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Executive summary

Background

The introduction of personalised medicine has manifested the need for preclinical models which can generate accurate and predictive data. This has meant a shift in translational research away from just a demonstration of efficacy and towards developing more sophisticated preclinical models which aim to integrate the increasing molecular categorisation of diseases to define proof of mechanism and predicting patient selection. There is a need for more robust resources for validating biomarkers, identifying the suitable pre-clinical model and subsequently demonstrating clinical utility of the stratified approach. The identification of bottlenecks and challenges of pre-clinical methods is a first step in defining a shared personalised medicine development strategy and can lay the foundation for more successful clinical trials across the sector.

The focus of the WP5 scoping review is pre-clinical methods (animal, cellular, organoid and in silico) for translational development of stratified therapies and treatment selection. The scope was a broad focus on the preclinical methodologies, highlighting advantages and disadvantages of the existing pre-clinical model systems used for personalised medicine, as well as the emerging models proposed to replace the traditional animal models. In addition, the methods were assessed for relevance, validity, predictive value and interpretation of the models in the context of personalised medicine. Two case models were chosen: oncology as being the most advanced in the field of personalised medicine, and brain disorders, in particular mental, neurodegenerative and neurodevelopmental diseases.

Research questions

The main research questions addressed for oncology were:

- Which pre-clinical models are currently used to provide validity data prior to therapeutic clinical trials of personalised medicine in oncology?
- What are the pros and cons of the various pre-clinical methods in oncology?
- Are the current pre-clinical models predictive for personalised medicine trial outcome in oncology?

The main research questions addressed for brain disorders were:

- Which pre-clinical models are currently used to provide validity data prior to therapeutic clinical trials of personalised medicine in brain disorders?
- What are the pros and cons of the various pre-clinical methods in brain disorders?
• How many drugs have been developed/are currently under development based on multi-omics profiling programs? What is the estimated success rate of the trial using this approach?
• What information was collected at the pre-clinical stage to inform the clinical study design?

In addition, in order to map patient stratification strategies currently undertaken by industry without any focus on a specific case model, a survey has been developed and distributed to experts in experimental and translational medicine, in both large pharma companies and SMEs. The results of the survey will be discussed during a workshop with field experts and key stakeholders, with the aim to develop recommendations for the establishment of industry-based translational strategies in personalised medicine.

Methods

A study protocol reporting all methodological details was uploaded in the Zenodo repository before conducting the present scoping review, which has been performed following the methodological framework suggested by the Joanna Briggs Institute and using the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews). Two separate search strategies, for oncology and brain disorders, were performed in several databases between March-June 2020.

Research papers and reviews which describe the use of preclinical methods in the broad context of personalised medicine development and assess the validity, reliability and predictive value of the methodologies were included, congress reports and abstract as well as articles with focus on only one disease, and articles that did not focus on personalised medicine methodology were excluded. No restrictions in terms of types of publications were included. During the data extraction phase, the main feature of each paper considered eligible, as providing information of a given aspect covered by one or more research questions, was summarised in tables by one reviewer and checked by a second reviewer to ensure data quality.

Results

In oncology, a total of 1292 records were screened from the literature search, and an additional 14 records were identified through hand searching. After removal of duplications, 1158 records remained, and 895 of these were excluded, leaving 263 articles for full text evaluation. A final total of 63 studies
met the inclusion criteria and were reviewed for quantitative and qualitative analysis. The insights into the pathophysiology of the disease has highlighted the importance of inter- and intra-tumour heterogeneity, the critical role of the tumour microenvironment, and the involvement of the immune system, but there is a lack of fully developed and reliable preclinical technologies that can navigate the complex variables in therapeutic responses and diagnostic accuracy. The future development of more sophisticated preclinical methods, such as microfluidic systems and in silico modelling, might close the gap in preclinical research in the future, but this is reliant on technologies which are still not developed.

In brain disorders, we identified 1516 articles through the literature search and 13 additional records have been identified through hand searching. Of the 1473 unique articles, the full text of 263 articles was reviewed, along with an additional 9 articles identified through hand searching. Most excluded studies (n=91) were abstracts from congresses and/or conferences. A total of 94 studies (54 reviews and 40 research papers) met the inclusion criteria and were included in the qualitative synthesis. Despite the large use and development of pre-clinical models in brain disorders, their application for personalised medicine approaches is not a reality yet. Among the articles considered for this review, none were focused on applying pre-clinical models for patient stratification. In fact, to date there are fundamental gaps that prevent their broad implementation in personalised SNC illness management. Important drawbacks are the lack of knowledge in the biology of these diseases, the model incapability to fully recapitulate the human pathologic phenotypes, the limited validity and reproducibility as well as ethical and regulatory issues.

However, our results highlighted more fundamental issues in preclinical research. Lack of methods reporting is a major problem, in three systematic reviews of in vivo PDX models only one included study reported following the recommended ARRIVE guidelines, and due to the large variation in methodology and reporting across the studies no quantitative analysis of bias could be performed in any of these reviews. Another problem is the failure to systematically validate the model systems, both in terms of internal validity (the experiments ability to identify causal relationships) and external validity (the model’s predictive power). The lack of systematic reviews is also a problem, only three systematic reviews were identified in the oncology search and none in the brain disorders search, as the use of systematic reviews to judge the reliability and validity of biomedical research can improve the success and reproducibility of subsequent translational clinical studies in this era of personalised medicine. A relevant point to be addressed is also the low availability of negative data. Negative results are not appealing for publication, meaning that the results of thousands of experiments that fail to confirm the reliability of pre-clinical models do not see the light of day.

Gaps identified
In order to allow for the implementation of personalised medicine, there has to be availability of appropriate preclinical models which can be relied upon to generate accurate and predictive data. In order to achieve this, the following gaps in preclinical methods must be addressed:

- Lack of good experimental models
- Lack of methods reporting
- Lack of standardised protocols
- Lack of external and internal validation
- Lack of systematic reviews and meta-analysis
- Reporting of negative results
- Lack of regulation of the above issues

These gaps will be object of analysis during the “Gap analysis workshop”, scheduled in December 2020.
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Document log

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</tbody>
</table>
Table of Contents

EXECUTIVE SUMMARY ........................................................................................................1
BACKGROUND ......................................................................................................................7
APPROACHES (METHODS) ................................................................................................14
RESULTS ............................................................................................................................16
SUMMARY OF FINDINGS AND NEXT STEPS .................................................................39
REFERENCES .....................................................................................................................42
APPENDIX I – SEARCH STRATEGY ..................................................................................52
APPENDIX II. DATA EXTRACTION FORM ........................................................................58
APPENDIX III – FULL SCOPING REVIEW PROTOCOL ....................................................60
Background

The introduction of personalised medicine has manifested the need for preclinical models which can generate accurate and predictive data. This has meant a shift in translational research away from just a demonstration of efficacy and towards developing more sophisticated preclinical models which aim to integrate the increasing molecular categorisation of diseases to define proof of mechanism and predicting patient selection. There is a need for more robust resources for validating biomarkers, identifying the suitable pre-clinical model and subsequently demonstrating clinical utility of the stratified approach. The identification of bottlenecks and challenges of pre-clinical methods is a first step in defining a shared personalised medicine development strategy and can lay the foundation for more successful clinical trials across the sector. The focus of the WP5 scoping review is pre-clinical methods for translational development of stratified therapies and treatment selection, and two case models were chosen: oncology as being the most advanced in the field of personalised medicine, and brain disorders as the non-oncology medical field.

Introduction

The concept of personalised medicine is going to impact how pharmacological treatments are discovered and developed, how patients are diagnosed and treated, and how health care systems allocate their resources to maximize patient benefits.

Personalised medicine may be considered an extension of traditional approaches to understanding and treating disease. Ideally, it could serve to take clinical decisions based on a patient’s profile (often molecular, but the concept is broader) to minimise harmful side effects, ensure a more successful outcome, and possibly help contain costs compared with a “trial-and-error” approach to disease treatment [1].

Personalised medicine stems on the broad concept that managing a patient's health should be based on the individual patient's specific characteristics, including age, gender, height/weight, diet, environment, etc. Different understandings of personalised medicine exist, in which three main positions can be identified [2]:

(a) personalised medicine is not a new concept as medicine has always been individualized;
(b) personalised medicine is holistic health care, centred around the needs of the individual patient;
(c) personalised medicine is treatment targeted at stratified subgroups (e.g. pharmacogenetics).
Even when the focus is restricted to the third position, there is not a unique definition of personalised medicine, nor a straightforward terminology to define this concept. While “personalised” emphasizes the notion of individualized—“this is exclusively designed for you”, other more scientifically rigorous terms such as stratified medicine refer to the identification of groups or strata of patients with specific molecular characteristics or other determining factors which predict susceptibility to disease, disease prognosis, and/or response to therapy. Some authors suggested that rather than considering personalised medicine as a precise scientific concept, it should be understood as an open and negotiable ideal that accounts for a plurality of visions, depending on people, reasons and interests behind these alternative conceptions [3].

Regarding the terminology, in the European context, the term personalised medicine is preferred, as this term best reflects the ultimate goal of effectively tailoring treatment based on an individual’s ‘personal profile’, as determined by the individual’s genetic and phenotypical characteristics. Other terms are widespread, for instance stratified medicine, mainly used in the UK, or precision medicine mostly used in US and broadly referred to the 4 P (preventive, predictive, personalised and participatory) medicine. While there may be small nuances in the literal meanings of these terms, they usually refer to the same concept when applied in practice [4].

A recent review reported that the literature about personalise
ded medicine usually refers to two different semantic approaches. Firstly, patients’ stratification, that is grouping individual patients in subpopulation according to their probability to have a therapeutic benefit from a drug or regimen. Secondly, treatment tailoring, that is the individual status of a patient (i.e., disease characteristics or subject’s genotype/phenotype) is the rationale basis for drug choice [5].

A broad community of stakeholders, including funders and professionals involved in medical research and care, are increasingly concerned with ensuring that the right patient receives the right therapy, at the right dose and at the right time. The identification of markers of mechanistic pathways or multiple variables characterising clusters of subjects that might inform meaningful disease stratification may have different clinical applications in the context of personalised medicine. Broadly, stratification may be applied at the diagnosis level (e.g., to identify a particular pathophysiological/clinical stratum within a heterogeneous patient population for diagnostic purposes), to predict disease course (prognostic value), the development of a disease (predictive value), or the response to therapy (theragnostic value).

Regardless of the application, any approach to personalised medicine should undergo different phases: discovery, validation and definition of usefulness from a clinical perspective. Robust
methodological approaches are needed to deal with the complexity and heterogeneity of the process, as well as the range of possible applications to stratification using multidimensional data (what is meant by “molecular profiling” among other terms).

**Personalised medicine research**

This series of scoping reviews mapped the general concept of methods for personalised medicine, to set the basis for the discussion on robustness and reproducibility of personalised medicine development programmes. The final goal is the identification of standards and needs in terms of methodology of data generation, management, analysis and interpretation to improve clinical studies in personalised medicine.

The group of authors agreed on a common operational definition of personalised medicine research: a set of comprehensive methods, (methodological, statistical, validation or technologies) to be applied in the different phases of the development of a personalised approach to treatment, diagnosis, prognosis, or risk prediction. Ideally, robust and reproducible methods should cover all the steps between the generation of the hypothesis (e.g., a given stratum of patients could better respond to a treatment), its validation and pre-clinical development, and up to the definition of its value in a clinical setting.

The process leading from the hypothesis to the clinic is complex and not always linear. The Medical Research Council in UK recently developed a framework for the development, design and analysis of stratified medicine [6] that is structured in six themes:

- **Theme 1:** Framing the Question/Defining the Population
- **Theme 2:** Designing Stratum Discovery Studies; selecting variables, defining response and powering
- **Theme 3:** Assay Design; managing complexity and variability
- **Theme 4:** Defining Strata; data integration, linkage to existing knowledge, linkage to outcome
- **Theme 5:** Stratum Verification
- **Theme 6:** Progression Towards Clinical Utility

Any attempt for classifying the phases of personalised medicine may appear as an oversimplification. However, a typical research programme in personalised medicine would include: first a stratification cohort (in many cases a retrospective study reusing data and bio samples from existing cohorts) with extensive multimodal data on which stratification algorithms are run, then a validation cohort, normally prospective, that assesses the reproducibility, robustness and validity of the clustering in another sufficiently large patient sample. Thirdly, a translational step is often necessary. In some cases, the use of pre-clinical
models (cellular, in-silico, organoid) might be useful to give confidence in the allocation of patients to specific treatment arms as identified through clustering. Alternatively, the multi-omics profiles from clinical samples can lead to the identification of new disease categories, prediction of disease prognosis, exploration of drug sensitivity and dose selection. Finally, treatment options should be tested in the subgroups of patients in the context of clinical studies, ideally randomised clinical trials, to generate evidence informing regulatory, clinical and coverage decisions.

