study certain aspects of the immune system. The availability of new financial resources, such as those previously provided by the National Growth Fund and the establishment of the Center for Animal-Free Biomedical Translation (Utrecht), will further accelerate this development. To achieve the transition goals described in this vision, additional funding at the national level and from health organizations is indispensable. Therefore, it would be beneficial if more personal fellowships and grants were developed by funding agencies for early-career and experienced scientists dedicated to developing or using animal-free test models. Funding for large consortia is also important.

Validation and standardization. Validation and standardization of test models are crucial and make it possible to repeat and apply studies in different laboratories. One of the current challenges is obtaining sufficient funding for this. It is important to also consider the needs of

companies, so that a natural and complementary collaboration can be established. This also applies to good consultation with regulatory authorities, such as the European Medicines Agency (EMA). When developing animal-free methodologies, it is important to consider their eventual application for the approval of new drugs and therapies on the market. This is to prevent the development of a model that is not suitable for potential end users, for example because it is too complex, not reproducible enough, or too expensive, resulting in a lack of further investment due to insufficiently reliable results. Possible obstacles that need to be removed include: lack of clarity about the validation process, differing interests of researchers and end users, and the duration of the validation process. These can create significant risks for both researchers and end users in further developing or using a model. Because both animal models and animal-free innovations only mimic part of biology, involving end users in the validation process is very important. Their input can ensure that the innovation meets needs and is applicable to specific research questions.

A crucial solution to bridge this gap is to make additional funding available for the validation of existing animal-free models (in addition to developing new innovations). It is important to do this as much as possible in collaboration with industry, for example supported by programs from the Samenwerkende GezondheidsFondsen (SGF) and Health Holland. Preferably, regulatory authorities should also be involved in this process at an early stage, so that the process of acceptance of these new innovations is clearer and faster. It is expected that validation of animal-free models will stimulate their use by companies and research institutions, thereby reducing the use of laboratory animals for similar purposes. In addition to the development and validation of animal-free models, it is important to (continue to) provide financial resources for the refinement and improvement of existing animal models.

5. Integrate the development of animal-free models into legislation, regulations, and policy

Human studies. Research with volunteers or patients is becoming increasingly feasible thanks to the growing availability of sensitive, non-invasive, and routine analytical techniques. It is important that knowledge about the possibilities of this type of research is actively shared and encouraged. Equally crucial is simplifying ethical restrictions and bureaucratic procedures, where responsible. In addition, it is very important that the preparation and execution of research in humans, including phase 0 studies, is actively supported in regulations. This will allow human approaches to be better used as a full alternative to animal-based methods.

Legally required research. Legally required toxicity and safety studies are not the direct focus of the NVvI and Dutch scientific immunological research. Nevertheless, these studies also deserve attention in this step-by-step plan. Until recently, it was mandatory to test all drugs for possible toxicity in animals. Recently, it has become possible to replace animal studies for formal toxicity studies with new test methods without the use of animals, provided these are validated and actually deliver reliable results. These are supported by EURL ECVAM (EU Reference Laboratory for alternatives to animal testing), the European reference laboratory committed to the development, validation, and acceptance of animal-free methods. It is part of the Joint Research Centre (JRC) of the European Commission and promotes the 3Rs in research and regulation.

This is an important change in regulations that makes it possible to achieve a reduction in the use of laboratory animals for legally required studies. This shift will not be realized overnight, as animal-free test methods are still in development and must be validated before they can be recognized by regulatory authorities as valid replacements. By adjusting requirements in international regulations, it is possible to achieve a paradigm shift in legally required research, where laboratory animals are no longer the standard. Good cooperation between researchers, regulatory authorities, and government is essential. In this context, it is important that there is more awareness and guidance on this topic within universities and research institutes. At an early stage of research design, contact should be made with the

‘scientific advice’ department of the EMA or Food and Drug Administration (FDA). This contact can be used to discuss what is needed for future market approval and to resolve possible barriers that may arise from the use of animal-free test methods. It should be realized that legally required research is only a part of all animal experimental research.

6. Facilitate education in the field of animal-free research models and reduction and refinement of animal models

Role of universities and research institutes. To reduce and, where possible, eventually phase out the use of laboratory animals in immunology, education and training at the start of the scientific career is essential. Upcoming researchers should be structurally informed about animal-free and other innovative research models during their bachelor’s, master’s, and postdoctoral training. This education should provide a realistic picture of the possibilities and limitations of both animal models and animal-free test models. Universities play a key role by offering education on animal-free test models such as 3D cell cultures, organoids, and organ-on-a-chip. This also applies to refined and innovative animal models with improved translation (such as humanized mouse models and animal models in which natural human commensals and pathogens are integrated). This enables young scientists to consciously and critically consider model choice in relation to the research question and translatability to humans or the target animal from the start of their careers. To implement this structurally, universities and research institutes, partly through government funding, will need to invest in developing comprehensive educational programs on this theme.

