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Advanced Non-animal Models in Biomedical Research

Autoimmune Diseases

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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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Advanced Non-animal Models in Biomedical Research

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TABLE OF CONTENTS

	Abstract	1
	Acknowledgments	2
1	Introduction	4
2	Methodology	7
3	Study results	10
3.1	Distribution of peer-reviewed articles by autoimmune disease	10
3.2	Autoimmune disease features covered by non-animal models	11
3.3	Research areas where human-based models are applied	12

3.4 Distribution of peer-reviewed articles by type of models used 12
3.5 *Ex vivo* models to study autoimmune disease with skin affection 14
3.6 Cell types and cultures employed 16
3.7 Throughput and biological relevance of models 19

4 Conclusions 22

References 25

List of abbreviations and definitions 30

Annex: inclusion and exclusion criteria applied to the systematic search 33

Abstract

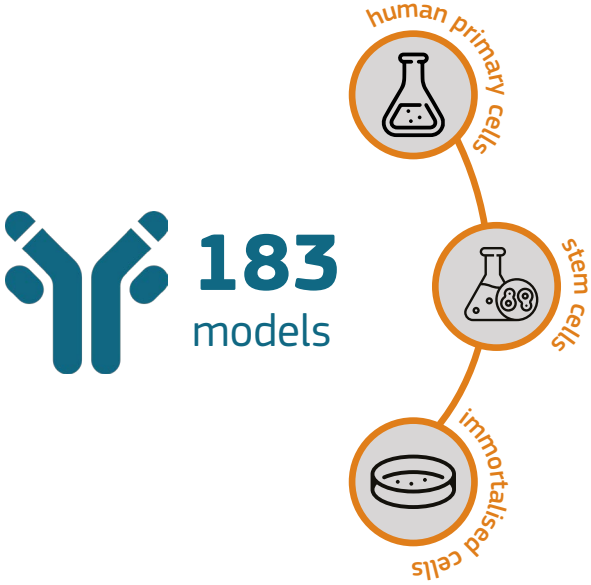
The prevalence of autoimmune disorders is increasing worldwide. Whilst some treatments to manage symptoms do exist, there is a lack of effective options to stop and/or reverse the progression of the majority of these diseases.

This is due in part to the fact that the causes and mechanisms of these conditions are difficult to identify as they include a mix of genetic, environmental and lifestyle factors, and that often they are studied in animal models which cannot fully replicate the complex biological interactions at play in these multifactorial diseases in humans.

Human-based alternative methods potentially represent more efficient options to elucidate disease mechanisms and discover potential drug targets. This study was conducted therefore, to systematically review non-animal models employed in the field of autoimmune disease research, and published in peer-reviewed journals between January 2014 and March 2019.

A total of 183 advanced models were identified and described. A notable observation was the sharp increase in the number of articles employing them between 2016 and 2018, compared to the 2014-2015 period. The majority of these models are based on the use of cells, particularly primary cells, and certain models are preferentially exploited for certain types of conditions, such as in the case of ex-vivo models like biopsies for skin diseases, and stem cells in the case of type 1 diabetes.

This study has produced a unique and highly curated knowledge base freely available to a variety of stakeholders in the research community and beyond.



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1 Introduction

For decades, clinical observations have suggested that the prevalence of autoimmune diseases is increasing. One of the latest statistics show that they affect 3–5% of the population, with autoimmune thyroid disease and type I diabetes (T1D) being the most common conditions (Wang L. et al. 2015).

Between 2003 and 2014, 148,947 new cases of nine autoimmune diseases were recorded in seven European healthcare databases from four countries (Willame C. et al. 2021).

Nowadays, over 100 diseases are classified as being autoimmune in nature, however the cause of many of them remains unknown as both genetic and environmental factors are likely to play a role in their aetiology. Some treatments are available, but definitive cures have yet to be discovered. The chronic and debilitating nature of these disorders, which can lead to high medical costs and reduced quality of life, is a burden for patients and surrounding families and communities.

In the past decade, there have been significant advances in diagnosis and disease classification, as well as improvements in prognosis, achieved through both the development of novel technologies in molecular immunology and sophisticated evidence-based clinical laboratory testing.

Treatment wise, some patients are able to use regular drugs to treat mild symptoms, like aspirin and ibuprofen for reducing pain. Others, with more severe symptoms, may need prescription of alternative drugs to help relieve pain, swelling, depression, anxiety, sleep problems, fatigue, or rashes. For other patients, surgery might be the only option.

Some treatment strategies include the administration of vital substances that the body can no longer make, like in the case of insulin injections for T1D and thyroid hormone replacement therapy for thyroid disease, or the use of drugs to suppress harmful immune system activity. For example, low-dose chemotherapy can control inflammation or in the case of people with lupus, anti-TNF therapy can prevent kidney failure. Despite the existence of these treatments, there is still a crucial need for novel therapeutic options.

There are currently more than 9,098 trials registered for autoimmune diseases. Unfortunately, this research field is riddled with many problems, one of which being the availability of reliable disease models. So far, animal models (mainly mouse and rat) have proved the most used way to probe disease mechanisms and test therapeutic strategies (Morel 2004). However, directly translating data from animal models to human diseases can be unreliable.

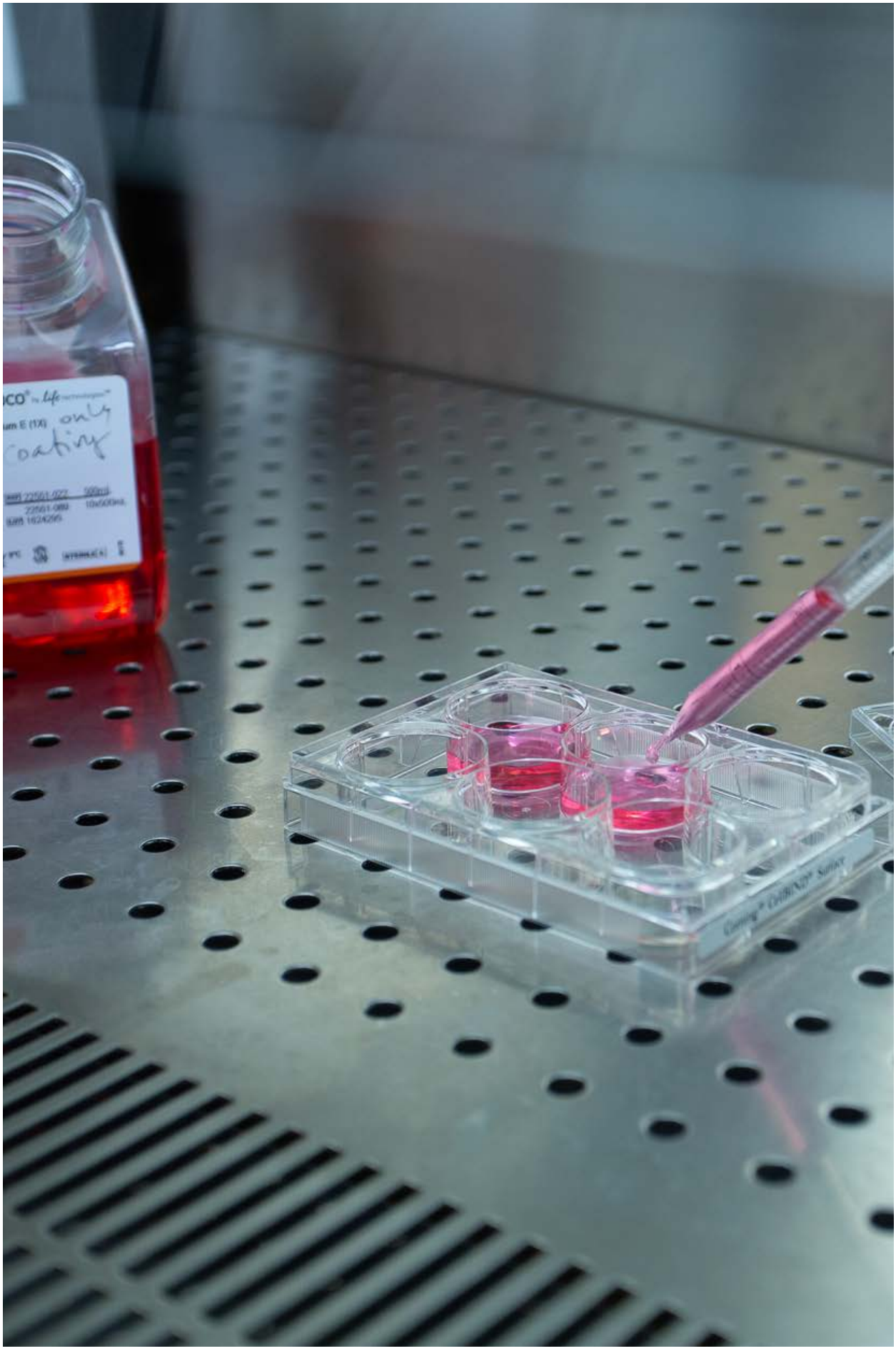
Most human autoimmune diseases show extremely heterogeneous clinical features which cannot be replicated in animals. In addition, a number of differences exist between the immune systems of humans and rodents (e.g., among Fc receptors, Ig isotypes, and immunoglobulin class switching) due to variations in the expression of epitopes (Mestas and Hughes 2004; Sinmaz et al. 2016).

These differences may result in failure to recognise certain antigens and can cause problems in translating mechanistic insights from animal models into therapeutic advances for patients. A report previously showed how multiple sclerosis studies using mouse models have failed to identify interesting therapeutic candidates, and pointed out again the limitations of animal models and their cost (Baker and Amor 2015).

Recently, human stem cell-derived models have emerged as a highly promising tool to study autoimmune diseases in a human context, providing an exciting addition to existing animal-based methods. Indeed, stem cells can be genetically modified, retain a high degree of developmental control, and maintain the capability to replicate, allowing the generation of the high amount of tissue required for high-throughput experiments.