However, many alternative pathways can be proposed. In some case, the stratification provides detailed information on the mechanism of disease and strong indications on the treatments to be tested in each patient cluster. This is for instance the case where identification of driver somatic mutations in cancer cells suggests the targeted treatment to be tested. In other cases, the stratification cohort includes data on response to an established treatment, making the translational step less necessary. Research programmes may be limited to the stratification step, in particular when no treatment is available – this is the case for instance for taxonomy studies in neurodegenerative disorders, aiming at identifying homogeneous clusters of patients. In any case, personalised medicine research is a complex programme, with multiple steps and lasting many years.

We consider out of the scope of this review the methods used for the clinical implementation of personalised medicine, the manufacturing and use of individualized treatments, and the pragmatic approach to individual patient care, such as n-of-1 trials.

Considering this framework outlined by Figure 1, the scoping reviews approached personalised medicine research focusing on four main phases:

1. Methods for stratification and validation cohorts
2. Methods for machine learning applied to stratification
3. Pre-clinical methods for translational development of stratified therapies and treatments selection
4. Methods for clinical trials in personalised medicine
Figure 1. Main steps in personalised medicine research programmes
Specific rationale for the WP5 and identification of the research questions

In the personalised medicine translational development process, we can identify 3 different scenarios (Figure 2):

1. Multi-omic profile identified from clinical research that has to be validated in a clinical setting
2. Multi-omic profile identified from clinical research that provide new biological insights, new hypotheses and experimental research projects
3. Drug sensitivity screening and preclinical testing (toxicity, pharmacodynamics) in clinical samples for identifying responders and non-responders, dose ranges, decision making in best treatment opportunity and other aspects relevant for regulation.

In the first scenario preclinical models are not needed, while they play a key role in the scenarios 2 and 3.

The scope was a broad focus on the preclinical methodologies (animal, cellular, organoid, *in silico*), highlighting advantages and disadvantages of the existing pre-clinical model systems used for personalised medicine, as well as the emerging models proposed to replace the traditional animal models. In addition, the methods were assessed for relevance, validity, predictive value and interpretation of the models in the context of personalised medicine. Two case models were chosen: oncology as being the most advanced in the field of personalised medicine, and brain disorders, in particular mental, neurodegenerative and neurodevelopmental diseases.

For the oncology case model the main research questions addressed were:

- Which pre-clinical models are currently used to provide validity data prior to therapeutic clinical trials of personalised medicine in oncology?
- What are the pros and cons of the various pre-clinical methods?
- Are the current pre-clinical models predictive for personalised medicine trial outcome in oncology?

For the brain disorders case model the main research questions addressed were:

- What are the pre-clinical models preferentially used in brain disorders for personalised medicine?
- What are the pros and cons of the various pre-clinical methods used in brain disorders?
• How many drugs have been developed/are currently under development based on multi-omics profiling programs? What is the estimated success rate of the trial using this approach?
• What information was collected at the pre-clinical stage to inform the clinical study design?

In addition, in order to map patient stratification strategies currently undertaken by industry a survey has been developed. The target of the survey are experts in experimental and translational medicine, working for large pharma companies and SMEs. The results of the survey will enable a better understanding of the challenges and opportunities in the translational development phase of personalised medicine clinical trials in the industry. The retrieved information will be discussed during a workshop in which field experts and key stakeholders will develop recommendations for the establishment of industry-based translational strategies in personalised medicine.

The reason for using brain disorders as a case model was that this therapeutic area is included in the FDA Table of Pharmacogenomic Biomarkers in Drug Labelling as one of the most represented after oncology [7]. In particular, we focused on mental, neurodegenerative and neurodevelopmental diseases. According to the WHO definition, "mental disorders" comprise a broad range of problems, with different symptoms, generally characterised by some combination of abnormal thoughts, emotions, behaviour and relationships with others, examples are schizophrenia, depression, intellectual disabilities and disorders due to drug abuse [8]. Neurodegenerative diseases are a type of disorders in which cells in the central nervous system stop working or die, examples of neurodegenerative disorders include Alzheimer's disease and Parkinson's disease [9]. Neurodevelopmental disorders are neurologically based conditions that can interfere with the acquisition, retention, or application of specific skills or sets of information, examples include autism spectrum disorders [10].
Approaches (Methods)

We conducted a scoping review following the methodological framework suggested by the Joanna Briggs Institute [11]. The framework consists of six stages:
1) identifying the research questions,
2) identifying relevant studies,
3) study selection,
4) charting the data,
5) collating, summarising and reporting results,
6) consultation.
We will perform the last step through a workshop with partners of the PERMIT project planned on December 1-2, 2020.
A study protocol reporting all methodological details was uploaded in the Zenodo repository before conducting the present scoping review [12] (see Appendix III). We used the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews) checklist to report our results [13].

Study identification
We searched PubMed, EMBASE, the Cochrane Library, Web of Science, and PsycInfo (search dates: March-June 2020) for research papers and (systematic) reviews in the fields of brain disorders and oncology to first identify the most common methodological approaches. The methods team (IRFMN) defined the search strategies with the support of the review team for identifying relevant keywords.
We limited our search from 2005 to April 2020. We restricted inclusion to English, French, Spanish, Italian and German languages. In addition, a review of grey literature was also conducted to obtain further information. Search strategies are presented in Appendix I.

Eligibility criteria
No restrictions in terms of types of publications were included.

Oncology case model
Research papers and reviews which describe the use of preclinical methods in the broad context of personalised medicine development and assess the validity, reliability and predictive value of the methodologies were included. References with focus on only one disease, or not focusing on a personalised approach of the methodology were excluded.

Brain disorders case model

PERMIT has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N. 874825
We included research papers and reviews describing preclinical models (i.e. cellular based assay, organoids, animal models) and assessing the validity, reliability and predictive value of the methodologies in the mental disorder field. We also included reviews in personalised medicine. Congress reports and abstract as well as articles which did not focus on methodology were excluded.

**Study selection**

The title and abstracts of records identified by the literature search were screened by two independent reviewers. The full text publication of the relevant articles were retrieved and checked for confirming eligibility. Discrepancies were solved by discussion among the review team and the methods group. During the data extraction phase, the main feature of each paper considered eligible, as providing information of a given aspect covered by one or more research questions, was summarised in tables by one reviewer and checked by a second reviewer to ensure data quality.

**Charting the data**

We designed a data extraction form using an Excel file (Appendix II). General study characteristics extracted were as follows: first author name, title of article, year of publication and type of publication. In addition, for each pre-clinical model referred to in the paper, we collected information on its definition, the pre-clinical model type, methodology, advantages, disadvantages face and predictive validity, and applications in personalised medicine.

Full data extraction was conducted by two reviewers working independently in two use cases for all included articles. In the case of disagreements, consensus was obtained through discussion.

Since many narrative reviews have been published about pre-clinical models, we decided to extract data first from reviews, adding relevant missing information from the remaining research papers.

In the present document, we report the results obtained by extracting data from reviews and research articles.
Results

Stratified medicine development in oncology

A total of 1292 records were screened from the literature search, and an additional 14 records were identified through hand searching. After removal of duplications, 1158 records remained, and 895 of these were excluded, leaving 263 articles for full text evaluation. A final total of 63 studies met the inclusion criteria and were reviewed for quantitative and qualitative analysis. The study selection process is outlined in Figure 3.

Figure 3. PRISMA flow diagram for the oncology search in task 2.4

Which pre-clinical models are currently used to provide validity data prior to therapeutic clinical trials of personalised medicine in oncology?

The animal model was the most commonly described and 27 references were included, of which there were three systematic reviews, 19 reviews and five primary studies. 18 (67%) of the references described mouse
models, and of these 15 related to patient-derived xenograft models, the rest described various other animal models, either specifically or in general.

For **cellular models**, 10 references were included, all of which were reviews. Of these, five described the development of microfluidic organ-on-chip platforms, and the other five reviews each described: 3D cellular models, 3D bioprinting, and exploring patient-derived cellular models from circulating tumour cells, tissue microarrays and tumour samples.

For the **organoid model**, 13 references were included. Of these 11 were reviews, one was a perspective and one was a primary study.

For the **in silico model**, 19 records were initially identified, and 12 of these were primary studies. It was far beyond the scope of this report to evaluate the validity of novel *in silico* methodologies, therefore the primary studies were excluded, and seven reviews of various *in silico* approaches in preclinical oncology research was included.

In addition, there were six references which described preclinical models in relation to drug development.

![Figure 4. Preclinical models for personalised medicine in oncology](image)

**What are the pros and cons of the various pre-clinical methods for the development of personalised medicine in oncology?**

**Animal models in oncology**

Animal models are the cornerstone of preclinical methods for cancer research, also often referred to as *in vivo* (within the living) model.

**Rodent models**

Traditional mouse models used in basic and translational oncology are based on *in vivo* inoculation of long-term cultured tumour cell lines, which can be injected ectopically (mostly subcutaneously), orthotopically to mimic tumour growth in its organ of origin or systemically (mostly intraperitoneally, intravenously or intracardially) to study metastatic spread. Despite being by far the most commonly used mouse models,
and the fact that most of our current understanding of cancer and its hallmarks is based on research in these models, cell-line derived xenografts fail to capture the heterogeneous nature of human cancers and their ongoing evolution, and lack the ability to predict human efficacy for most therapies targeted to cancer-driving proteins [14,15]. In line with the increase in knowledge pertaining to the complexity of cancer, mouse models that better represent human cancer patients have been developed. These models will be described in a broad context in relation to their application towards personalised cancer medicine, detailed analysis about specific methods is beyond the scope of this report.

Genetically engineered mouse models (GEMM) can be produced by introducing human-relevant cancer mutations into mice, allowing de novo tumour formation that recapitulate molecular and histopathological features of human disease in a native immune-proficient microenvironment [14,15]. These models have been used for co-clinical trials, which is an approach to stratify the patient population in a clinical trial and involves the testing of new drug entities in both the clinic and the GEMMs developed for that cancer simultaneously, stratification is then based on the response seen in each of the GEMM classes. The disadvantages of this approach are that the GEMMs take a very long time to develop, and the transition of the data to the clinic is slow, which means that the pharmaceutical companies must delay the clinical trials [16]. Other challenges with GEMMs are that they are challenging to work with, the tumours developing in these mice demonstrate a reduced clonal heterogeneity compared with human tumours, and as these are tumours are of murine origin, the biomarkers which are discovered in these models must be validated in human samples[15,17].

Patient derived xenografts (PDX) models are generated by implanting sectioned patient tumour fragments into immunodeficient mice, subcutaneously or orthotopically [17]. Thus, PDX models have the ability to recapitulate the inter-patient and intra-tumour heterogeneity that is inherent to human cancer, and these models are increasingly utilised in several ways; as PDX population “xenopatient” trials for therapeutic screening, as co-clinical avatar studies in individual patients, and for biomarker development. In addition, several large-scale PDX repositories have been implemented, including the EurOPDX consortium, the US National Cancer Institute (NCI) repository of patient-derived models, the Public Repository of Xenografts (PRoXe), the Children’s Oncology Group (COG) cell culture and xenograft repository, the Pediatric Preclinical Testing Consortium (PPTC) and the Novartis Institutes for Biomedical Research PDX Encyclopedia (NIoBR PDXE) [18]. One of the main limitations of PDX models relates to their immunodeficient status, a prerequisite to facilitate xenotransplantation, but which limits the evaluation of immunological effects. To overcome this, various methodologies are employed to generate a competent human immune system in these models, so called humanised PDX (huPDX), leading to differing degrees of immune reconstitution [19]. Currently, the major limitation of this approach is the durability and quality of engraftment of the human immune system [18]. Another consideration is the ability to study metastasis. Subcutaneous
engraftments almost never produce metastasis, but endogenous metastases can occur when using an orthotopic method of engraftment [20]. Non-invasive *in vivo* imaging modalities are necessary to monitor orthotopic models [21].

The main advantages and disadvantages of PDX models are summarised in table 1, limitations include the variable engraftment rate, the fact that the human stroma is gradually replace by murine tissue, in addition to high cost and the need for specialised skills. Another important consideration is the validity of the PDX tumours in representing the human tumour heterogeneity, which is particularly important as these mice can develop human tissue lymphomas if exposed to the Epstein-Barr virus [17]. Systematic studies have reported on a genomic discordance from the original patient samples [22,23], others have found that the fidelity is preserved [24,25]. This highlights the need for a systematic approach to these models, both in terms of internal validity, i.e. standardised methodological procedures to ensure reproducibility, and for external validity, i.e. how well observations in the model translate to clinical practice. There are efforts to implement such standards for PDX models [26], but currently these are not being employed in most studies, the lack of reporting was a consistent finding in three systematic reviews [20,22,24].