Transition workshops should also be encouraged, where scientists working with animal methods and wishing to transition to animal-free models can become acquainted with animal-free methods and networks. In this way, they can continue their research without the use of laboratory animals (see www.helpathonhotel.org).

Role of the NVvI. The NVvI plays a central and connecting role by structurally putting animal-free and animal-friendly innovations on the agenda during national and international meetings, such as the aforementioned annual Spring and Winter meetings and within European networks. For PhD students and young researchers, additional course days and symposia can be organized in which both innovative animal-free models and refined animal models are discussed. This is already being done, for example, by the Netherlands Respiratory Society with an annual 'Advanced Technology in Lung Research' Symposium. Both refined animal models and innovative animal-free immunological models can be presented and discussed here. At the same time, it is important that established researchers also stay informed of the latest developments through symposia, webinars, and publications, so that innovation is widely supported within the research community.

International position. It is important that young researchers receive training in good communication with a broad and international audience, so they can clearly explain why research is conducted with laboratory animals, but also why there is a strong focus on developing and validating alternatives. By structurally investing in up-to-date and coherent education about research models, and facilitating researchers in making well-founded choices, animal-free alternatives will be chosen as much as possible. This will lead to a natural decrease in animal use, without loss of scientific quality. In this way, Dutch immunological research can remain internationally leading and at the same time play a pioneering role in animal-free and animal-friendly innovation, benefiting science, patients, and laboratory animals alike.

Objectives and Final Recommendations

The exponential growth of new and innovative animal-free models will continue in the coming years. These models will become increasingly important and more widely implemented. Nevertheless, there are still significant challenges to making animal-free methods the dominant model for immunological research within the next ten years. It is crucial to invest substantially in the validation of these models. When an innovative animal-free model performs comparably to an animal model, the use of such a new model in future research should be encouraged through efficient transfer of the developed technology. Achieving this will require investments. Some of these investments will require considerable effort, while others are already underway.

Five-Year Objective. The five-year objective should first be to invest in the education and training of young researchers within universities, university medical centers, and institutions. This will accelerate the development and application of animal-free methods and the innovation of animal models based on the 3Rs principle. Much attention should be given to the development of animal-free immune-competent organoid models and multi-organ models.

Additionally, the NVvI aims to make significant progress in the next five years in both the development of animal-free immunological test methods and in the reduction and refinement of animal models. The NVvI should position itself as a coordinating and connecting factor for knowledge sharing, collaboration, and agenda-setting for animal-free and animal-friendly innovations at both national and international levels. The annual NVvI Spring and Winter meetings offer excellent opportunities for this, for example through thematic workshop sessions or an award for animal-free innovation.

Another important focus is to invest in the systematic collection of datasets from patient cohorts. It is essential that the supply of ex vivo tissues for researchers is facilitated.

Finally, it is crucial to stimulate validation studies and parallel studies between animal models and animal-free models. Ultimately, only these studies can demonstrate which models are suitable for studying processes or developing drugs for certain diseases. Therefore, the first five years can be seen as an investment in the future.

Ten-Year Objective. The ten-year objective encompasses the implementation phase of many animal-free models and models with less or refined use of laboratory animals. Both immune-competent and multi-organ human models should be further developed and implemented. Such development should enable reliable systemic, chronic, biodistribution, and safety studies. Through Open Science and access to large amounts of (multi-omics) datasets, there will also be a shift from in vitro and in vivo to in silico research. As a result of these developments, the use of laboratory animals in the Netherlands and worldwide can decrease significantly.

Final Recommendations for the Transition. Funding will always be a key driver of the transition to animal-free test methods. This is based on critical evaluations of research projects, determining whether the proposed animal study or animal-free test method is the best way to answer the research questions. Funding from national institutions (e.g., NWO, ZonMw, National Science Agenda) and health funds is indispensable to achieve the transition goals described in this vision. Training and education of scientists at the start of their careers is the key to transition. Therefore, it would be beneficial if more personal fellowships and grants were developed by funding agencies and if funding were provided for large consortia to initiate collaborations and offer career opportunities.

Finally, we live in a world that is increasingly critical of research into human diseases and their treatment. New areas of focus are emerging for improving health research. The influence of, for example, ethnicity, lifestyle, air quality, and environmental factors are becoming increasingly important factors in research and require new approaches that are difficult to study in animal models and for which innovative animal-free approaches are needed. This is the era in which a boost will be given to research on humans in relevant human models,

in vitro, ex vivo, in vivo, or in silico, while refinement and reduction of existing animal models will continue to deserve attention.