In recent years, convincing human stem cell models have been generated to recapitulate disease-relevant phenotypes and therapeutic response for myasthenia gravis (Julius A. Steinbeck et al. 2016), multifocal motor neuropathy (Harschnitz et al. 2014) and auto-demyelination (Clark et al. 2017). With appropriate development, these methods will play a leading role in the study of the molecular mechanisms of pathogenicity of autoimmune diseases, and ultimately in identifying and testing potential novel therapeutic strategies.

Here we present the results of a systematic literature review of 183 scientific peer-reviewed articles, published from January 2014 to March 2019, that used non-animal models to study autoimmune diseases. The articles were retrieved from PubMed, Scopus and Web of Science databases as well as from grey literature sources.



2 Methodology

To systematically review all studies published in peer-reviewed journals between January 2014 and March 2019, describing or dealing with *in vitro* human models/methods or assays or test systems in the field of autoimmune diseases research, searches were performed on 30 April 2019 using the following platforms:

- PubMed/MEDLINE (*https://pubmed.ncbi.nlm.nih.gov*);
- Web of Science (WoS) (*https://www.webofscience.com/wos/woscc/basic-search*);
- Scopus (*https://www.scopus.com/search/form.uri?display=basic#basic*).

Additionally, we also considered scientific articles describing or dealing with any *in silico* model, such as algorithm, mathematical, computational or simulations, and monitored the following grey literature sources to retrieve news and/or highlights on non-animal models in the field:

- MS Society (*https://www.mssociety.org.uk*);
- European Society for Blood and Bone Marrow Transplantation - Autoimmune Diseases Working Party (ADWP) (*https://www.ebmt.org/working-parties/autoimmune-diseases-working-party-adwp*);
- Autoimmune research foundation (*http://autoimmunityresearch.org*);
- Relent (*http://www.relent.eu*);
- American Autoimmune Related diseases association (*https://www.aarda.org*).

The search strategy was tailored to the different databases, and was structured using the appropriate Boolean operators as shown in Table 1, while eligibility and exclusion criteria are provided in Annex.

Search results from the abovementioned databases were combined, and a list of abstracts was compiled. This initial search yielded a total of 2,970 potentially relevant articles which were screened based on the title and the abstract to evaluate whether they met the eligibility criteria.

After removal of manuscripts not meeting these criteria, 242 scientific articles were retrieved for full-text selection.

This resulted in a collection of 183 articles – avaiable from the EURL ECVAM collection in the JRC Data Catalogue ([1]) – from which all the identified data were extracted and analysed to produce this technical report and the executive summary.

1 *https://europa.eu/!WDjTjH*

Table 1: Search phrases and filters used in each database (PubMed, WoS, and Scopus) to retrieve articles on autoimmune disease published between January 2014 and March 2019.

DATABASE	SEARCH
PubMed	Search autoimmune AND (model* OR assay* OR "test* system*") NOT (child OR case OR mouse OR mice OR veterin* OR equine* OR dog* OR canin* OR rat OR rats OR trial* OR review* OR overview* OR association* OR correlation* OR infection* OR trial* OR crystal* OR retrospective OR follow-up) Filters: Journal Article; published in the last 5 years; Humans; English; Field: Title/Abstract
Scopus	autoimmune AND (model* OR assay* OR "test* system*") AND NOT (child OR case OR mouse OR mice OR veterin* OR equine* OR dog* OR canin* OR rat OR rats OR trial* OR review* OR overview* OR association* OR correlation* OR infection* OR trial* OR crystal* OR retrospective OR follow-up) AND (LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014)) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (LANGUAGE , "English"))
WoS	TS=(autoimmune) AND TS=(model* OR assay* OR "test* system*") NOT TS=(child OR case OR mouse OR mice OR veterin* OR equine* OR dog* OR canin* OR rat OR rats OR trial* OR review* OR overview* OR association* OR correlation* OR infection* OR trial* OR crystal* OR retrospective OR follow-up). Refined by: PUBLICATION YEARS: (2019 OR 2017 OR 2016 OR 2015 OR 2018) AND LANGUAGES: (ENGLISH) AND DOCUMENT TYPES: (ARTICLE) Timespan: Last 5 years. Databases: WoS, BIOSIS, CABI, CCC, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC. Search language=Auto

3 Study results

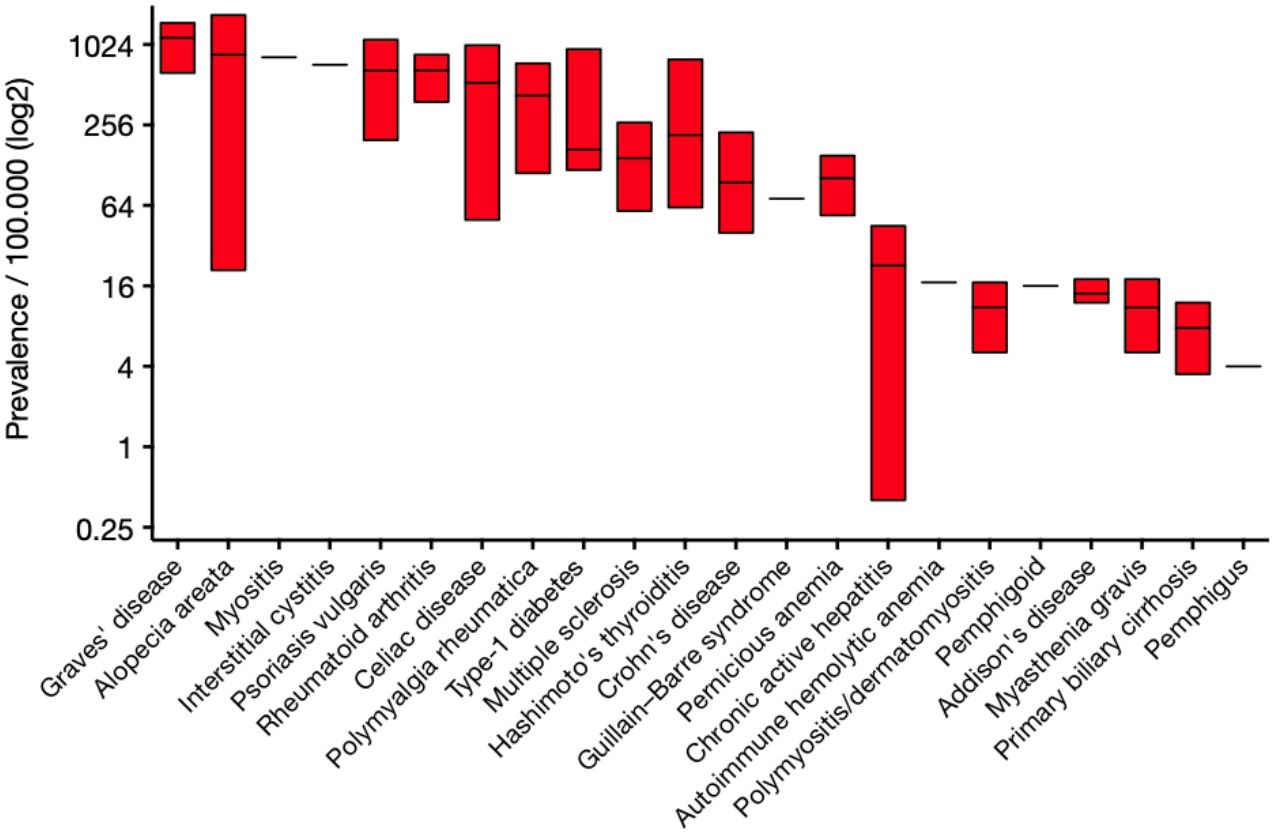
3.1 Distribution of peer-reviewed articles by autoimmune disease

We initially focused our search on the most prevalent autoimmune diseases. To accomplish this objective, we calculated the autoimmune disease prevalence based on the published literature (Cooper, Bynum, and Somers 2009; Cooper and Stroehla 2003; Hayter and Cook 2012).

Our estimation showed that the 12 most prevalent autoimmune disorders are (Figure 1; Eaton et al. 2007): 1) Grave's disease, 2) alopecia areata, 3) myositis, 4) interstitial cystitis, 5) psoriasis vulgaris, 6) rheumatoid arthritis, 7) celiac disease, 8) polymyalgia rheumatica, 9) type-1 diabetes, 10) multiple sclerosis, 11) Hashimoto's thyroiditis, 12) Crohn's disease.