Table 1. Summary of advantages and disadvantages of PDX models

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<th>Disadvantages</th>
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<td>PDX recapitulate the intra- and inter-tumour heterogeneity of human cancer</td>
<td>Engraftment induced molecular divergence from original tumour</td>
<td>[16,17,29–31,18,20,22–25,27,28]</td>
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<tr>
<td>Suitable for biomarker discovery</td>
<td>Lack of stromal and immune compartments</td>
<td>[16–18,27,29,30]</td>
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<tr>
<td>Can do personalised drug screening of individual patient tumours</td>
<td>Variable and unpredictable engraftment rates</td>
<td>[15–18,24,28–30]</td>
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<td>Can establish large PDX biobanks for drug discovery</td>
<td>High cost, technically challenging</td>
<td>[15–18,24,28,29]</td>
</tr>
<tr>
<td>Can establish large collaborative PDX platforms</td>
<td>Lack of standardised protocols</td>
<td>[15,18,20,22,26,27,30,31]</td>
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**Other animal models**

*Chicken chorioallantoic membrane* (CAM) is a highly vascularized extra-embryonic membrane of the chick embryo which is accessible for experimental manipulation and engraftment of fresh patient neoplastic tissue has been performed in several studies. Disadvantages include tumour interaction with the developing avian
immune system, the development of nonspecific inflammatory response after 15 days of incubation, and the stromal environment of CAM xenografts is not characterised [32].

**Zebrafish embryos** is a fast and relatively inexpensive model for cancer, with emerging precision oncology applications. However, this model lacks an immune-system, is only available for short-term assays, and require lower physiological temperatures than human tissues [33].

**Transgenic pig cancer models** have been suggested as a suitable *in vivo* model to further advance efficacy testing of promising compounds selected in preclinical studies before moving to human clinical trials, but only limited models are available [34].

**Comparative oncology** is the study of naturally occurring cancer in companion animals, and a proof of concept study performed by the NCI initiative “Comparative Oncology Trials Consortium” has validated bioinformatics analyses on canine tumour genomic data, suggesting dogs with cancer as an ethically attractive model for clinical evaluation of novel personalised medicine approaches. One limitation is the challenges in translation of genomic signatures across species [35,36].

An important aspect when it comes to animal models is the concept of the 3Rs, referring to replacement, reduction, and refinement in the use of animals, and the emphasis on developing alternative methods. Some claim that animal models are not representative for human disease, and should be replaced by novel surrogates, some of which are outlined below [37].

**Cellular models in oncology**

Cellular models are often referred to as *in vitro*, meaning “in an artificial environment”.

The traditional cellular models utilised in preclinical cancer research are cancer cell lines derived from human patients and which are cultured as monocultures or co-cultures in specialised media in plastic flasks or dishes to form two-dimensional cell layers. The advantages of these simple cell culture models are that they offer easy production for high throughput screening procedures. However, this static model does not exhibit morphology, phenotype, metabolism and functionalities representative of human tissues, and therefor lack predictive value for human patients [38].

**Three-dimensional (3D) cell cultures** have been developed in an attempt to overcome some of these limitations. Several different methods can be employed to generate 3D cellular cultures; suspension culture (stir or rotation method), non-adherent surface methods, hanging drop methods and scaffold-based culture [39]. These cancer models are also referred to as tumour spheroids, and they recapitulate the *in vivo* tumour architecture more closely than 2D models, including cell morphology, growth kinetics, signalling pathways and drug response. However, the limitations are that they are more technically challenging and less consistent to culture, and it is difficult to supply oxygen and nutrients to the spheroids [40].
Microfluidic cell culture technologies have emerged as a tool for growing cells in native-like microenvironments. These systems consist of four components; a chip which has microstructures connected to an irrigation system, living 2D or 3D biomaterial, specialised culture media, and sensors for measuring responses to experimental challenges [38]. Describing the details of this micro-engineered technology is beyond the scope of this report, but is covered in recent reviews [38,41]. These organ-on-chips has the potential to facilitate assessment of pharmacological and toxicological effects [42]. Microfluidic tumour models have the ability to replicate the tumour microenvironment in a physiological relevant manner by incorporating a vascular system, co-culturing with relevant cell types, mimic elevated interstitial fluid pressure and shear stresses [39]. Personalised drug therapy is the ultimate goal for tumour-on-chip models and could be achieved by incorporating primary cancer cells in a 3D environment along with other cell types from the same patient, or human induced pluripotent stem cells (iPSC), in order to obtain a true bio-mimicking cancer model [43]. But organ-on-chips are a long way from modelling the human body, there are still many technical and ethical challenges to overcome before patient-matched, tumour-derived, organ chips are available for clinical evaluation for personalised medicine.

3D bioprinting technology provides the possibility to develop in vitro tissue models with physiological relevant cell composition, material properties, complex microstructures and proper vascularisation, ultimately generating a human-on-chip. However, great challenges remain, with the need for further technological and innovative developments. To apply 3D printed tissue models to personalised drug screening and disease modelling, patient specific cell sources, as well as human iPSCs, must be used in development, representing further challenges both in differentiation of stem cells and ethics [44].

Other cellular methods involving patient tissues

Tissue microarray (TMA) is a histopathological method to study and validate cancer biomarkers in various defined cancer patient cohorts. Patient samples are assembled into the same paraffin block and sections can be investigated for biomarker expression through immunohistochemistry or in situ hybridization. The main limitation of TMA is the representativeness of sample, which might not capture the intra-tumour heterogeneity of the patient sample [45].

Patient-derived explants are freshly resected human tumour tissue fragments, without deconstruction of the tumour, that are cultured ex vivo and used for drug studies. The advantage of this method is the use patient-relevant material that retains the original architecture and proliferative capacity of the primary sample, in addition to inclusion of tumour-stroma interactions. The main disadvantages are the availability of samples, which must be cultured immediately and only allows short-term evaluation, as well as the lack of standardised protocols [46].

Circulating tumour cells / disseminated tumour cells can be harvested and used to establish primary cell lines in order to perform functional studies and drug sensitivity testing. One of the main disadvantages of this method is that these cells are usually only available from patients with advanced disease [47].
Organoids in oncology

There is currently no consensus definition of organoids, but the term generally refers to growing cells in 3D to generate cellular units that resemble an organ (=organoid) in both structure and function [48], or as defined by three characteristics; self-organisation, multicellularity and functionality [49]. Organoids can be established to form healthy organs through stem cell initiation and have the potential to provide disease modelling for infectious disease, genetic disease, personalised medicine, drug discovery (screening and toxicology) and regenerative medicine [49,50]. In the field of oncology, primary or metastatic cancer cells are used to develop tumour organoids [51]. It is beyond the scope of this report to comment on the specifics of the various culture conditions and protocols of cancer organoids.

The main advantages and disadvantages of the organoid model based on data extraction performed in the broad context of personalised medicine is summarised and described below in Table 2. Patient-derived organoids (PDO) and organoid tumour biobanks have been established from numerous malignancies, proposing a potential personalised tumour model for drug screening and drug development [50,52,53]. A proposed workflow for a personalised clinical organoid screen includes tumour biopsy, organoid generation and growth, drug panel treatment, response assessment, and optimal drug selection for patient treatment, also incorporating molecular imaging of the organoid [54]. PDOs have been used as a tool to predict chemotherapy response in individual patients, however the main disadvantage of the personalised approach is the inconsistency of the organoid growth rate, and the possibility of overgrowth of non-tumour cell populations [55]. The absence of an immune system is also a limiting factor, excluding any immunotherapeutic assays [48]. The lack of standardised procedures in organoid development, response criteria, and the fact that it is not yet a validated method are also currently limiting the implementation of this method.

Organoids have also been established from circulating tumour cells (CTCs), potentially allowing closer study of metastasising cancer cells. However, CTCs have their own limitations in terms of validation, as well as the other organoid limitations [56]. In future developments of organoids it has been suggested to incorporate organoids with microfluidics technology, creating organoids-on-chip, which might be a prospect to overcome some of the current limitations [38].

Table 2. Summary of advantages and disadvantages of the organoid model

<table>
<thead>
<tr>
<th>ORGANOID models in personalised cancer medicine</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Can be generated from individual cancer patients</td>
<td>Inconsistent, variable growth rate, overgrowth of normal epithelial cells.</td>
<td>[48,50,51,55,57–59]</td>
</tr>
</tbody>
</table>
### PDOs are cellular and molecular representative of parent tumour

<table>
<thead>
<tr>
<th>Lack of stromal and immune compartments, lack of perfusion</th>
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</thead>
<tbody>
<tr>
<td>[48–52,55,57,58,60]</td>
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</table>

### Can do drug screening of individual tumours

<table>
<thead>
<tr>
<th>Primary tumour directly exposed to drug, representative for in human?</th>
</tr>
</thead>
<tbody>
<tr>
<td>[49–54,57]</td>
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### Can establish biobanks of organoids for drug discovery

<table>
<thead>
<tr>
<th>Low throughput, medium requirements limiting factor</th>
</tr>
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<tbody>
<tr>
<td>[50,52,55,57,58,61]</td>
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### Can be transplanted for in vivo screening

<table>
<thead>
<tr>
<th>Not validated to replace existing systems</th>
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<tbody>
<tr>
<td>[52,54,58,59]</td>
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### Less expensive than PDX-mouse models

<table>
<thead>
<tr>
<th>Technically challenging, expensive, access to tumour material</th>
</tr>
</thead>
<tbody>
<tr>
<td>[51,52]</td>
</tr>
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</table>

### In silico models in oncology

The term **in silico** refers to as performed on computer or via computer simulation, and the techniques can be summarised as the process of integrating computational approaches to biological analysis and simulation.

In cancer research, **in silico** modelling has been applied in several aspects:

**In silico drug repositioning** is the application of computational methods to available big datasets to identify new drug targets, drug response biomarkers, drug indication and drug mode of action [62]. The extensive molecular characterisation of tumours, coupled with large available multi-omics datasets, have enabled the development of various computational methods which aim to integrate various types of data to improve the accuracy of drug response predictions and drug prioritisation [63].

**Virtual clinical trial** is a concept based on the development of mechanistic computational models of large scale cellular signal transduction networks for predicting drug effects, and functional responses based on patient-specific multi-level omics profiles. However, the main hurdle is the validation and reliability of predictions. Some researchers are using established preclinical models, such as patient derived xenograft mouse models, as a platform to refine and validate the **in silico** model predictions [64].

**Systems biology** refers to a systems-level perspective of disease, taking into account the interaction between relevant physiological signalling processes and molecular mechanisms, and aims to provide an integrated understanding of complex disease mechanisms with targeted therapy action [65]. This model is often used in combination with other **in silico** methods to predict drug effects, however, given the complexity of molecular biochemistry in comparison with the sparse data available, such models have great limitations [66].

**Pharmacometrics**, defined as the science of developing and applying mathematical and statistical methods to (a) characterise, understand, and predict a drug’s pharmacokinetic and pharmacodynamic behaviour, (b) quantify uncertainty of information about that behaviour, and (c) rationalise data-driven decision making in drug development process and pharmacotherapy [67].
One of the main advantages of *in silico* methods in cancer research is the possibility of refining experimental programs of clinical and biomedical studies involving laboratory work, resulting in a reduction of animal experiments [68]. The main challenge with developing *in silico* methodology is that the model is only as good as the data available to initialise it, and the large number of unknown parameters in the model affects the accuracy of prediction [64]. Also, there is a need for harmonisation of the different data resources [63], and importantly, a lot of the data are derived from preclinical models, which bring with them their own limitations to interpretation of results (as discussed previously).

**Are the current preclinical models predictive for personalised medicine trials in oncology?**

Anticancer drug development suffers high attrition rates during the later phases of clinical development, with less than 5% of compounds reaching the market [69]. Retrospective analysis of the preclinical data used to support a failed clinical program should be published to help advance the field [70,71]. An example of this is enzastaurin, a protein kinase inhibitor, which failed to have an impact in late-stage clinical trials, despite promising preclinical data in patient derived xenograft models. The authors call for critical evaluation of preclinical data, and biomarker research alongside preclinical studies [72].

Advancing drug development and biomarker research in the era of personalised medicine is highly dependent on choosing the right preclinical model for the right question [73]. There is also a question whether the more advance models fit within the established drug development paradigm, calling for a rethink of the existing anticancer drug discovery pipeline [74].
Pre-clinical models for personalised clinical decision-making in brain disorders.

Results of the study selection and general characteristics of reports

The flow of information through the different phases of our systematic review are summarised in the PRISMA flow diagram (Figure 5). It maps out the number of records identified, included and excluded, and the reasons for exclusions.

We identified 1516 articles through the literature search and 13 additional records have been identified through hand searching. Of the 1473 unique articles, the full text of 263 articles was reviewed, along with an additional 9 articles identified through hand searching. Most excluded studies (n=91) were abstracts from congresses and/or conferences. A total of 94 studies (54 reviews and 40 research papers) met the inclusion criteria (1) and (2) for this scoping review and were included in the qualitative synthesis.