During various phases of the development of this research, additional input was provided by members of a sounding board group consisting of immunologists from different disciplines and at different stages of their careers.

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Dr. Jeffrey Bajramovic, UMC Utrecht (until May 31, 2025)
Dr. Peter Boross, Genmab BV, Utrecht
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Dr. Rory de Vries, Erasmus MC Rotterdam
Dr. Michiel van der Vlist, UMC Utrecht
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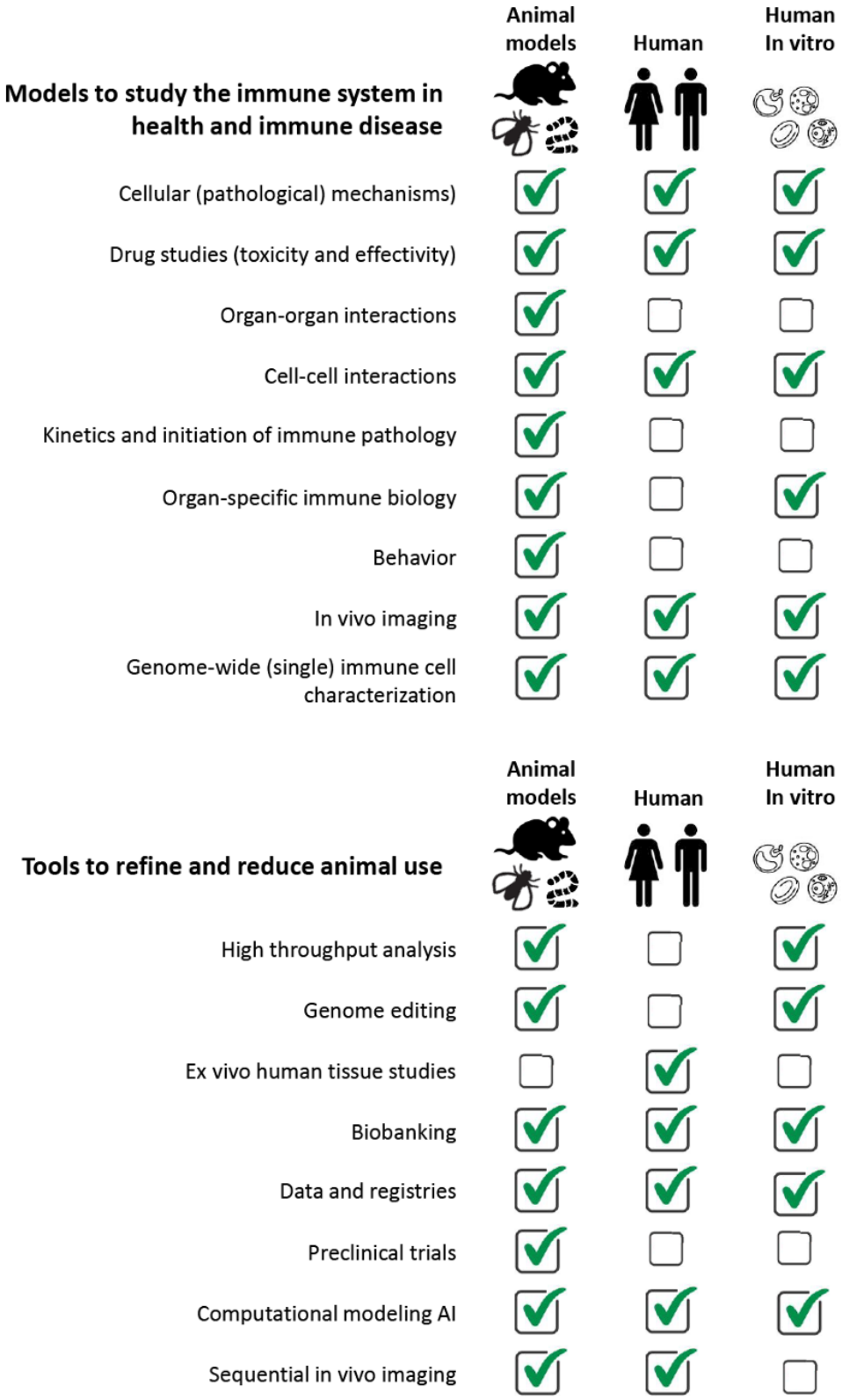
Appendix



- Figure 1 | Available models for immunological analyses (adapted from Van der Velden et al., Cardiovascular Research 2022; DOI: [10.1093/cvr/cvab370](https://doi.org/10.1093/cvr/cvab370))