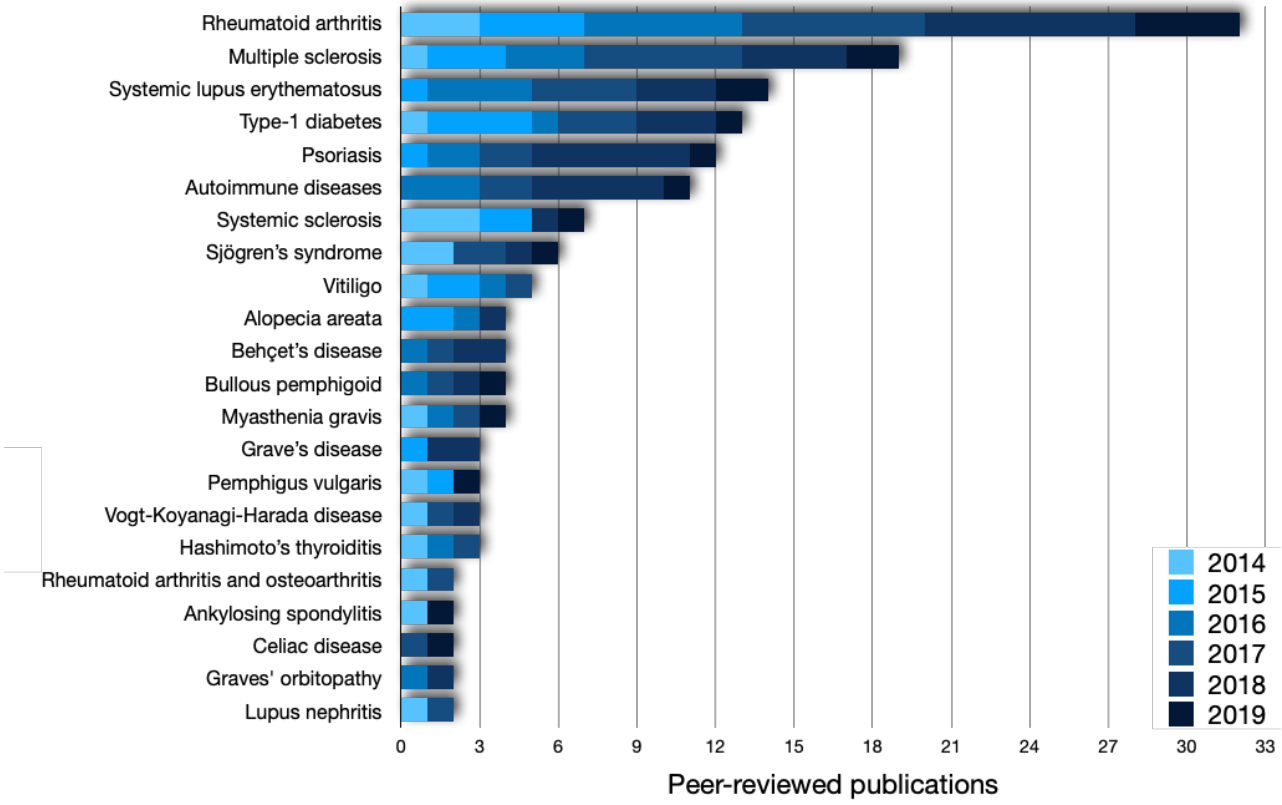
Our systematic search retrieved 183 peer-reviewed articles from January 2014 to March

Figure 1: Estimate of the prevalence* of selected autoimmune diseases.



* Prevalence is shown as number of affected people per 100,000 people (log2 scale); values represent mean (black line) +/- standard deviation (SD, red bars). Six diseases do not present SD since their respective prevalence was reported in only one previous study (Eaton et al. 2007.

Figure 2: Distribution of peer-reviewed articles using non-animal models by autoimmune disease and year of publication. Only autoimmune diseases reported in at least two peer-reviewed publications are shown.



2019 reporting the use of non-animal models in autoimmune disease research (Figure 2). However, the analysis of these articles showed a low correlation (R^2 = 0.07) between the estimated literature-based prevalence and the prevalence of the autoimmune disease studied.

Indeed, the five most frequent diseases investigated by the manuscripts did not cover all the five most prevalent at population level, being rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, T1D, and psoriasis.

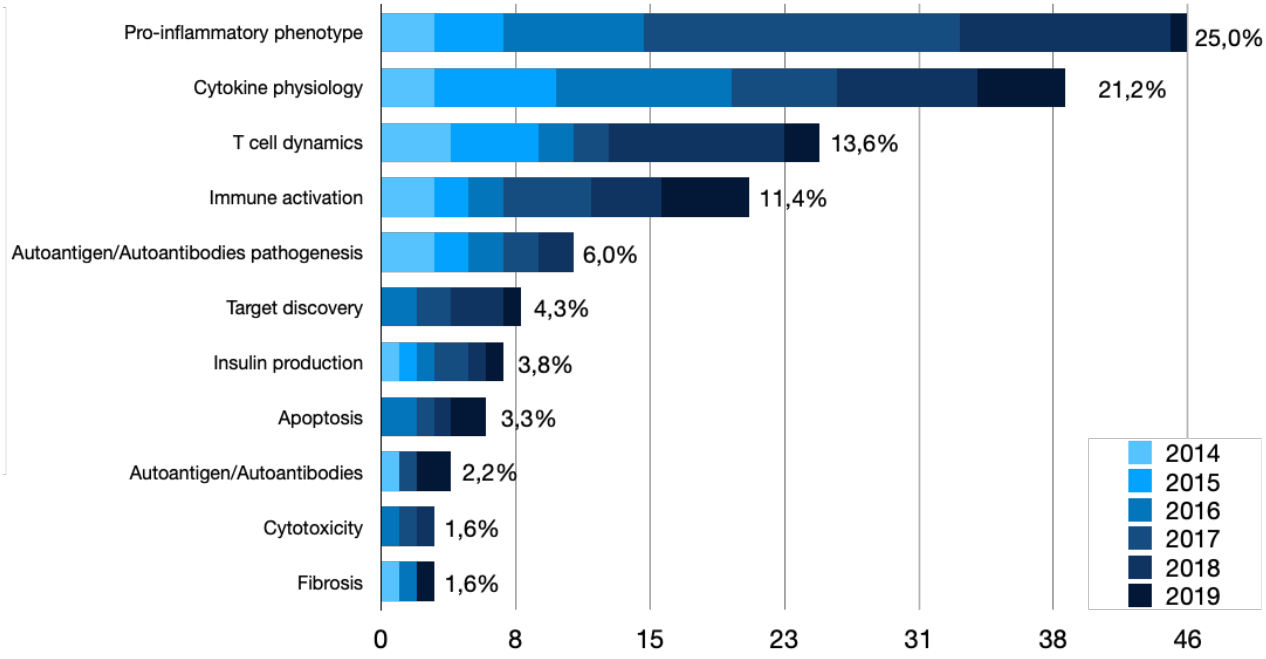
The articles' distribution in the period of study was relatively homogenous from 2014 to 2018, however the number of publications increased by a factor between 1.5 and 2 in the few months of 2019 analysed here.

3.2 Autoimmune disease features covered by non-animal models

The majority of retrieved articles employing human-based models to study autoimmune diseases (59.8%) aimed to model pro-inflammatory phenotypes, cytokine physiology and T cell dynamics (Figure 3). These models are also used to study immune activation, autoantigen and autoantibodies pathogenesis in 17.4% of publications, whilst 11.4% of them were applied to search disease therapeutic targets (target discovery: 4.3%). Others aimed to investigate insulin production (3.8%), apoptosis (3.3%), cytotoxic events (1.6%) and fibrosis (1.6%; Figure 3). The remaining 6% modelled other 11 specific features of autoimmune diseases and only one scientific article was retrieved for each of them ([2]).

([2]) *https://europa.eu/!WDjTjH*

Figure 3: Distribution of articles by disease feature modelled by non-animal models and year of publication.



3.3 Research areas where human-based models are applied

Our analysis of the 183 scientific articles identified five main areas of applications for non-animal models in the autoimmune research field (Figure 4). The majority of studies fell into two categories: those investigating disease mechanisms, and those analysing therapeutics approaches, with each category representing 40% of all papers. The remaining publications concerned the development and testing of drug candidates (23%), the analysis of possible therapeutic targets (disease therapy development, 17%), and the qualification of human-based models and methods (17%).

In addition, the temporal analysis showed an increase of the number of papers qualifying the use of the models from 2014 to 2018, indicating an increased interest in the use of alternative methods in this field. Finally, 2% of the selected articles describes the use of these models to characterise diagnostic methods of autoimmune diseases.

3.4 Distribution of peer-reviewed articles by type of models used

In vitro models were employed in 91% of the studies, whereas *in silico* approaches represented 9% of them (Figure 5).

The classification for mathematical and computational models was based on the information 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).

A sharp increase in the number of studies making use of *in vitro* approaches was observed from 2014 (19 articles) to 2018 (39 articles). In addition, the fact that 18 articles meeting the criteria of our search were published in just the first quarter of 2019 suggests that this trend might continue into the future (Figure 5).

A detailed analysis of *in vitro* models showed that the majority of them were cell-based

Figure 4: Total number and percentage of articles published by research area and year.

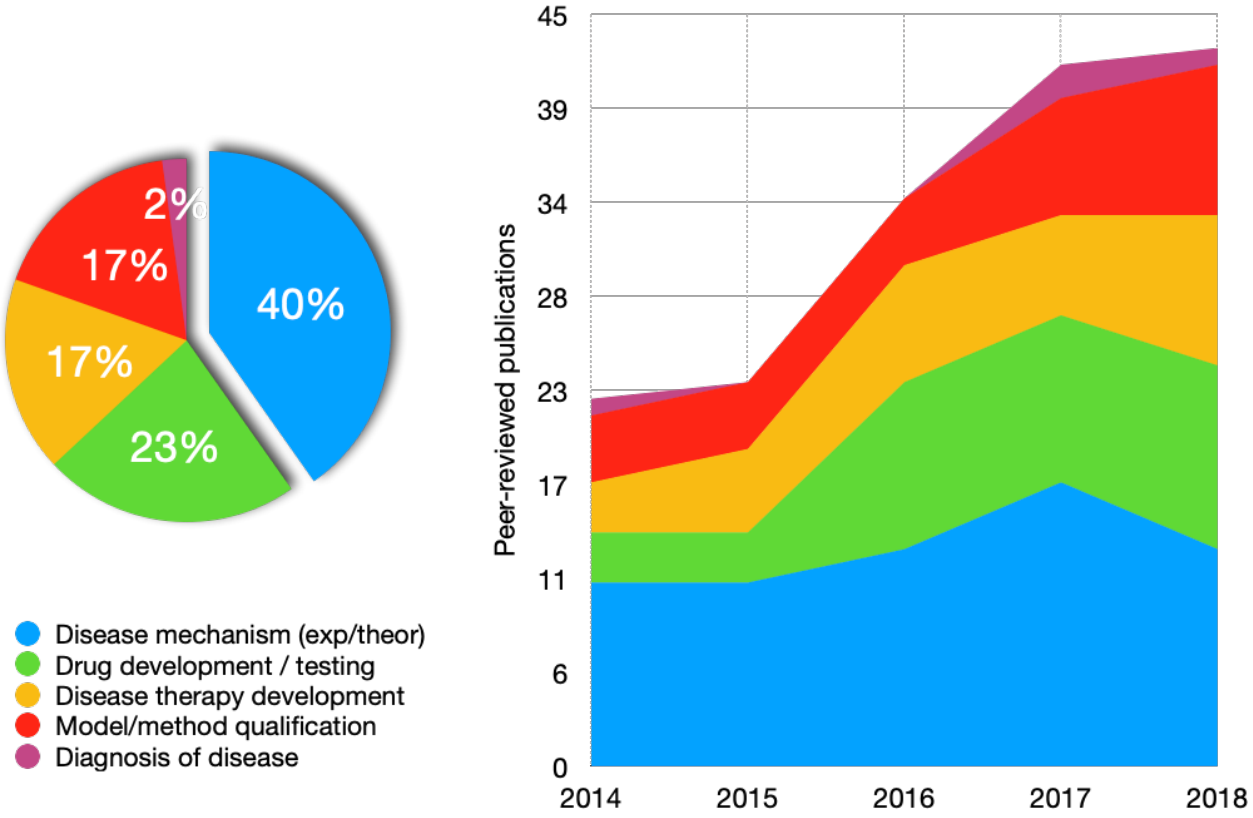


Figure 5: Total number and percentage values of peer-reviewed articles by type of non-animal models used (*in vitro* and *in silico*) and by year of publication.