Figure 5 Brain disorders case. PRISMA flow diagram

What are the pre-clinical models preferentially used in brain disorders for personalised medicine?
What are the pros and cons of the various pre-clinical methods used in brain disorders?

Most studies and reviews were focused on psychiatric disorders (n=59), followed by neurodegenerative diseases (n=33) and neurodevelopmental disorders (n=8) (Figure 6).
Pre-clinical models preferentially used in this context are animal models (n=45) and cellular models (n=21). The use of organoids (n=2) and in silico models (n=5) appeared to not be well developed in the mental disorder field (Figure 7). This result is confirmed when we analyse the use of different preclinical models in mental disorders areas (Figure 8). Among the articles considered for this scoping review, none was focused on applied pre-clinical models for patient stratification (Figure 5).

Figure 6. Brain disorders areas represented in the systematic review

Figure 7. Preclinical model used in brain disorders field

* 5 reviews about psychiatric and neurodegenerative diseases
Animal models in brain disorders field

Although over the past years, the ease of developing rodent and invertebrate models by genetic manipulation or other means increased, modelling of human brain disorders in animals is extremely challenging. Despite the limitations of animal models to fully capture human brain complexity, aetiology and neurobiological mechanisms, whole animal systems remain the gold standards for studying brain disorders.

Rodents

Different aspects have made rodents the most extensively used animal tool and model in psychiatric disorders field. Rodents, in particular mice, have been used because of their high genome conservation with humans (~99%), their possibility for advanced genetic manipulation, and availability of complex behavioural assays [75]. Moreover, mice show a broad range of behavioural traits associated with mental diseases. In fact, these animals exhibit human-like attitudes, such as reciprocal social interactions, perseverance in spatial tasks, and motor stereotypies [76]. Mice are also a relevant tool for testing new therapeutic candidates in drug discovery [77]. Against their considerable advantages, the use of mice in psychiatric disorders field has multiple limitations. Among them, the high costs in fosterage and maintenance, their inability to fully recapitulate human disease phenotype and the difficulties for studying multiple organ systems and comorbidities. Because of the cost and difficulty of assays and the number of animals required it is not convenient to use rodents in medium- or large-scale drug screening and, in addition, these models are limited in predicting treatment efficacy in human disorders.

Rodents dominate approaches to animal modelling of human neurodegenerative diseases too. In particular, genetic and transgenic models are the most developed ones, followed by seeding and spontaneous models.
Genetic and transgenic mice are extremely effective at modelling neurodegenerative mechanisms, such as amyloid or tau processes in Alzheimer’s diseases. For this reason, they are useful for studying some aspects of the disease and for preclinical evaluation of specific treatments directed at Aβ or tau pathology. However, no treatment has been successfully developed so far based on those models. Similar considerations can be made for rodent models for Huntington’s and Parkinson’s diseases, where models mimic only some of the aspects associated with the disorders, but do not fully recapitulate the human pathology or their progressive nature [83–85]. Development of small animal imaging and identification of novel biomarkers are additional considerable advantages of rodent models use. Despite these advantages, the lack of understanding of the biology and biomarkers as well as the species-to-species differences are the main limitations of these models [78,80,85,86].

Rodent models have advanced our understanding of specific aspects of disease pathology in neurodevelopmental disorders field. In particular, based on the strong genetic evidence for autism spectrum disorders (ASD), mice with targeted mutations in homologous genes have been generated as translational research tools. Although, studies performed on these models have significantly contributed to formulate working hypotheses about the onset, progression and possible treatment of ASD, some challenges remain to be addressed. Similar to other brain disorders, ASD diagnosis depends heavily on the patient’s verbal history of illness, such as reports of subjective feelings around social interactions, deficits in communication, and stereotyped repetitive behaviors, features that cannot be replicated in animals. This aspect, together with the lack of appropriate endpoints for evaluation of changes in social behavior, leads to a limited translatability animal to humans [87–93] (Table 3).

### Table 3. Summary of advantages and disadvantages of the rodent models in brain diseases

<table>
<thead>
<tr>
<th>Disease type</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
</table>
| Psychiatric disorders   | • Essential to study causal links between a detected psychiatric pathology and affected molecular pathways.  
• For studying the side effects of chronic drug administrations.  
• Biomarkers development.  
• High genome conservation with humans.  
• Amenability to advanced genetic manipulation, and availability of complex behavioural assays.  
• For drug discovery | • Expense in husbandry and maintenance.  
• Difficult to obtain statistically significant sample sizes and perform large-scale analysis.  
• Difficult to study multiple organ systems and comorbidities.  
• They cannot be used in medium- or large-scale drug screening because of the expense and difficulty of assays.  
• Lack of distinct endophenotypes of psychiatric disorders.  
• Limited in their ability to fully capture human brain complexity, aetiology and neurobiological mechanisms.  
• Limited in predicting treatment efficacy in human disorders. | [75,85,102–111,94,112–120,95–101] |
| Neurodegenerative disorders | Extremely effective at modelling the amyloid OR tau pathology.  
- Development of small animal imaging modalities.  
- Useful for preclinical evaluation of treatments directed at pathologic pathway level.  
- Biomarkers development.  
- Recapitulate multiple aspects of the pathology (exhibit cognitive deficits, show progression, motor impairments)  
- They have yielded novel hypotheses that have advanced into clinical development.  
- Occurrence of senescence-related cognitive decline and behavioural alterations linked to neurochemical and morphological alterations.  
- Allow preclinical evaluation of symptomatic efficacy of cholinomimetics.  
- Do not fully recapitulate human disease  
- Lack of biology understanding  
- Lack of validated biomarkers  
- Not reproduce the full spectrum of human phenotypes  
- Species-to-species differences (timing of intervention, dose equivalency, duration of treatment, genetic background, genetic contribution to the pathophysiology…)

| Neurodevelopmental disorders | Important for isolating specific aspects of the disease pathology  
- Development of behavioural assays that can be used to evaluate drug efficacy at the behavioural level.  
- To improve bases of research and new therapeutic strategies.  
- To optimize drug safety and efficacy.  
- Human ranges in presentation is hard to duplicate in an animal model without knowing the exact cause of the disease.  
- Extreme phenotypic variability.  
- Lack of validated biomarker.  
- Lack of appropriate endpoints for evaluation of changes in social behaviour.  
- Limited translatability animal to human.  
- High costs.

### Other animal models

**Zebrafish (Danio rerio).** In order to overcome the previously mentioned limitations of rodent models, such as high cost and the relatively low throughput, zebrafish (Danio rerio) has emerged as new promising model for studying various brain disorders. Due to the balance between evolutionary conservation with humans (in some cases closer than the mice one), the experimental tractability, the quick reproductive cycle as well as the possibility of a large range of genetic manipulations, both transient and permanent, the use of zebrafish as model for studying CNS disorders is increasing. Moreover, zebrafish allows imaging of neurons easily because of its relatively simple nervous system[78]. This creates the opportunity to perform real-time neurological imaging studies, visualising specific neuronal proteins of interest and investigating neurological processes in detail [75,78,100,121,122]. The external development allows easily to control environmental factors and to perform molecular-genetic and pharmacological experiments. As well, it is a hugely powerful tool for small-molecule screening, for large-scale forward genetic screens and for primary toxicity tests [78].
Although this model mimics various stereotypic behaviours that can be powerful indicators of gene function, as other animal models, it may not phenocopy human behaviour and presents limitations in modelling the human condition especially regarding emotional and cultural aspects [75,100,121–123]. Finally, the pharmacological modifications assessment made by adding the desired compound in water is unpredictable, as chemicals can be absorbed randomly by the fish, through the gills and skin [75,78,100,121,122].

**Fruit fly (Drosophila melanogaster).** This is a versatile model organism for brain disorders. *Drosophila* is easy and relative inexpensive to maintain in a laboratory setting, has a short generation cycle (<2 weeks) and genetic study is facilitated by unique aspects of fly biology. In fact, it is a prime organism to perform unbiased genetic screens and can thus shed new insight into certain pathological processes. However, there are still drawbacks to address when it comes to use fruits flies for studying brain disorders. These conditions are more complex, with a complicated underlying genetic architecture and heterogenous symptoms that can be difficult to model in *Drosophila*. Furthermore, this invertebrate shows low levels of conservation with mammals, including brain and nervous system structures that are significantly different from those in vertebrates. These are the reasons why fly models might be used as a tool for investigating specific disease phenotypes only [75,78].

**Nematode (Caenorhabditis elegans).** It is characterized by a nervous system composed of 302 neurons, utilising most of the known neurotransmitters in the mammalian nervous system and allowing functional analysis of various neuronal regulatory pathways in the context of an intact animal. The possibility to easily perform genetic manipulations, the fact it is one of the best systems to utilize RNAi for rapid screens,11 and that fact that approximately 50% of human disease genes have an equivalent in *C. elegans*, make this worm an excellent organism model for studying brain diseases. Although the nematode models do not fully recapitulate the pathophysiology of this human neurodegenerative disease, it mimics neurodegenerative features – as the tau hyperphosphorylation - and many of the neurotoxic effects of the disease-related proteins. The main disadvantages of working with *C. elegans* is its limited complexity compared to human and the difficulty to perform drug screens as chemicals can be selectively taken up by their intestines, requiring application of very high drug doses [75,78,100,124]

**Non-human Primate Models.** In order to bridge the gap between worms, fruit flies, zebrafish, rodents and humans, non-human-primate models have been developed. Genetic-engineering and imaging development over the past decade allowed the generation of non-human primate models that might play a crucial role in the brain illness field, in particular for translational studies. In this regard, current therapeutic methods, such as deep brain stimulation and transcranial magnetic stimulation will also need to be tested in non-human primates prior to human trials. Despite the close relationships between monkeys and humans, whether complex environmental factors can be applied to monkeys is a critical question to address. Moreover, given ethical reasons, most of the approaches available for other animal model are not practical in primates. In these models identifying novel compounds, pharmacological studies and other investigations are highly
dependent on a cost-effective. These aspects make the application of non-human primate models in brain illness field challenging [85,125].

**Molluscs.** Molluscs have robust neurons in long-term culture and are often used for electrophysiological studies related to learning and memory, mapping circuits and linking them to a behaviour, as well as for ecotoxicological studies. In addition, the genome of the gastropod mollusc *Lymnaea stagnalis* is useful for investigating the molecular mechanisms related to neurodegenerative diseases. Molluscs may offer to translational medicine a powerful new tool to study the CNS and identifying new molecular targets for the development of innovative therapeutic strategies. However, tools for manipulating the genome and husbandry for raising these animals in the laboratory lag behind those of other laboratory organisms [75,126].

**Frogs.** *Xenopus laevis* is the only vertebrate utilized for both whole-animal and biochemical studies. This frog possesses many qualities that make it a powerful model to study disorders of the developing nervous system. In addition, *X. laevis* played a key role on the discovery of a molecular target for lithium in psychiatric illness field. The major disadvantages of the frog model are the long generation time and the fact that this species is tetraploid, the difficulties of genetic manipulations, in particular for knockouts [75,127,128].

**Dogs.** They spontaneously develop plaque pathology and some species even exhibit tauopathies, sometimes accompanied by cognitive decline, they have been pointed out as a model for research around human brain ageing and neurodegenerative diseases, especially AD. Given the phylogenetic proximity to humans, the in-depth knowledge of canine behaviour and neurology as well as the histopathological and molecular similarities dogs have been used in AD preclinical studies. However, there are many challenges to address when using these animals. These are their limited availability, economical and ethical reasons [78,129].

**Table 4.** Summary of advantages and disadvantages of other animal models in brain diseases

<table>
<thead>
<tr>
<th>Animal models in brain disorders fields</th>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
</table>
|                                        | Zebrafish | • Balance between evolutionary conservation with humans and experimental tractability - In some cases, closer conservation with humans than mice.  
• It can be used for a range of genetic manipulations, both transient and permanent.  
• Powerful tool for small-molecule screens  
• Possibility to measure the behavioral effects of pharmacological treatment and genetic manipulations in early stages.  
• Because the development is external, it is easy to control environmental factors and to perform molecular-genetic and pharmacological experiments. | • No human complexity.  
• No phenocopy human behaviour.  
• Pharmacological modifications assessment. | [75,100,121,122] |
|                                        | Fruit fly | • Short generation time .  
• Genetic study is facilitated by unique aspects of fly biology. | • No human complexity.  
• Differences in the subcellular localization of peptides. | [75,78,87,89] |
### Animal models for personalised medicine in brain disorders field

The animal models previously described provide an important instrument for understanding pathogenic mechanisms, identifying drug targets, and developing new therapeutic approaches for CNS diseases. Still they present important limitations. Major weaknesses are their incapability to fully recapitulate the human phenotypes and to discriminate between successful and unsuccessful treatments likewise their lack of validity and reproducibility among labs. Increasingly, it is clear that human heterogeneity within clinically defined brain disorders, and between patients with the same genetic mutations, significantly impacts disease presentation and, potentially, therapeutic efficacy. Despite many decades of research and development, it remains a translational gap, as only few treatments have reached the clinic [92,130–135].