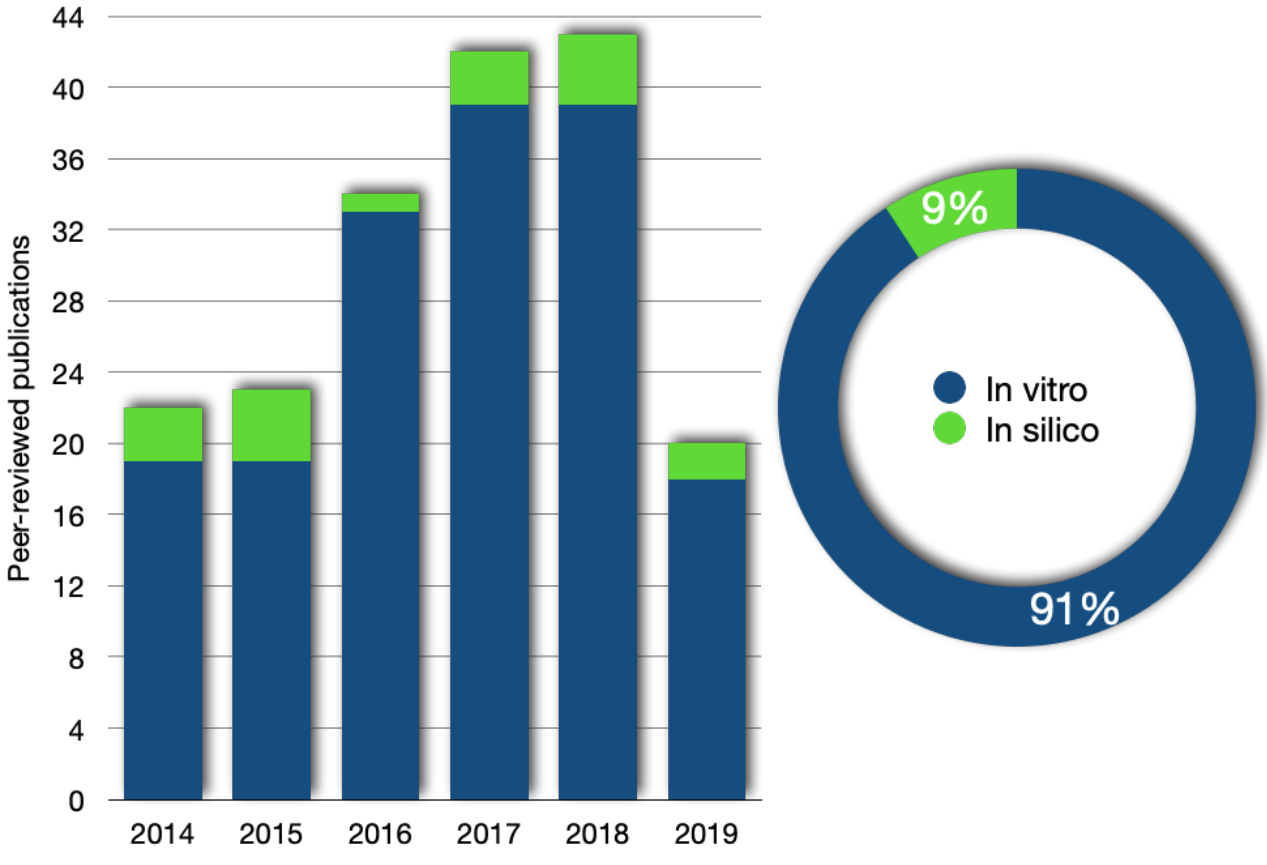
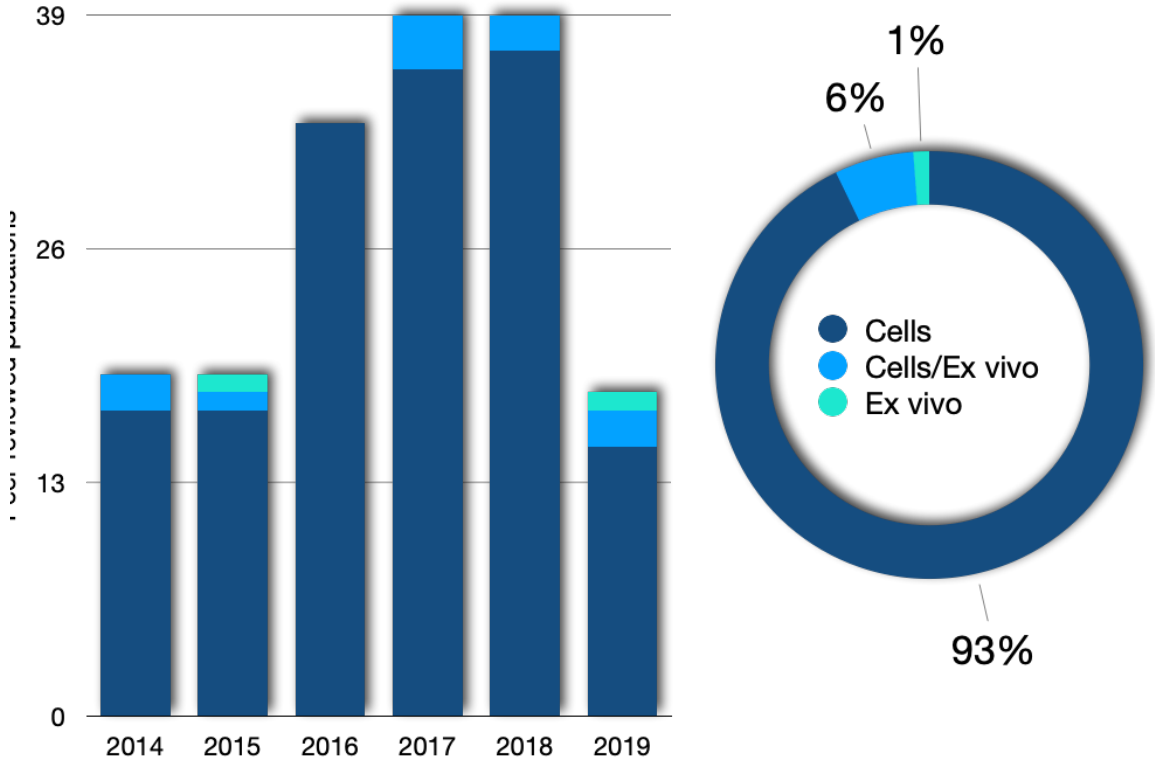


Figure 6: Total number and percentage values of peer-reviewed articles by type of *in vitro* non-animal models used (cells only, cells/ex-vivo, ex-vivo only) and by year of publication.



(93%), whereas 6% employed combined approaches (cells and *ex vivo* models) and only 1% were based on *ex vivo* methods only (Figure 6). This indicates that human cell-based models might be driving the increase in the use of *in vitro* methods recorded between 2014 and 2018.

We found 17 articles describing *in silico* approaches (mathematical = 12; computational = 4 and algorithm = 1; Figure 7). Among the mathematical approaches, those modelling cytokine physiology and autoantigen/autoantibodies pathology were the most frequent (8 articles out of 12), whereas 33% of mathematical models and computational approaches combined were employed for studying T cell dynamics, fibrosis and immune activation.

We also retrieved a study describing an algorithm for immune activation in alopecia areata (Chen et al. 2015).

3.5 *Ex vivo* models to study autoimmune disease with skin affection

Ex vivo models were mostly used to study autoimmune diseases with skin affection, such as bullous pemphigoid, epidermolysis bullosa acquisita, pemphigus, psoriasis, vitiligo and systemic sclerosis.

Nine articles reported the use of human skin scalp samples as organ slice model to study the disease mechanism (Figure 8).

Two other articles employed biopsies to study type-1 diabetes (D'Addio et al. 2015) and bullous pemphigoid diseases' mechanism (de Graauw et al. 2018), whilst one study used a whole organ model of placenta to test therapeutic strategies (Roy et al. 2019; Figure 8).

Figure 7: Number of peer-reviewed publications by type of *in silico* models and by disease feature analysed.

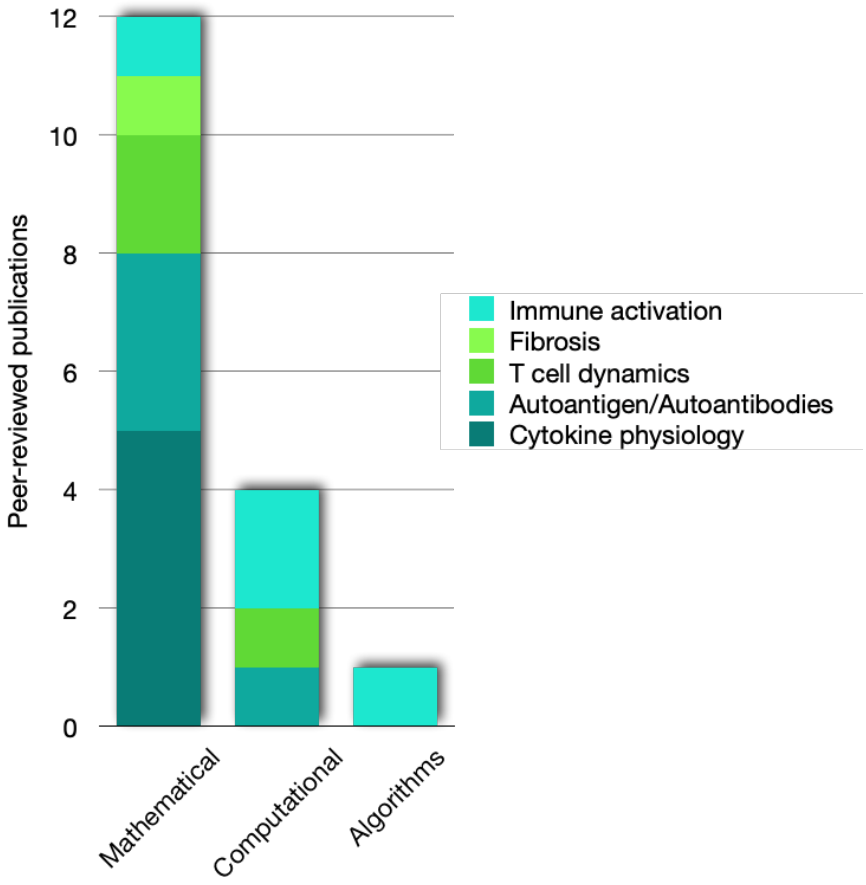
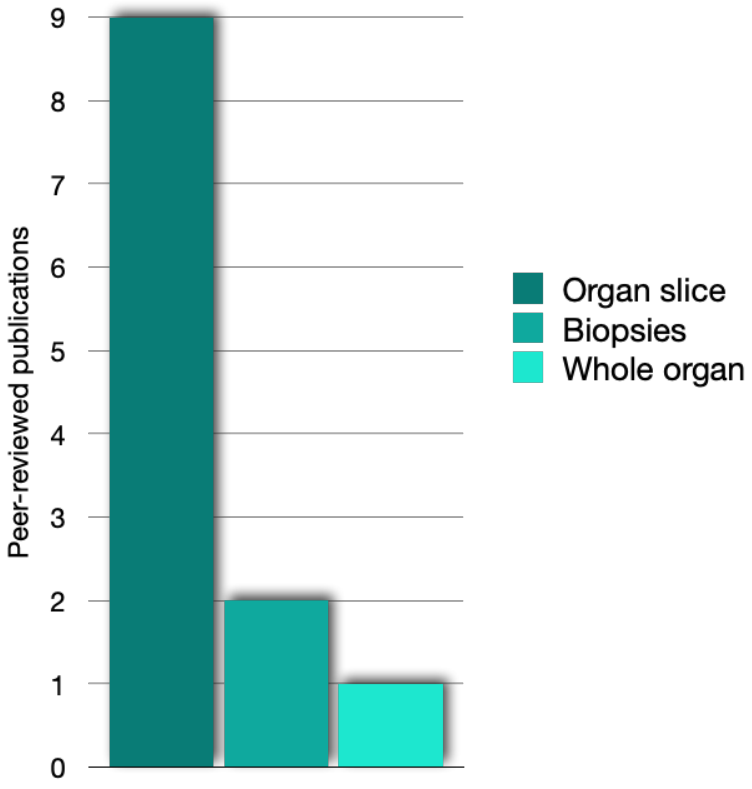


Figure 8: Number of peer-reviewed publications classified by type of *ex vivo* models (organ slice, biopsies, whole organ).



3.6 Cell types and cultures employed

We categorised the cell-based models used in autoimmune disease research in three sub-classes: primary cell cultures, immortalised cells and stem cells.

In our analysis, we found that 80% of articles reported the use of primary cell cultures, and that the number of publications steadily increased from 2016 to 2018 (Figure 9).

They were implemented as the only models in 68% of the articles, whereas 10% of studies reported using them in combination with immortalised cell lines, and 1% (three articles) in combination with stem cells. Of these, two articles employed them in co-culture models (Piatek et al. 2018; Julius A Steinbeck et al. 2016), and one as two separate individual models (Seren et al. 2018; Figure 9).

The second most reported cellular model was represented by immortalised cell lines (12%), followed by human stem cells (8%, 13 articles) (Figure 9). Of these, nine publications reported using pluripotent or pluripotent-derived cells and 2 publications used somatic stem cells-based models (Coppola et al. 2017; Pringle et al. 2019).

The use of primary culture of human peripheral blood mononuclear cells (PBMCs) and different sub-type of T cells was reported for 31.6% of non-animal models.

Other types of used human immune cells were dendritic cells (DCs; 3.3%), B cells (3.3%), neutrophils (2.9%), monocytes (2.9%) and various others (Figure 10). In addition, human non-immune cells were also employed, namely fibroblasts (9.0%), fibroblast-like synoviocytes (8.2%), keratinocytes (6.1%) and endothelial cells (4.5%; Figure 10).

Figure 9: Total number and percentage values of peer-reviewed articles by type of cells used and year of publication.

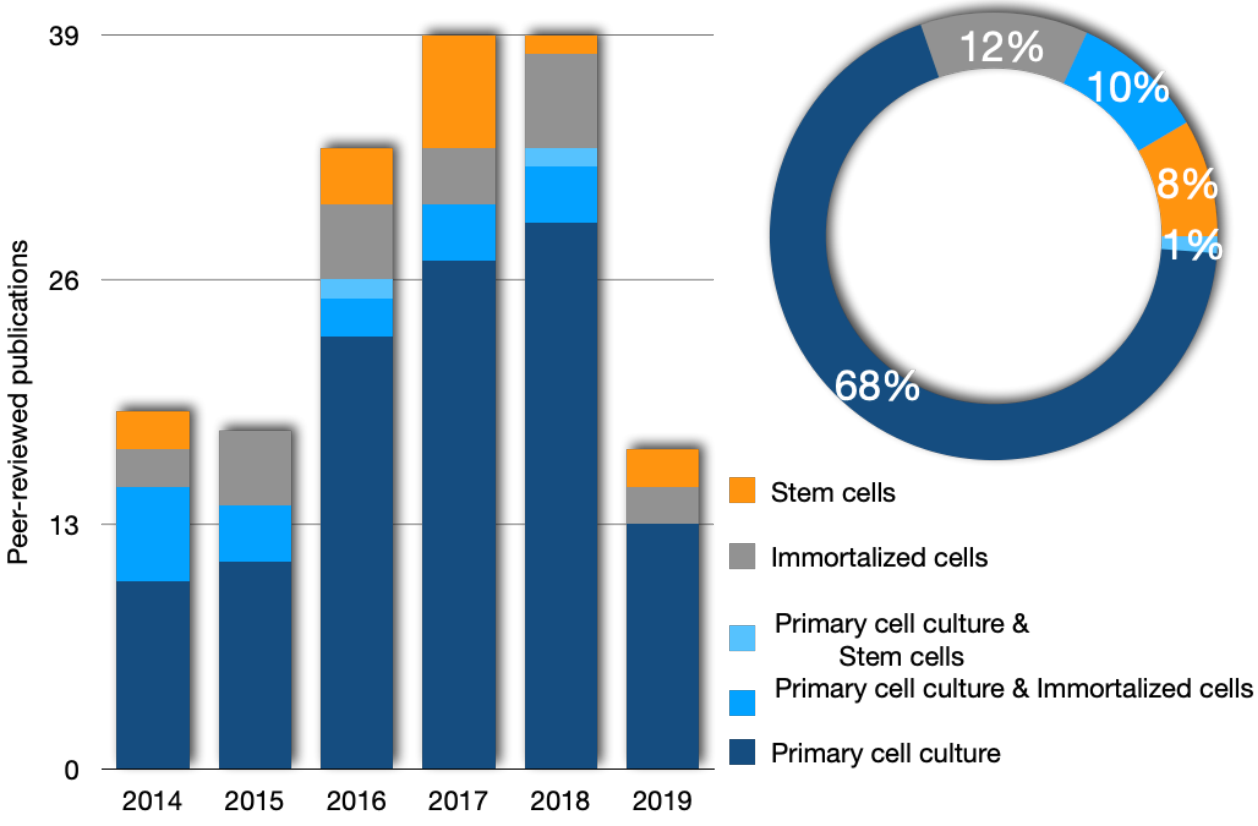
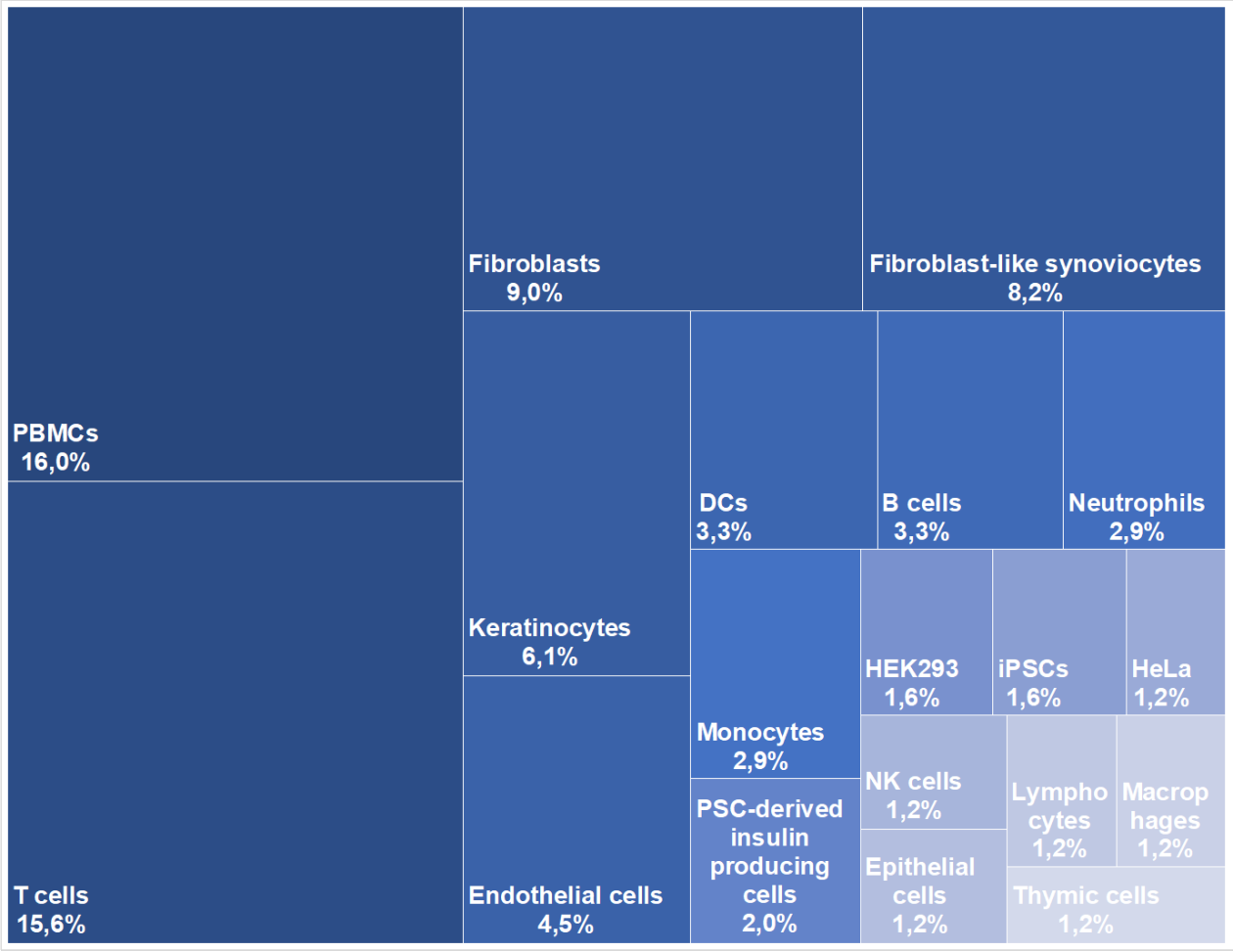