In the context of personalised medicine, the concept of stratifying patients according to genetics, lifestyle, disease presentation, ethnicity, and other parameters have been developed and brought to the concept of humanised animal models. Although there is a great potential of this type of models, it is very challenging to generate them for modelling psychiatric, neurodegenerative and neurodevelopmental illnesses. In fact, they

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
</table>
| Nematode     | Detailed characterization at the genomic and cellular levels.  
               • One of the best systems to utilize RNAi for rapid screens.  
               • For rapid and functional analysis of various neuronal regulatory pathways in the context of an intact animal.  
               • As in mammals, neural sensory mechanisms gauge environmental conditions and coordinate myriad behavioral and physiological responses.  
               • Exhibition of tau hyperphosphorylation in AD models  
               • Limited organ similarity to humans.  
               • Drug screens can be tricky because of an impermeable outer cuticle and because drugs can be selectively taken up by their intestines, requiring application of very high doses. |
| Non-human Primate Models | Bridge the gap between rodents and humans.  
                           • Help untangle high heterogeneity of mental diseases by focusing on its core, evolutionarily conserved root.  
                           • High costs.  
                           • Ethical issues. |
| Molluscs      | Neurons are robust in long-term culture and for electrophysiological readings  
               • Facilitate mapping circuits and linking them to a behaviour.  
               • Do not fully recapitulate the human features. |
| Frog          | For whole-animal and biochemical studies  
               • Key role on the discovery of a molecular target for lithium  
               • Long generation.  
               • Difficulties in genetic manipulations. |
| Dog           | Phylogenetic proximity to humans.  
               • In-depth knowledge of canine neurology.  
               • Histopathological and molecular similarities between clinical AD and the canine variant.  
               • Suited for longitudinal studies.  
               Availiability.  
               Ethical issues.  
               Economical (based on long lifespan) reasons  
               [75,100]  
               [75,127,128]  
               [78,129] |
are complex diseases with multifactorial causes, often still unknown, not attributable to a single highly penetrant mutation. In addition, the shortage of validated biomarkers, ethical and economic reasons are further limitations to address. The lack of knowledge about animal models for patient stratification in brain disorders shows that personalised medicine is still largely unrealized in this field.

**Cellular models in brain disorders field**

Cellular models results are summarised in table 5. Several *in vitro* approaches have been developed to understand the aetiology and pathogenesis of CNS diseases. In this section, we discuss different cerebral models, from traditional cell cultures to human induced pluripotent stem cells (iPSCs), focusing on their advantages and disadvantages.

**Mammalian Cell Culture.** Mammalian cell culture has been useful for investigating genetic and pharmacological effects at the cellular level, including toxicity and side effects. This type of cell lines offers the advantages of large numbers of cells, homogeneous cell populations, and controlled physiological environments. However, primary cells are often difficult to culture, as they are heterogeneous and often develop immortality or genomic instability after continued passage, resulting in different behaviour from primary cells [75].

**Primary Neuronal Culture.** The use of primary cultures for studying brain diseases is desirable because they are more likely to recapitulate the properties of neuronal cells *in vivo*. In addition, they allow the definition of cellular phenotypes in a controlled environment without the complexity of *in vivo* inputs. However, unlike cell lines that provide unlimited supplies of homogeneous cells, the preparation and culture of primary cells is much more challenging and this is especially true for neuronal cells. Primary cell cultures are not immortal and hence the number of cells available for experiments is much more limited. Plasticity studies are limited by the difficulties to cultivate neurons *in vitro* for long periods and by the disruption of connectivity. Two-dimensional growth of neurons in dishes cannot reflect *in vivo* conditions, *in primis* the complex anatomical brain structure. As brain is the only source for primary neuron culture, it has been challenging to access the human material. Therefore, these derive from animal tissues and for this reason it is necessary to separate them, as much as possible, from astrocytes and oligodendrocytes as well as from other cell types [75,97,105,136].

**Patient-derived peripheral blood cells (PBMCs).** PBMCs are an important and a powerful tool for research in brain diseases, because of their efficient and easy isolation, processing and analysis. Blood collection is simple, economic, and less invasive than lumbar puncture, allowing for repeated sampling. These cells are potential novel screening platforms for drug profiling and are important for novel biomarkers identification. Evidences show that PBMCs provide a window into the CNS, not directly accessible to evaluation [136–138]. They reflect inflammatory mechanisms in a more specific way compared with the serum/plasma, but it is
debatabile if changes detected in PBMC are actually associated to the diseases happening in the CNS or are due to other reasons [136–138].

**Human lymphoblastoid cell lines (LCL).** These cells are considered the most reliable, inexpensive, and convenient representation of cells from unrelated individuals for *in vitro* research [75,105,136,139–142]. They arise from peripheral B lymphocytes infected *in vitro* with the Epstein-Barr virus (EBV), a process that immortalizes them. This is made possible by EBV genes that, when expressed in human cells, inhibit apoptosis. The genomes of human LCLs remain stable during subsequent cell divisions and these cell lines are the best resource for representing large cohorts of individuals – including their genomes, transcriptomes, proteomes, metabolomes and *in vitro* phenotypes that can be accurately measured in these cell lines. Large diseases cohorts are stored in large public biobanks and their preparation is simple and low-cost. However, as other *in vivo* models, they do not fully recapitulate *in vivo* complexity [75,105,136,139–142].

**Human induced pluripotent stem cell (iPSC).** iPSC are a type of pluripotent stem cell that can be generated directly from a somatic cell. After 2006, year of the Yamanaka’s paper about the possibility of reprogramming mature cells, rapidly growing field of research has advanced iPSC generation and allowed for their derivation from patient fibroblasts, keratinocytes, hair follicles, peripheral blood and likely most other cell types. Once isolated from patients, iPSC can be differentiated into neurons, affording a way to study human cellular phenotypes in the context of the patient's genetic background. In fact, iPSC technology successfully models different complex diseases *in vitro*, allowing the investigation of pathophysiologic pathways, it is a good platform for high-throughput screening (HTS) development for drugs, likewise for toxicology tests. In addition, it grants the identification of genetic predictors of drug responses and associating cellular abnormalities with clinical phenotypes in a human context. Given that, this technology offers great opportunities to overcome many obstacles in brain illness fields and provides a great potential to better understand the molecular and pathophysiological defects underlying neuropsychiatric, neurodegenerative and neurodevelopmental disorders. Some potential disadvantages of iPSCs include the high cost and the long labor-intensive process for generating specific iPSCs lines. In fact, actual protocols often result in heterogeneous populations and/or neurons not fully mature, with a development altered during fetal stages. Reprogramming of the iPS cells “re-sets” the epigenome, and that other phenotypes associated with cellular aging -mitochondrial function and telomere length are returned to a “juvenile-like” state, preventing studies in neurodegenerative conditions such as AD [75,88,148–152,97,105,136,143–147]. Most of the reported studies refer to small sample sizes and lack of validated protocols lead to a lack in reproducibility. Although it is challenging, iPSC technology offers new opportunities to model disease-relevant neural cells from patients, as long as there is a careful selection of patient cohorts [75,88,148–152,97,105,136,143–147].
Table 5. Summary of advantages and disadvantages of cellular models in brain diseases

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
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</table>
| Mammalian Cell Culture             | • Useful for investigating psychiatric genetic and pharmacological effects at the cellular level (toxicity, side effects).  
• Large numbers of cells.  
• Homogeneous cell populations.  
• Controlled physiological environments. | • Difficult to culture.  
• Heterogeneity.  
• Often develop immortality or genomic instability after continued passage, resulting in different behaviour from primary cells.  
• Do not reflect in vivo conditions. | [75] |
| Primary neuronal culture           | • Allow the definition of cellular phenotypes in a controlled environment without the complexity of in vivo inputs.  
• Recapitulate the properties of neuronal cells in vivo.  
• Provide unlimited supplies of homogeneous cells. | • Number of cells available.  
• Difficult to culture.  
• Plasticity studies are limited - difficulties to cultivate neurons in vitro for long periods and by the disruption of connectivity.  
• Do not reflect in vivo conditions. | [75,97,105,136] |
| Patient-derived PBMC               | • Efficient and easy isolation, processing and analysis.  
• Simple, economic, and low invasive.  
• Novel screening platforms for drug profiling  
• For novel biomarkers identification. | • Do not reflect in vivo conditions.  
• Connection with CNS to be clarified. | [136–138] |
| Human lymphoblastoid cell (LCL)    | • The most reliable, inexpensive, and convenient representation of cells from unrelated individuals for in vitro research.  
• Affordable research tool  
• High value for disease and drug response biomarker discovery  
• Availability in large public biobanks  
• Low costs | • Do not reflect in vivo conditions. | [75,105,136,139–142] |
| Human induced pluripotent cell (iPSC). | • Isolated from patients  
• They can be differentiated into neurons, in the context of the patient's genetic background.  
• Successfully models different complex diseases in vitro.  
• Good platform for high-throughput screening (HTS) development for drugs, and for toxicology tests.  
• To overcomes inter-species differences.  
• Useful when no good animal model exists. | • High costs.  
• Long labor-intensive process.  
• Reprogramming often results in heterogeneous populations and/or neurons not fully mature, with a development altered during fetal stages.  
• Reset of epigenetic features.  
• Small sample sizes .  
• Lack of validated protocols.  
• Lack of reproducibility.  
• Careful selection of patient cohorts is critical. | [75,88,148–152,97,105,136,143–147] |
**Cellular models for personalised medicine in brain disorders field**

Cellular models could be successfully used to pre-select the most effective therapy for patients with brain disorders towards the application of personalised medicine. In fact, they have a huge potential to make personalised medicine feasible for heterogeneous and genetically complex CNS conditions. Human lymphoblastoid cell lines (LCLs) are an affordable research tool that can be of high value for disease and drug response biomarker discovery in the context of personalised medicine exploration and can be obtained from many human biobanks. In addition, iPSc technology allows researchers to develop more appropriate disease models using relevant cell types to understand the molecular basis of a specific disorder. iPSc offer the advantage of displaying specific cellular phenotypes for drug screening purposes to identify potential therapeutic drugs tailored to an individual, they can be used for drug prediction and for evaluation of drug efficacy *in vitro*. Ultimately, the selection of highly efficient drugs with low adverse effects will bring a deep impact on the cost and success of pre-clinical trials. Despite the progresses in the field, there are still unresolved issues that bring to some of the current concerns for employing cellular models for personalised medicine research in brain diseases area. Among them, improvements in genetic manipulation technology, validated and advanced protocols are required. These should be viewed as aspects to work on, rather than as disadvantages of this innovative research tool [88,137,144–146,153]

**Organoids in brain disorders field**

Organoids results are summarised in table 6. The complex architecture of the human brain has made preclinical model development very challenging. Monolayered culture systems lack tissue structure and tissue environment and this prevent them to fully mimic the CNS diseases phenotypes. In order to overcome these limitations, efforts for improving suspension culture of iPSCs led to the organoid systems generation of different regions of the human brain. Organoids, obtained from individuals, facilitate investigation of phenotypes specific to the human context by combining the human genome and the associated human developmental timeline. Moreover, they reflect the 3D structure, organization, composition, and connectivity of the CNS hence, a powerful tool for modelling of human neurodevelopmental and neurological disorders. Although progresses in organoid technologies led to more refined in vivo-like tissue, there remain some challenges to be faced. Evidences showed that some cell types display broader transcriptomic profiles and often lack of maturity and show limitations in the cellular composition. One other clear drawback of organoid systems is the lack of inter-organ communication. In addition, developing organoids is costly and requires time and effort. Finally, this systems require *in vivo* validation and protocol harmonisation across labs in order to increase result reproducibility [128,148,154].
Table 6. Summary of advantages and disadvantages of organoids in brain diseases

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
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<tr>
<td>• Allow discovery and characterization of multicellular phenotypes.</td>
<td>• High variability.</td>
<td>[128,148,154]</td>
</tr>
<tr>
<td>• Dimensional complexity.</td>
<td>• Do not recapitulate the precise organization of</td>
<td></td>
</tr>
<tr>
<td>• Model the 3D structure, organization, composition, and connectivity of</td>
<td>the brain.</td>
<td></td>
</tr>
<tr>
<td>the human brain.</td>
<td>• Human brain- lack of maturity and limitations in</td>
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<tr>
<td>• Resemble the early developing human brain also with respect to gene</td>
<td>the cellular composition.</td>
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<td>expression programs.</td>
<td>• High costs.</td>
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<tr>
<td>• Exhibit human specific cellular diversity, histological layers, and</td>
<td>• Lack of validated protocols.</td>
<td></td>
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<tr>
<td>migration patterns.</td>
<td>• Lack of reproducibility.</td>
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</table>

Organoids for personalised medicine in brain disorders field

Organoids have potential in the personalised medicine context, in which they can be used to predict response to drugs. In fact, “personalised organoids”, directly derived from the patient, together with drug testing, would represent an effective clinical usage of this technology. Although improvements have been made, in the brain disorders field the organoids systems are not translated in a personalised clinical setting yet. A multidisciplinary approach will yield new insights and has the most potential to drive the organoids forward.