Figure 10: Cell-type employed in the retrieved articles. The percentages are calculated on the number of citations per cell-based model. Only cell types reported in more than 1% of publications are shown.



Most models used primary cells in single culture condition to compare diseased cells from patients with those from healthy individuals, (74% of the scientific articles using *in vitro* cell-based models; Figure 11).

An equal proportion of studies employed cell-based models only in co-culture conditions, or in both culture and co-culture (10% each; Figure 11). A smaller percentage (7%) implemented microphysiological system technologies (MPS) as their methods of choice (Figure 11).

The use of MPS increased from one article per year, from 2014 to 2016, to three articles per year in 2017 and 2018 (Figure 11). Furthermore, three articles were retrieved for the first quarter of 2019. In these type of articles, organoids and spheroids were reported eight (Bouchi et al. 2014; Chen et al. 2018; Kim et al. 2016; Loomans et al. 2018; Pringle et al. 2019; Tao et al. 2019; Thomas et al. 2017; Wang, Jin, and Ye 2017) and two times (D'Addio et al. 2015; Manzar, Kim, and Zavazava 2017) respectively.

Regardless of the culture condition (culture or co-culture), cells were cultivated in bi-dimensional (2D) conditions in 79% of the selected articles (Figure 12). The observed increase of *in vitro* models in autoimmune disease research during the period 2016-2019, was mostly due to 2D cellular models. Considering the overall period of study, models with more than two dimensions were employed in 20% of articles (2D/2.5D: 7%; 2.5D: 2%; 2D/3D: 3%; 3D: 8%; Figure 12).

Figure 11: Total number and percentage values of peer-reviewed articles by type of cell culture condition (culture, co-culture, MPS: microphysiological system) and by year of publication.

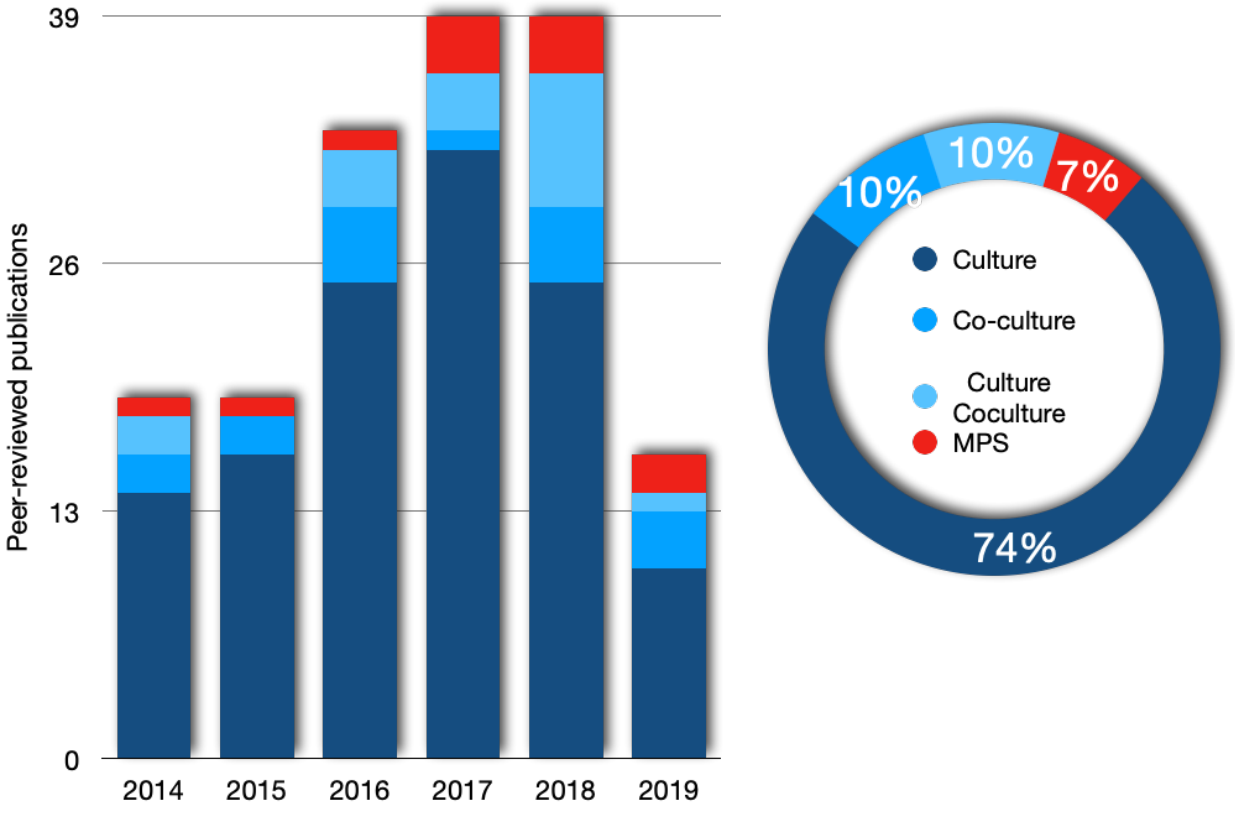
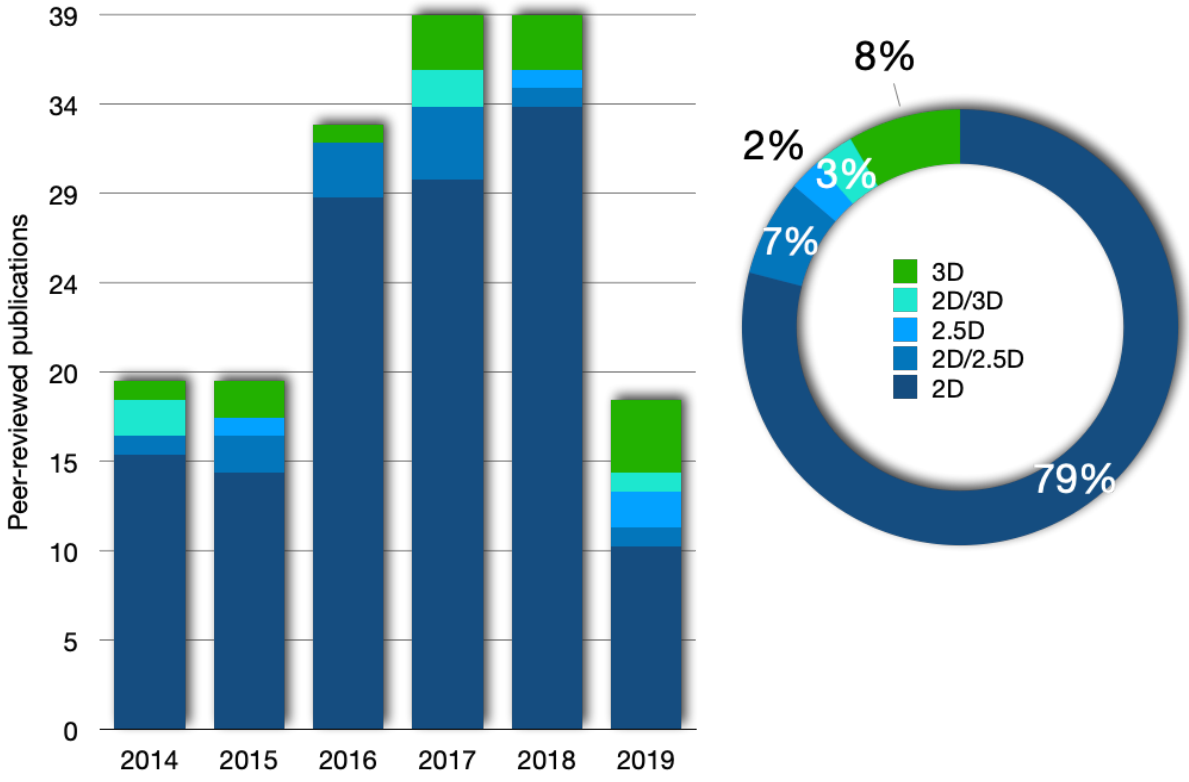
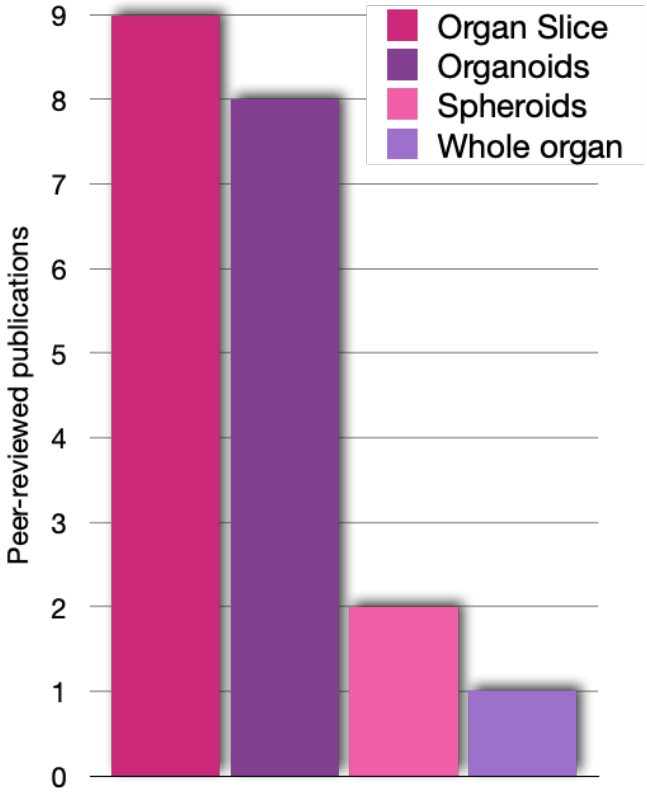


Figure 12: Total number and percentage values of peer-reviewed articles by number of culture dimensions (2D/2.5D/3D) and by year of publication.



3D culture conditions were used in 20 publications, including nine making use of organ slices as model system, eight employing organoids cultures, two using spheroids, and one article reporting the use of whole placenta organ (Roy et al. 2019; Figure 13).

Figure 13: Number of articles published from 2014 to March 2019 divided by type of human *in vitro* 3D model used.



3.7 Throughput and biological relevance of models

We also analysed the use of human-based models in autoimmune disease research considering model throughput ([3]) and the quantity of biological information they produced ([4]). The majority of publications (152 or 83%) reported a low model throughput and a low information content, whereas 7.5% of publications (14) described high-throughput and high-content aspects (Table 2).

On one hand, 49% of studies (91 articles) employed human-based models commonly used in research (Figure 14), while on the other 20% of publications reported proof-of-concept models. Additionally, 14% of articles made use of models qualified by the same research group in previous publications (qualified internally; Figure 14), whilst 10% of them used the ones already published by others (qualified externally; Figure 14).

The articles made use of non-animal models to investigate several disease features, as shown in Figure 15. The majority of these studies (89%) employed models which had a central and primary role in modelling these features, and therefore had a direct biological relevance in achieving the study's aims. Only 11% of them had instead a supportive role since other models were employed to address the study's hypothesis.

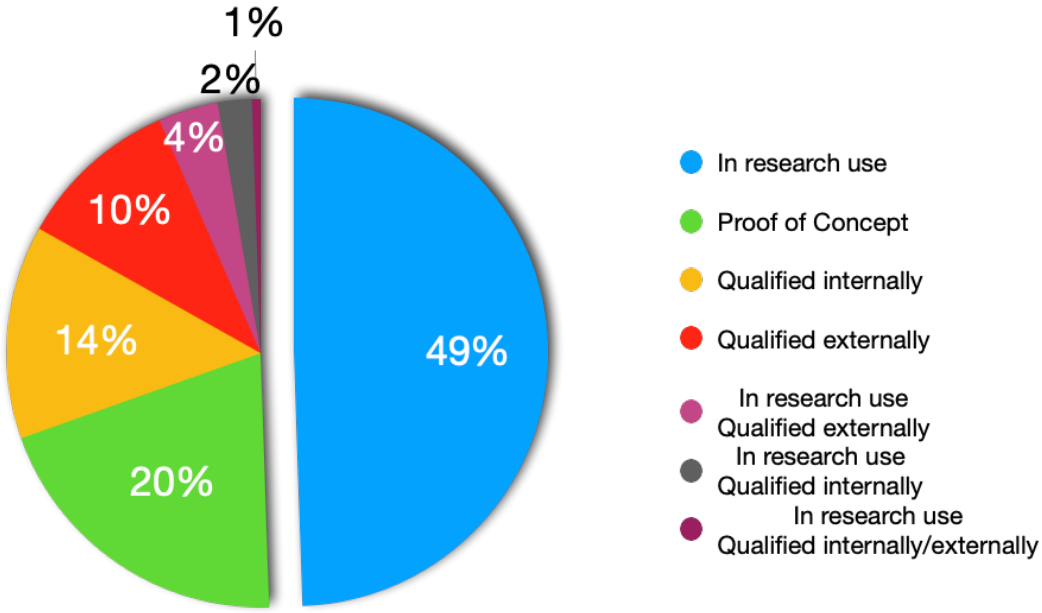
Table 2: Classification of the articles based on the content and throughput levels (low, medium, high) of the models employed.

		THROUGHPUT		
		Low	Medium	High
CONTENT	High	2	0	14
	Medium	1	1	3
	Low	152	5	3

([3]) Throughput is defined as the number of samples that can be processed in parallel.

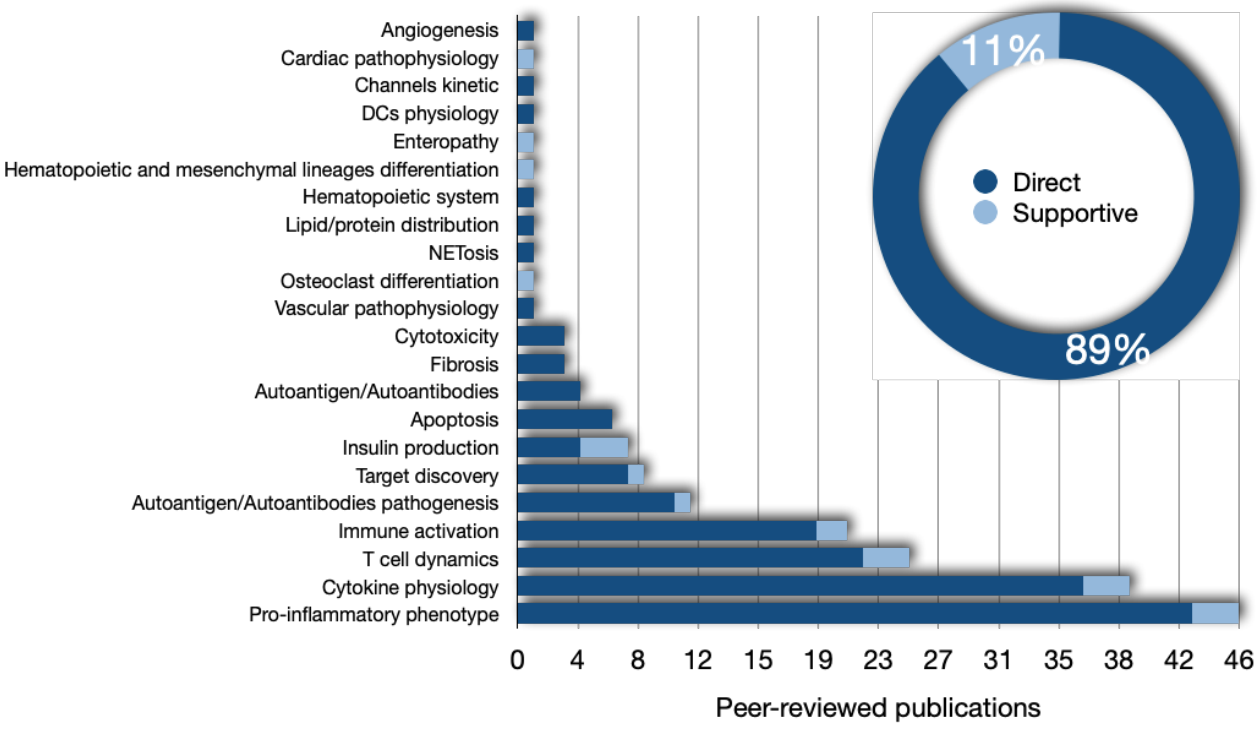
([4]) Content is defined as the quantity of information retrieved by each sample with a single analysis or method.