In silico models in brain disorders field

The goal of in silico modelling is the detailed understanding of the function of molecular networks as they appear at different biology levels. This is achieved by using a level of mathematical abstraction that needs a minimum of biological information to capture all physiologically relevant features of a cellular network. In silico models of disease combine the advantages of both in vivo and in vitro experimentation. They are able to include a huge number of parameters, which render the results more applicable to the organism as a whole. With these systems it is possible to investigate biological processes, predict changes disease-related and perform simulations in a short time and avoiding ethical issues. However, in silico models present limitations. Taking into account every single interaction leads to an unmanageable model, as it requires many parameters. Therefore, parameters must be carefully determined, but this is not always easy. Data can come from both in vivo and in vitro experiments, and results might diverge in the two settings.

Although application of in silico models for better investigating brain illness is appealing, obtaining appropriate parameters of a CNS disease is rooted in a deep knowledge of its pathophysiology. Therefore, due to the lack of the understanding of such disorders, this is not always possible. In addition, assuming the parameters for in silico modelling can be identified, giving a reasonable biological interpretations to the results from the complex interaction of all variable considered is also a big challenge [155–158]
In silico models for personalised medicine in brain disorders field

In silico models are a unique tool to personalise CNS disease management and care. Indeed, they could couple clinical data with mathematical methods to create subject-specific brain models to design new, personalised and more optimal protocols, as well as to allow patient stratification. Departing from different patient-specific parameters they can capture inter- and intra-patient variability, the difference between patients and the evolution of patient condition. Although this is great advance in health care, to date there are not translatable in silico models for brain disorders. The inadequacy of key sensitivity parameters, the absence of defined guidelines for obtaining high quality clinical data, and the lack of model validation to ensure that the outputs are safe, accurate and clinically relevant are key challenges to be address before in silico models will be applied in a personalised medicine context.

How many drugs have been developed/are currently under development based on multi-omics profiling programs? What is the estimated success rate of the trial using this approach? What information was collected at the pre-clinical stage to inform the clinical study design?

These questions could not be answered by the findings in the Literature review. We would expect to have more information further in the consultation process during the “Gap analysis workshop”, scheduled in December 2020. Moreover, we could retrieve this information from the Industry survey.
Summary of findings and next steps

In order to allow for the implementation of personalised medicine, there has to be availability of appropriate preclinical models which can be relied upon to generate accurate and predictive data. Despite the large use and development of pre-clinical models in brain disorders, their application for personalised medicine approaches is not a reality yet. Among the articles considered for this review, none were focused on applying pre-clinical models for patient stratification (Figure 5). In fact, despite the enormous progresses in developing more and more reliable and powerful systems, to date there are fundamental gaps that prevent their broad implementation in personalised SNC illness management. Important drawbacks are the lack of knowledge in the biology of these diseases, the model incapability to fully recapitulate the human pathologic phenotypes, the limited validity and reproducibility as well as ethical and regulatory issues.

In oncology, the insights into the pathophysiology of the disease has highlighted the importance of inter- and intra-tumour heterogeneity, the critical role of the tumour microenvironment, and the involvement of the immune system, but there is a lack of fully developed and reliable preclinical technologies that can navigate the complex variables in therapeutic responses and diagnostic accuracy. The future development of more sophisticated preclinical methods, such as microfluidic systems and in silico modelling, might close the gap in preclinical research in the future, but aside from the fact that this is reliant on technologies which are still not developed, there are other more fundamental issues in preclinical research.

Lack of methods reporting is a major problem, and not a new issue in preclinical research [159]. Only three systematic reviews were identified in the oncology search, all on in vivo PDX models, and of the studies included in these reviews, only one reported following the recommended ARRIVE guidelines, developed to improve the standard of reporting for animal experiments, which was published in 2014 and endorsed by more than 1000 journals. Due to the large variation in methodology and reporting across studies no quantitative analysis of bias could be performed in any of the reviews.

Another problem is the failure to systematically validate the model systems, both in terms of internal validity (the experiments ability to identify causal relationships) and external validity (the model’s predictive power). The lack of systematic reviews is also a problem, as the use of systematic reviews to judge the reliability and validity of biomedical research can improve the success and reproducibility of subsequent translational clinical studies in this era of personalised medicine.

A relevant point to be addressed is also the low availability of negative data. Negative results are not appealing for publication, meaning that the results of thousands of experiments that fail to confirm the reliability of pre-clinical models do not see the light of day. This results in a waste of time and resources from
other scientists in repeating negative findings and in a consequent deceleration of the translational pipeline. This is even more true in an industry setting where in-house data are not generally published for reasons of competitiveness. Therefore, the scientific community should address this issue, sensitising to the richness of negative results in research.

**Identified gaps – a description of the areas identified through the analysis that could benefit from standards – priority areas for recommendations**

These gaps will be object of analysis during the “Gap analysis workshop”, scheduled in December 2020.

- Lack of methods reporting
- Lack of standardised protocols
- Lack of external and internal validation
- Lack of systematic reviews and meta-analysis
- Reporting of negative results
- Lack of regulation of the above issues

Other important points to be addressed:

- **Regulatory and Ethical issues**
  As with many emerging technologies, the enthusiasm surrounding personalised medicine is tempered by uncertainties in ethical and regulatory aspects. As the shift to personalised medicine is younger than the laws that otherwise regulate the medical and research fields, there are gaps between technology and oversight, in particular, in the preclinical phase.

- **Lack of standardised guidelines**
  One of the biggest gaps identified in the development of preclinical research in personalised medicine is the lack of quality and reproducibility. This is due to the absence of standardised protocol and guidelines. Every lab develops its own in-house protocol and it is challenging to reproduce results in another setting. Since reproducibility is a cornerstone of scientific research, we call for more reproducible research.

- **Talk about another case → cardiovascular diseases?**
  In this report we chose to focus on two case model that are at the extremes of personalised medicine development in preclinical research: oncology and brain disorders. It might be interesting investigate the level of development of preclinical models in patient stratification in another case model in order to better understand the state of art and identify common issues and challenges.

- **Industry survey results**
  In order to map patient stratification strategies currently undertaken by industry a survey has been developed. The target of the survey are experts in experimental and translational medicine, working for large pharma
companies and SMEs. The results of the survey will enable a better understanding of the challenges and opportunities in the translational development phase of personalised medicine clinical trials in the industry.

The next step is to integrate the report with the results of the Industry Survey, aiming to map patient stratification strategies currently undertaken by industry. The results of the survey will enable a better understanding of the challenges and opportunities in the translational development phase of personalised medicine clinical trials.

The results from this review will be discussed in a workshop with partners of the PERMIT project, planned on December 1-2, 2020, and based on this gap analysis we will identify key external experts representing the various aspects of preclinical development for personalised medicine.

After discussing the findings and conclusions from the scoping review with the team, a revised, filtered and shortened text version of the resulting recommendations would be made available to external experts (with the possibility to provide more detailed information on topics of interest upon request).

External experts can be identified both within EATRIS network and beyond, priority will be given to key representatives of the pharmaceutical industry, stakeholders including regulatory bodies, and investigators from academia. The experts will have diverse backgrounds and in-depth knowledge of translational medicine and will be invited to join a workshop, with the aim of identifying the main issues and challenges of preclinical research in both academia and industry, to lay the foundation of innovative and common recommendations for translational strategies to improve personalised medicine clinical trials.
References


33. Astone M, Dankert EN, Alam SK, Hoeppner LH. Fishing for cures: The allURE of using zebrafish to develop precision oncology therapies. npj Precis Oncol. 2017;1. doi:10.1038/s41698-017-0043-9


62. Jean-Quartier C, Jeanquartier F, Jurisica I, Holzinger A. In silico cancer research towards 3R. BMC
PERMIT has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N. 874825


102. Heilig M, Sommer WH, Spanagel R. The need for treatment responsive translational biomarkers in


118. Belzung C. Innovative drugs to treat depression: Did animal models fail to be predictive or did clinical


147. Prilutsky D, Palmer NP, Smedemark-Margulies N, Schlaeger TM, Margulies DM, Kohane IS. IPS-


### Appendix I – Search strategy

#### Pubmed

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**BRAIN DISORDERs: 23/03/2020**

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PsycInfo 23/3/2020

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Embase

**ONCOLOGY – 1/04/2020**

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474356

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## Appendix II. Data extraction form

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**Language**

- English
- French
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- Spanish
- Italian

**Type of publication**

- Research article
- Systematic review
- Review
- Commentary/ Editorial
- Book chapter

**Disease**

- Type of cancer
- Other than oncology

**Topic**

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**Type of model**

- Animal
  - Animal species
  - Type of animal model
  - Origin of tumour
  - Immune status
- Cellular model
  - Type of cellular model
  - Origin of cells
- Organoid model
  - Origin of organoid
- In silico models
  - Type of in silico model
  - Type and source of data

**Advantages**

Advantages of the preclinical model

**Disadvantages**

Disadvantages of the preclinical model

**Validity**

Has the model been validated (external and internal validation)

**Personalised medicine**

Consideration related to personalised medicine
## BRAIN DISORDERS:

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<tr>
<td>Predictive validity</td>
<td>Can the model successfully discriminate between successful and unsuccessful treatments for the human disease condition?</td>
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Appendix III – Full scoping review protocol
Methodological approaches for personalised medicine: a series of scoping reviews
Protocol V.2 – 29 April 2020

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Table of Contents

Introduction .......................................................................................................................... 3
Personalised medicine research ............................................................................................. 4
Review methods ..................................................................................................................... 6
  Identification of the research questions .............................................................................. 7
Scope & Research Questions ................................................................................................. 7
  1. Methods for stratification and validation cohorts ............................................................ 7
  2. Methods for machine learning applied to stratification ................................................. 8
  3. Pre-clinical methods for translational development of stratified therapies and treatments selection ............................................................................................................. 9
  4. Methods for clinical trials in personalised medicine ...................................................... 9
Study identification ............................................................................................................... 10
Eligibility Criteria ................................................................................................................ 10
  1. Methods for stratification and validation cohorts ............................................................ 10
  2. Methods for machine learning applied to stratification ................................................. 11
  3. Pre-clinical methods for translational development of stratified therapies and treatments selection ............................................................................................................. 11
  4. Methods for clinical trials in personalised medicine ...................................................... 12
Study selection .................................................................................................................... 12
Data extraction .................................................................................................................... 12
Study quality ....................................................................................................................... 13
Plan for presenting the results .............................................................................................. 13
Consultation exercise .......................................................................................................... 13
Funding ................................................................................................................................. 14
References ............................................................................................................................ 14
Introduction

The concept of personalised medicine is going to impact how pharmacological treatments are discovered and developed, how patients are diagnosed and treated, and how health care systems allocate their resources to maximize patient benefits. Personalised medicine may be considered an extension of traditional approaches to understanding and treating disease. Ideally, it could serve to take clinical decisions based on a patient’s profile (often molecular, but the concept is broader) to minimise harmful side effects, ensure a more successful outcome, and possibly help contain costs compared with a “trial-and-error” approach to disease treatment (1).

Personalised medicine stems on the broad concept that managing a patient's health should be based on the individual patient's specific characteristics, including age, gender, height/weight, diet, environment, etc. Different understandings of personalised medicine exist, in which three main positions can be identified (2):

(a) personalised medicine is not a new concept as medicine has always been individualized;
(b) personalised medicine is holistic health care, centred around the needs of the individual patient;
(c) personalised medicine is treatment targeted at stratified subgroups (e.g. pharmacogenetics).

Even when the focus is restricted to the third position, there is not a unique definition of personalised medicine, nor a straightforward terminology to define this concept. While "personalised" emphasizes the notion of individualized— “this is exclusively designed for you”, other more scientifically rigorous terms such as stratified medicine refer to the identification of groups or strata of patients with specific molecular characteristics or other determining factors which predict susceptibility to disease, disease prognosis, and/or response to therapy. Some authors suggested that rather than considering personalised medicine as a precise scientific concept, it should be understood as an open and negotiable ideal that accounts for a plurality of visions, depending on people, reasons and interests behind these alternative conceptions (3).

Regarding the terminology, in the European context, the term personalised medicine is preferred, as this term best reflects the ultimate goal of effectively tailoring treatment based on an individual’s ‘personal profile’, as determined by the individual's genetic and phenotypical characteristics. Other terms are widespread, for instance stratified medicine, mainly used in the UK, or precision medicine mostly used in US and broadly referred to the 4 P (preventive, predictive, personalised and participatory) medicine. While there may be small nuances in the literal meanings of these terms, they usually refer to the same concept when applied in practice (4).

A recent review reported that the literature about personalised medicine usually refers to two different semantic approaches. Firstly, patients’ stratification, that is grouping individual patients in subpopulation according to their probability to have a therapeutic benefit from a drug or regimen. Secondly, treatment tailoring, that is the individual status of a patient (i.e., disease characteristics or subject’s genotype/phenotype) is the rationale basis for drug choice (5).

Box 1 reports a collection of definitions, along with their references.

A broad community of stakeholders, including funders and professionals involved in medical research and care, are increasingly concerned with ensuring that the right patient receives the right therapy, at the right dose and at the right time. The identification of markers of mechanistic pathways or multiple variables characterising clusters of subjects that might inform meaningful disease stratification may have different clinical applications in the context of personalised medicine. Broadly, stratification may be applied at the diagnosis level (e.g., to identify a particular pathophysiological/clinical stratum within a heterogeneous patient population for diagnostic purposes), to predict disease course (prognostic value), the development of a disease (predictive value), or the response to therapy (theragnostic value).
Regardless of the application, any approach to personalised medicine should undergo different phases: discovery, validation and definition of usefulness from a clinical perspective. Robust methodological approaches are needed to deal with the complexity and heterogeneity of the process, as well as the range of possible applications to stratification using multidimensional data (what is meant by “molecular profiling” among other terms).

**Personalised medicine research**

This series of scoping reviews will map the general concept of methods for personalised medicine, to set the basis for the discussion on robustness and reproducibility of personalised medicine development programmes. The final goal is the identification of standards and needs in terms of methodology of data generation, management, analysis and interpretation to improve clinical studies in personalised medicine.

The group of authors agreed on a common operational definition of **personalised medicine research**: a set of comprehensive methods, (methodological, statistical, validation or technologies) to be applied in the different phases of the development of a personalised approach to treatment, diagnosis, prognosis, or risk prediction. Ideally, robust and reproducible methods should cover all the steps between the generation of the hypothesis (e.g., a given stratum of patients could better respond to a treatment), its validation and pre-clinical development, and up to the definition of its value in a clinical setting.

The process leading from the hypothesis to the clinic is complex and not always linear. The Medical Research Council in UK recently developed a framework for the development, design and analysis of stratified medicine (6) that is structured in six themes:

- **Theme 1: Framing the Question/Defining the Population**
- **Theme 2: Designing Stratum Discovery Studies; selecting variables, defining response and powering**
- **Theme 3: Assay Design; managing complexity and variability**
- **Theme 4: Defining Strata; data integration, linkage to existing knowledge, linkage to outcome**
- **Theme 5: Stratum Verification**
- **Theme 6: Progression Towards Clinical Utility**

Any attempt for classifying the phases of personalised medicine may appear as an oversimplification. However, a typical research programme in personalised medicine would include: first a stratification cohort (in many cases a retrospective study reusing data and biosamples from existing cohorts) with extensive multimodal data on which stratification algorithms are run, then a validation cohort, normally prospective, that assesses the reproducibility, robustness and validity of the clustering in another sufficiently large patient sample. Thirdly, a translational step is often necessary. In some cases, the use of pre-clinical models (cellular, in-silico, organoid) might be useful to give confidence in the allocation of patients to specific treatment arms as identified through clustering. Alternatively, the multi-omics profiles from clinical samples can lead to the identification of new disease categories, prediction of disease prognosis, exploration of drug sensitivity and dose selection. Finally, treatment options should be tested in the subgroups of patients in the context of clinical studies, ideally randomised clinical trials, to generate evidence informing regulatory, clinical and coverage decisions.

However, many alternative pathways can be proposed. In some case, the stratification provides detailed information on the mechanism of disease and strong indications on the treatments to be tested in each patient cluster. This is for instance the case where identification of driver somatic mutations in cancer cells suggests the targeted treatment to be tested. In other cases, the stratification cohort includes data on response to an established treatment, making the translational step less necessary. Research programmes may be limited to the stratification step, in particular when no treatment is available – this is the case for instance for taxonomy studies in
neurodegenerative disorders, aiming at identifying homogeneous clusters of patients. In any case, personalised medicine research is a complex programme, with multiple steps and lasting many years.

We consider out of the scope of this review the methods used for the clinical implementation of personalised medicine, the manufacturing and use of individualized treatments, and the pragmatic approach to individual patient care, such as n-of-1 trials.

Considering this framework outlined by Figure 1, the scoping reviews will approach personalised medicine research focusing on four main phases:

- Methods for stratification and validation cohorts
- Methods for machine learning applied to stratification
- Pre-clinical methods for translational development of stratified therapies and treatments selection
- Methods for clinical trials in personalised medicine

Figure 1: Main steps in personalised medicine research programmes
Review methods
We aim to perform a set of scoping reviews investigating various aspects of the methodology applied in personalised medicine research programmes as outlined in the Scope & Research Questions section.

Scoping reviews are used to present a broad overview of the evidence pertaining to a topic; they are useful to examine areas that are emerging, to clarify key concepts and identify gaps. Scoping reviews have great utility for synthesizing research evidence and are often used to map existing literature in a given field in terms of its nature, features, and volume. They differ from standard systematic reviews that are usually aimed to answer a specific question or series of questions according to a rigid set of a priori eligibility criteria. Scoping reviews have a broader approach, generally with the aim of mapping literature and addressing broader research questions. Due to the iterative nature of scoping reviews, deviations from the protocol are expected, differently from what happens in systematic reviews. Anyway, the discrepancies from the protocol will be clearly detailed and justified in the ‘Methods’ section of the scoping review report, if and when they occur.

To ensure the transparency and reproducibility of the review process, we will follow the methodological guidance for the conduct of scoping reviews suggested by the Joanna Briggs Institute (7, 8). The main steps of the process are summarised in Figure 2.

Figure 2: Main steps in the preparation of scoping review (in grey optional steps).

This overall process will be applied to the four themes outlined below by a dedicated review team supported by a methods team. Each step may require small adaptations given the nature of the research questions and scope defined and the type of literature/data that will be retrieved.

The four reviews will be part of a unique report covering the different aspects of methodology to inform the gap analysis and the subsequent phases of the PERMIT project (https://permit-eu.org/).
Identification of the research questions

The first step of any scoping review is to define the objective and research questions of interest. For the purpose of these reviews, four main aspects of the general concept of personalised medicine have been identified and will be the focus of this analysis:

- Methods for stratification and validation cohorts
- Methods for machine learning applied to stratification
- Pre-clinical methods for translational development of treatment options and treatments selection
- Methods for clinical trials in personalised medicine

Through several rounds of joint discussion and one face-to-face meeting (Paris, Jan 24, 2020), the four review teams had clarified the scope and defined research questions to the purpose of the scoping reviews. As the four topics are connected, this step also served to harmonise the four parts and avoid possible overlaps.

The outcome of this exercise is reported in the following section Scope & Research Questions.

Scope & Research Questions

1. Methods for stratification and validation cohorts

The scoping review will focus on:

- The characteristics of cohorts that have been used for patient stratification or validation of patient clustering obtained through stratification cohorts. Stratification cohorts of patients are used to create the clustering, and validation cohorts of patients are used to assess the reliability (robustness, reproducibility, etc.) of patient clustering.
- The different methods and tools used in design and management of stratification and validation cohorts (especially complex in multimodal approaches) to understand their limitations.

The review will not be restricted to a given type of data for stratification, i.e., genetic, metabolomics, gene expression, genomic, neuroimaging, etc.

General papers that describe methods and tools in the design and management of stratification and validation cohorts will be assessed irrespective of the diseases field. Case examples of biomarkers or multimodal data profiling in different medical fields and coming from different sources (omics, neuroimaging, genetics…) will be also analysed to explore the actual application of these methods and tools. Cancer, stroke and Alzheimer’s disease will be the three areas where informative examples will be collected, as they are complex conditions (many biological and environmental factors involved) and are representative of different approaches and degree of success in personalised medicine.

The main research questions addressed by the scoping review will be:

- What are the approaches to define the optimal size of stratification/validation cohorts?
- What are the differences, pros and cons of the prospective and retrospective nature of stratification and validation cohorts?
- What are the prerequisites and methods used for integration of multiple retrospective cohorts?
2. Methods for machine learning applied to stratification

The scoping review will focus on:

- Supervised and unsupervised machine learning methods for biomedical stratification using omics data. Few examples for other data modalities, e.g. imaging data, digital pathology and mobile sensor data will also be explored but not as the major focus.
- Cover both disease-based stratification (patient omics clustering, major focus) and drug-based stratification (clustering of drug-induced changes in patient-derived cells, minor focus)
- Methodologies that have been successfully validated and applied in clinical practice. New emerging approaches, which have not yet been sufficiently validated will also be explored but not as the major focus.
- Pros/cons, opportunities/limitations of different stratification methodologies and the associated validation approaches.
- Examples of successful applications.

Methodologies that have led to clinically validated biomarker signatures will be prioritised, as well as methodologies that have been cross-validated and externally tested on large sample sizes (preferably across multiple patient cohorts). Methods that lack statistical validation and a demonstrated biomedical application will be excluded.

The main research questions addressed by the scoping review will be:

- What are the main types of supervised and unsupervised machine learning methods for omics-based stratification in biomedicine (structured categorization)?
- What are the used and recommended workflows for supervised and unsupervised omics-based stratification (pre-processing, quality control, model building, model validation, model interpretation)?
- What are the specific strengths/weaknesses and opportunities/limitations of different types of omics-based stratification methods?
- Which validation methods exist for omics-based stratification models (assess accuracy, confirm biomedical relevance, test robustness) and what are their pros and cons?
- Which practical utility has been demonstrated for omics-based stratification and validation methods in real-world biomedical applications in the past (representative examples for previous success and/or failure stories, lessons learned)?
- What are the current gaps in standardization and methodological guidelines, and what is the outlook for the future of the field of omics-based machine learning stratification (new emerging approaches, new initiatives for data sharing, quality improvement, FAIRification)?
3. Pre-clinical methods for translational development of stratified therapies and treatments selection

The scoping review will focus on two aspects:

3.1. Personalised clinical decision-making based on pre-clinical models, aimed to explore drug sensitivity screening step (cellular based assay, organoids, PDX model) to predict therapy response and allocation of patients to different treatment arm, dose ranges and other aspects relevant for initiation of clinical trials. Suitable use cases will be selected in fields other than oncology, where clinical trials have been performed using pre-clinical models for personalised clinical decision-making.

The main research questions addressed will be:

- What are the fields of medicine other than oncology where pre-clinical models for personalised clinical decision-making have been applied?
- What are the pre-clinical models preferentially used in this context?
- How many drugs have been developed/are currently under development based on multi-omics profiling programs? What is the estimated success rate of the trial using this approach?
- What are the current gaps for broad implementation of pre-clinical testing for treatment selection?
- What information was collected at the pre-clinical stage to inform the clinical study design?

3.2. Stratified medicine development, to show which pre-clinical models (cellular, animal, organoid, in silico) are currently used as validation methods prior to personalised medicine clinical trials, both in academia and in industry. The example use case will be oncology.

As prospects, the review will discuss how to adapt the existing pre-clinical model systems to personalised medicine, and emerging models (such as in silico) which can replace the traditional animal models (3Rs). We will also perform a categorisation based on relevance and interpretation of models in the context of personalised medicine.

The main research questions addressed will be:

- Which pre-clinical models are currently used to provide validity data prior to therapeutic clinical trials of personalised medicine in oncology?
- What are the pros and cons of the various pre-clinical methods?
- Are the current pre-clinical models predictive for personalised medicine trials in oncology?

4. Methods for clinical trials in personalised medicine

The scoping review will focus on:

- Clinical trials, especially randomised trial designs, for personalised medicine.
- Trials evaluating a treatment in a subgroup of patients defined e.g. by a biomarker, in several clusters or subgroups of patients (e.g., basket or umbrella trials), trials comparing a personalised medicine strategy to a non-personalised strategy, or trials aiming at defining a subgroup of patients with enhanced response to treatment (e.g., adaptive enrichment design, adaptive signature design).
• Elements of clinical trial design applied to personalised medicine improving their appropriateness for HTA decision (e.g., external validity, choice of comparator, use of clinically meaningful outcome measure).
• Methodological reports (e.g., a scientific piece of work aiming at describing and evaluating the operational characteristics of a particular design) and guidance documents issued by regulatory or agencies for health technology assessment.
• Examples of published or ongoing trials in personalised medicine.

The review will not be restricted to a given medical field, although several examples in oncology are expected.