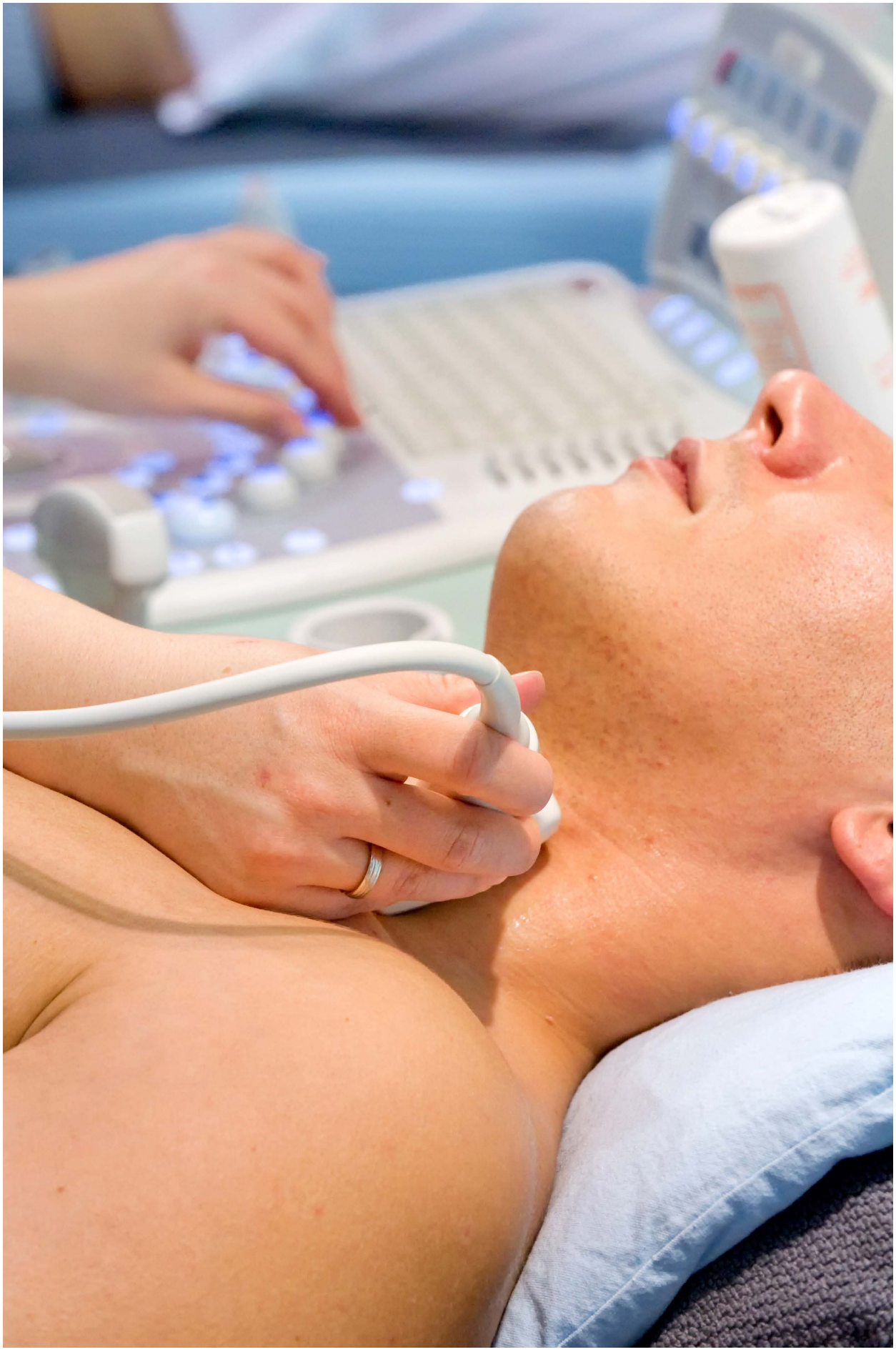
Figure 14: Classification of non-animal models based on their stage of development*.



* Proof of concept: new method/model description; in research use: method/model in use by the research community or commercial kits are used; qualified internally: the research group is referencing own previous article/s; qualified externally: the research group is referencing other previous article/s; in research use/qualified externally: method/model in use by the research community with external reference; in research use/qualified internally: method/model in use by the research community previously published by the authors; in research use qualified internally/externally: method/model in use by the research community with external and internal references.

Figure 15: Number of peer-reviewed publications by disease's feature investigated. For each feature, employed models were classified based on their biological relevance in modelling that feature (direct or supportive).





4 Conclusions

The immune system has essential physiological roles, acquired during evolution, to protect an organism from the invasion of external pathogens and to maintain homeostasis in response to internal stimuli. However, its intricate regulation can be altered by genetic, internal and/or environmental events to the point of no return. In these situations, the immune system's humoral and cellular effectors may damage the organism's own cells, and give rise to the phenomenon of autoimmunity, which at present causes more than 100 different types of pathological conditions worldwide (AARDA 2020).

The complexity of the human immune system, and the idiopathic nature of most of these diseases, hampers the bona-fide modelling of the pathology in animals, due to the profound interspecies genetic and epigenetic differences. However, animal models do exist, with several different ones often being used to study a single disease as they have been developed for different purposes, and following a "trial and error" methodology (Yu and Petersen 2018).

Although biomedical research on autoimmune diseases is highly fragmented, with each research group working on a specific disease using a specific model, at the end of 2019 a large European project funded by the Innovative Medicines Initiative (IMI) was started with the aim of studying the fundamental common mechanisms of autoimmune diseases, by using real world data (IMI 2019).

In our opinion, this project opens a new era for in humano and *in silico* modelling of autoimmunity. However, any findings provided by this project should also go through pre-clinical studies, since sponsoring clinical trials is highly expensive and there is the need for the research community to understand the underlying etiological molecular mechanism. In this context, human relevant and advanced *in vitro* models could represent an interesting platform to test clinical hypotheses *ex vivo*. Although modelling the complexity of the immune system *in vitro* requires elaborate R&D efforts, and will likely only be achieved by the development of several different models, considerable advances have already be been made concerning a human immune system-on-a-chip platform (Polini et al. 2019).

At present, the research field on autoimmunity is still struggling to understand the intricate network of malfunctioning interactions leading to the onset of most autoimmune diseases. This hampers the full exploitation of *in vitro* and *in silico* models. However, a variety of human-based models are used to reproduce specific aspects of these diseases, as we show in this systematic analysis of the literature. Indeed, we identified 183 articles using non-animal approaches to study 21 different aspects of 48 diseases. Of note, the autoimmune diseases investigated were not necessarily the ones most prevalent in the general population. This indicates that research efforts into this field are driven by factors other than disease prevalence.

Most models were employed to study the disease mechanism at cellular level by using human patient primary immune cells, highlighting the need of relevant human pathology models. Indeed, the use of stem cells and immortalised cells lines as models is hindered by the lack of understanding of the genetic or external causes triggering most of these conditions. Hence, using primary cultures of patient immune cells is of great importance in order to understand the immune cells behaviour of patients with particular interest in pro-inflammatory response, cytokine physiology and T cells' dynamics.

In addition, we observed a clear increase from 2015 to 2018 in the number of studies using non-animal models aimed at developing new therapeutic strategies and/or to qualify these models, indicating a positive trend in their usage in this field of research. Within this positive trend, 3D models played a small but important role and, in the few months of 2019 under our analysis, they were employed in more than 20% of the selected articles, suggesting a demand for advanced models to better mimic human diseases *in vitro*. This was also confirmed by the analysis of the model's stage of development, which showed that 54% of them were not already in research use.

Finally, the majority of the publications analysed, applied these models within a basic research context, resulting in low-throughput and low-information-content approaches with reduced insights. However, their implementation was directly addressing the scientific hypothesis of the authors, providing direct biological relevance to the model.

The main findings of this systematic review into the autoimmune disease research area highlight the need to potentiate projects developing the basic components (cells, tissues and organs) of the human immune system *in vitro* using integrated approaches, such as tissue/body-on-chip platforms. Furthermore, to increase the clinical relevance of these tools for researchers working with other *in vitro* and *in silico* methods, it would be of paramount importance to foster the implementation of high-throughput methods and high-content analyses in the context of non-animal models.

For the future, establishing a common meta-research platform to freely share in humano, *in vitro* and *in silico* data will be crucial to push forward the modelling of the highly complex human immune system and its disorders.

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List of abbreviations and definitions

2D/3D	2/3 Dimensional
AARDA	American Autoimmune Related Diseases Association
ADWP	Autoimmune Diseases Working Party
DCs	Dendritic Cells
Ig	Immunoglobulin
IMI	Innovative Medicine Initiative
MPS	Microphysiological system technologies
MS	Multiple sclerosis
NK cells	Natural killer cells
PBMCs	Peripheral blood mononuclear cells
PSCs/iPSCs	(induced) Pluripotent stem cells
SCs	Stem cells
SD	Standard deviation
T1D	Type 1 diabetes
TNF	Tumour necrosis factor
WoS	Web of Science

List of figures

Figure 1: Estimate of the prevalence* of selected autoimmune diseases. 10

Figure 2: Distribution of peer-reviewed articles using non-animal models by autoimmune disease and year of publication. 11

Figure 3: Distribution of articles by disease feature modelled by non-animal models and year of publication. 12

Figure 4: Total number and percentage of articles published by research area and year. . . . 13

Figure 5: Total number and percentage values of peer-reviewed articles by type of non-animal models used (*in vitro* and *in silico*) and by year of publication. 13

Figure 6: Total number and percentage values of peer-reviewed articles by type of *in vitro* non-animal models used (cells only, cells/ex-vivo, ex-vivo only) and by year of publication. 14

Figure 7: Number of peer-reviewed publications by type of *in silico* models and by disease feature analysed. 15

Figure 8: Number of peer-reviewed publications classified by type of *ex vivo* models (organ slice, biopsies, whole organ). 15

Figure 9: Total number and percentage values of peer-reviewed articles by type of cells used and year of publication. 16

Figure 10: Cell-type employed in the retrieved articles. The percentages are calculated on the number of citations per cell-based model. Only cell types reported in more than 1% of publications are shown. 17

Figure 11: Total number and percentage values of peer-reviewed articles by type of cell culture condition (culture, co-culture, MPS: microphysiological system) and by year of publication. 18

Figure 12: Total number and percentage values of peer-reviewed articles by number of culture dimensions (2D/2.5D/3D) and by year of publication. 18

Figure 13: Number of articles published from 2014 to March 2019 divided by type of human *in vitro* 3D model used. 19

Figure 14: Classification of non-animal models based on their stage of development*. 20

Figure 15: Number of peer-reviewed publications by disease's feature investigated. For each feature, employed models were classified based on their biological relevance in modelling that feature (direct or supportive). 20

List of tables

Table 1: Search phrases and filters used in each database (PubMed, WoS, and Scopus) to retrieve articles on autoimmune disease published between January 2014 and March 2019. 8

Table 2: Classification of the articles based on the content and throughput levels (low, medium, high) of the models employed. 19

Annex: inclusion and exclusion criteria applied to the systematic search

Inclusion criteria:

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

Exclusion criteria:

1. The study does not deal with autoimmune diseases.
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.

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