The main research questions addressed by the scoping review will be:

• What are the available designs for clinical trials applied to personalised medicine?
• What are the examples of current applications of these approaches?
• What are the pros and cons of the different approaches?
• What are the gaps in the current research on personalised medicine clinical trials?
• How is a personalised medicine strategy vs. non-personalised strategy evaluated?

Study identification

Relevant studies and documents will be identified balancing feasibility with breadth and comprehensiveness of searches.

Formal literature searches will be conducted on relevant databases (i.e., Medline, Embase, Cochrane Library) by the methods team. The keywords for the search strategy will be defined with the support of the review teams. Additional rounds of literature searches may be needed to refine specific aspects. The reference list of all identified reports and articles will be searched for additional studies.

To identify reports not published as scientific journal papers and unpublished (grey literature) information each review team will hand searching of relevant literature and websites (including conference meetings). Review teams may also contact relevant stakeholders to retrieve additional studies.

Documents published between 2005-2020 written in English, French, Spanish, Italian, German will be sought. Other specific time window, if deemed necessary by each review team, will be applied. Appropriate and clear justification for choices will be provided.

Appendix 1 reports examples of the search strategies planned for the four parts of this scoping review.

Eligibility Criteria

Each review team defined broad eligibility criteria based on the four “Scope & Research Questions” sections.

1. Methods for stratification and validation cohorts

We will include articles and other reports describing the methods applied to cohorts that have been used for patient stratification or validation of patient clustering obtained through stratification cohorts.

We will also include reports on methods to define the optimal size of cohorts, to design these cohorts, to integrate multiple retrospective cohorts, to evaluate risk of bias, and to manage data and analysis in personalized medicine. We are also interested in the quality of data and monitoring
of associated clinical data requirements and in the legal framework of data generated in personalized medicine.

Three case models will be explored: oncology, Alzheimer's disease and stroke.

These three fields were chosen for their big impact on society and individual health, because they are in three different phases of personalized medicine, which allows us to know different methods and strategies in different levels of development, and because they cover different kind of data to stratify patients. Oncology is the field where personalised medicine was firstly applied and where targeted therapies and diagnostics have been focused. Moreover, several applications of biomarkers for the successful stratification of patients with a given type of cancer exist, most of them based on molecular data, specially genomics. Alzheimer’s disease research in personalized therapies and diagnostics is nowadays giving its firsts results, based in imaging, cognitive and also molecular data. Stroke is currently opening up to personalized medicine, with some approaches and studies in more initial steps. Most of the data for patient’s stratification are imaging and molecular data. The review will cover a broad range of multimodal data profiling studies and biomarkers based on all kinds of data: genetic, metabolomic, genetic expression, genomic, or radiomic.

As general approach, we will search for (systematic) reviews to first identify the most common methodological approaches. Subsequent rounds of more specific searches will be conducted according to the results obtained from the scan of the reviews and to cover detailed aspects.

2. Methods for machine learning applied to stratification

We will include articles and other reports with methodology descriptions or reviews/opinion articles on supervised and unsupervised machine learning approaches and associated validation methods for omics-based stratification that have been tested on real-world biomedical data.

We will prioritize reports describing methodologies that have led to clinically validated biomarker signatures and those describing methodologies that have been cross-validated and externally tested on large sample sizes (preferably across multiple patient cohorts)

Articles reporting on methods that lack appropriate validation statistics and a demonstrated biomedical application will be excluded

There will be no restrictions in terms of types of publication or medical areas.

3. Pre-clinical methods for translational development of stratified therapies and treatments selection

3.1. Pre-clinical models for personalised clinical decision-making.

We will include articles and other reports describing methods (i.e. cellular based assay, organoids, animal models) used to assign treatment options to patient clusters. The case model will be mental disorders disease, chosen as non-oncology medical field. Indeed, this therapeutic area is included in the FDA Table of Pharmacogenomic Biomarkers in Drug Labelling as one of the most represented after oncology (9). Biomarkers in the table include but are not limited to germline or somatic gene variants (polymorphisms, mutations), functional deficiencies with a genetic etiology, gene expression differences, and chromosomal abnormalities; selected protein biomarkers that are used to select treatments for patients are also included.

3.2. Stratified medicine development in oncology

We will include articles and other reports describing translational medical approach, specifically pre-clinical validation methods applied prior to personalised medicine clinical trials. The case
model will be oncology, chosen as the field where personalised medicine was firstly applied and where targeted therapies and diagnostics have, for the most part, been focused.

The review will have a broad focus on the preclinical methodologies used for personalised medicine i.e. animal (mainly PDX), organoid, cellular models and in silico/computerised models, assessing the validity, reliability and predictive value of the various models. As general approach, we will include papers which describe the concept of the methods and exclude those which only deal with models applied to a specific type of cancer and original biomarker research.

Subsequent rounds of more specific searches will be conducted if needed, according to the results obtained from the scan of the first set of articles to cover detailed aspects.

There will be no restrictions in terms of types of publications included.

4. Methods for clinical trials in personalised medicine

We will include methodological and statistical articles and reviews describing or evaluating designs and validation of randomised controlled trials for personalised medicine, assessing both pharmaceutical and non-pharmaceutical interventions. We will also include articles reporting on personalised medicine trials and trial protocols, either published or available on trial registries. Finally, guidance documents issued by regulatory or health technology assessment agencies will be assessed.

There will be no restrictions in terms of types of publication or medical areas.

Study selection

The title and abstracts of records identified by the literature search will be screened by two independent reviewers. The full text publication of relevant articles will be retrieved and checked for confirming eligibility. Discrepancies will be solved by discussion among the review team and the method group if needed. An iterative approach to study selection is expected: each major change from what is reported in this protocol will be recorded and justified.

The screening process will be summarised in flow diagrams as suggested by the PRISMA guidelines for reporting scoping review (10).

Data extraction

The main feature of each report considered eligible, as providing information of a given aspect covered by one or more research questions, will be summarised in tables by one reviewer and checked by a second to ensure data quality. As we expect the reviews to include a variety of scientific articles and other documents, we will not develop a common pre-defined extraction form. However, the following information will be sought and summarised for each included report. This list will be adapted according to the needs of the different review teams.

- Author(s)/reference/title
- Year of publication
- Source origin/country of origin
- Type of publication (e.g. article, editorial, report, poster, etc.)
- Concept/Aims/purpose
- Study population and sample size (if applicable)
- Methodology/Study design
- Intervention type and comparator (if applicable)
- Duration of the intervention/time horizon (if applicable)
- Outcome measures (if applicable)
• Main results/findings
• Key findings that relate to the review question

This list will be adapted according to the needs of the different review teams.

Study quality
As general approach, we will not perform a formal assessment of methodological quality of the included studies as it is generally not performed in scoping reviews. However, the evaluation of risk of bias of clinical studies included as case examples may be considered.

Plan for presenting the results
The collected evidence will be assembled, summarized and reported to address the research questions defined in the Scope & Research Questions section. The format will be refined toward the end of the process when we will have the increased awareness of the contents of their included studies. Results will be discussed considering the gaps in methodology and the implications for policy, practice and research to inform the consultation exercise.

Consultation exercise
The activities of the review teams (WP3-WP6 in the PERMIT project, permit-eu.org/) will cover this aspect, through dedicated consultations and workshops with field experts. The discussion will involve PERMIT participants and associated partners, and the PERMIT project Scientific Advisory Board.
Funding
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 874825.

References
6. UK Medical Research Council Stratified medicine methodology framework.
8. Tricco AC. et al. A scoping review on the conduct and reporting of scoping reviews. BMC Medical Research Methodology 2016;16:15.
9. FDA. Table of Pharmacogenomic Biomarkers in Drug Labeling
10. Equator Network. Enhancing the QUALity and Transparency Of health Research
### Box 1: Main definitions of personalised medicine

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<tr>
<th>Proponent</th>
<th>Definition</th>
<th>Reference</th>
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<td>Horizon 2020 Advisory Group and Strategic Research and Innovation Agenda (SRIA) of PerMed</td>
<td>Personalised medicine is ‘a medical model using characterisation of individuals’ phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention.</td>
<td><a href="https://ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/personalised-medicine_en">https://ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/personalised-medicine_en</a> <a href="http://www.permed2020.eu">http://www.permed2020.eu</a></td>
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<tr>
<td>European Council conclusions on personalised medicine for patients (2015/C 421/03)</td>
<td>Medical model using characterisation of individuals’ phenotypes and genotypes, or tailoring the right therapeutic strategy for the right person at the right time, and to determine the predisposition to disease and/or deliver timely and targeted prevention.</td>
<td><a href="https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52015XG1217(01)&amp;from=EN">https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52015XG1217(01)&amp;from=EN</a></td>
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<tr>
<td>UK Medical Research Council</td>
<td>Stratified medicine is the identification of key sub-groups of patients within a heterogeneous disease population; these being distinguishable groups with differing mechanisms, risk or course of disease, or particular responses to treatments. Stratification can be used to: • Improve mechanistic understanding of disease processes and enable the identification of new targets for treatments • Develop biomarkers for disease risk, diagnosis, prognosis and response to treatment • Allow treatments to be developed, tested and applied in the most appropriate patient groups</td>
<td><a href="https://mrc.ukri.org/research/initiatives/precision-medicine/stratified-medicine-methodology-framework/">https://mrc.ukri.org/research/initiatives/precision-medicine/stratified-medicine-methodology-framework/</a></td>
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<td>Personalized Medicine Coalition (PMC)</td>
<td>Personalized medicine is an evolving field in which physicians use diagnostic tests to determine which medical treatments will work best for each patient. By combining the data from those tests with an individual’s medical history, circumstances and values, health care providers can develop targeted treatment and prevention plans. Personalized medicine is the tailoring of medical treatment to the individual characteristics of each patient. The approach relies on scientific breakthroughs in our understanding of how a person’s unique molecular and genetic profile makes them susceptible to certain diseases. This same research is increasing our ability to predict which medical treatments will be safe and effective for each patient, and which ones will not be. Personalized medicine may be considered an extension of traditional approaches to understanding and treating disease. Equipped with tools that are more precise, physicians can select a therapy or treatment protocol based on a patient’s molecular profile that may not only minimize</td>
<td><a href="http://www.personalizedmedicinecoalition.org/">http://www.personalizedmedicinecoalition.org/</a> <a href="http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/pmc_age_of">http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/pmc_age_of</a> PMC_factsheet.pdf</td>
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<td><strong>Precision Medicine Initiative (US NIH)</strong></td>
<td>Precision medicine is &quot;an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.&quot; This approach will allow doctors and researchers to predict more accurately, which treatment and prevention strategies for a particular disease will work in which groups of people. It is in contrast to a one-size-fits-all approach, in which disease treatment and prevention strategies are developed for the average person, with less consideration for the differences between individuals.</td>
<td><a href="https://ghr.nlm.nih.gov/primer/precisionmedicine/definition">https://ghr.nlm.nih.gov/primer/precisionmedicine/definition</a></td>
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<tr>
<td><strong>Food and Drug Administration</strong></td>
<td>Precision medicine, sometimes known as &quot;personalized medicine&quot; is an innovative approach to tailoring disease prevention and treatment that takes into account differences in people's genes, environments, and lifestyles. The goal of precision medicine is to target the right treatments to the right patients at the right time.</td>
<td><a href="https://www.fda.gov/medical-devices/vitro-diagnostics/precision-medicine">https://www.fda.gov/medical-devices/vitro-diagnostics/precision-medicine</a></td>
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<td><strong>Schleidgen et al.</strong></td>
<td>Personalized medicine seeks to improve stratification and timing of health care by utilizing biological information and biomarkers on the level of molecular disease pathways, genetics, proteomics as well as metabolomics.</td>
<td>BMC Medical Ethics 2013, 14:55</td>
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<td><strong>Sadée and Dai</strong></td>
<td>Pharmacogenomics is a harbinger of personalised medicine, a paradigm shift from the mindset of 'one-drug-fits-all' to 'the right drug for the right patient at the right dose and time.' This does not mean that each patient will be treated differently from every other patient, an economically untenable proposition. Rather, patients are divided into groups by genetic and other markers that predict disease progression and treatment outcome.</td>
<td>Human Molecular Genetics 2005;14(suppl_2):R207–R214</td>
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Appendix 1: Examples of search strategies

### 1. Methods for stratification and validation cohorts

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2. Methods for machine learning applied to stratification

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### 3. Pre-clinical methods for translational development of stratified therapies and treatments selection

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## 4. Methods for clinical trials in personalised medicine

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<td>9610086</td>
</tr>
<tr>
<td>#13</td>
<td>#11 AND #12</td>
<td>927</td>
</tr>
<tr>
<td>#14</td>
<td>#11 AND #12 AND ([english]/lim OR [french]/lim OR [german]/lim OR [italian]/lim OR [spanish]/lim)</td>
<td>9610086</td>
</tr>
</tbody>
</table>