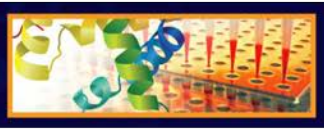


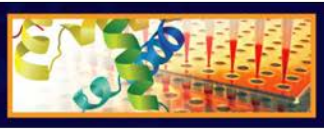


Complement Animal Research In Experimentation (Complement-ARIE)

Landscape Analysis Report 2024: New Approach Methodologies in Biomedical Research

National Institutes of Health
Office of Strategic Coordination–The Common Fund





This project was funded in part by the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH) under Contract No. HHSN273201500010C to ILS/Inotiv and under consulting agreement No. 15-199 to ICF in support of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and The NIH Common Fund Complement Animal Research In Experimentation (Complement-ARIE) Program.

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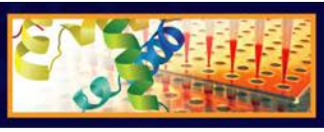


Complement-ARIE Landscape Analysis



Project Background

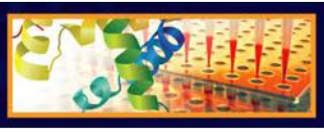
The NIH Complement-ARIE (Complement Animal Research In Experimentation) program will catalyze the development, standardization, validation, and use of human-based new approach methodologies (NAMs) that will transform the way we do basic, translational, and clinical science. The goals of the program include developing improved alternatives to traditional models for understanding human health and disease outcomes across diverse populations; developing NAMs that provide insight into specific biological processes or diseases states; validating/qualifying mature NAMs to support regulatory use and standardization; and complementing or replacing traditional models, making biomedical research more efficient and effective. To ensure that the Complement-ARIE program is focused on the areas of science with the greatest need, and which present the best opportunities for human-based model development, a landscape analysis was required to collect information on ongoing efforts in the NAMs space. The following landscape analysis is intended to provide a foundation on which to better define the scope of Complement-ARIE and inform upon coordination with existing programs. It includes a survey of *in vitro*, *in chemico*, and *in silico* approaches that have the potential to improve understanding of human health and disease mechanisms, reduce reliance on animal models, and make the use of animals more efficient. To ensure a rapid and comprehensive approach, we leveraged generative artificial intelligence (GenAI) and other computational methods, supplemented with subject matter expertise. In addition, a survey is presented of the requirements for data associated with and generated by NAMs to make the data Findable, Accessible, Interoperable, and Reusable (FAIR) and AI-ready. This survey includes considerations for a suitable data ecosystem and analysis of currently available infrastructure, including existing data centers and repositories, that can be leveraged.

Accordingly, this analysis focused on describing existing efforts, and highlighting gaps, challenges, and opportunities in the following primary areas of developing human-based models of health and disease:

- *In vitro* models (e.g., cell lines and organoids)
- *In silico* models (e.g., multiscale models and digital twins)
- *In chemico* cell-free models (e.g., biocomputers and high-throughput receptor-ligand screens)
- FAIRness of data needed to train, interpret, and use NAMs (FAIR = findable, accessible, interoperable, reusable) (e.g., findability/accessibility of datasets, data annotation and interoperability, artificial intelligence (AI)-readiness of training data, data ecosystem infrastructure requirements)

In these areas, the following questions are addressed:





- Current and past systematic efforts (e.g., Multiscale Modeling Consortium and Tissue Chip program) to develop and refine NAMs, including both success stories, scientific and technical challenges, and roadblocks to wider adoption. This includes, e.g., current efforts by the Food and Drug Administration (Regulatory Science Tools program) and the National Science Foundation (Reproducible Cells and Organoids initiative), as well as other federal agencies and non-federally supported initiatives (as relevant).
- Opportunities to validate mature NAMs to support their regulatory use and market adoption. This includes thinking beyond the NAM itself to see how it can be meaningfully leveraged in research and/or industrial settings.
- Requirements for data associated with and generated by NAMs to make the data Findable, Accessible, Interoperable, and Reusable (FAIR) and AI-ready. This includes considerations for a suitable ecosystem, as well as analysis of currently available infrastructure that can be leveraged without building new data centers or dedicated data repositories.
- The likely impact of NAMs on complementing and streamlining animal research, including methods to evaluate potential economic benefits.

Project Methods

The overall project methods are summarized in Figure 1 and further described in subsequent sections.

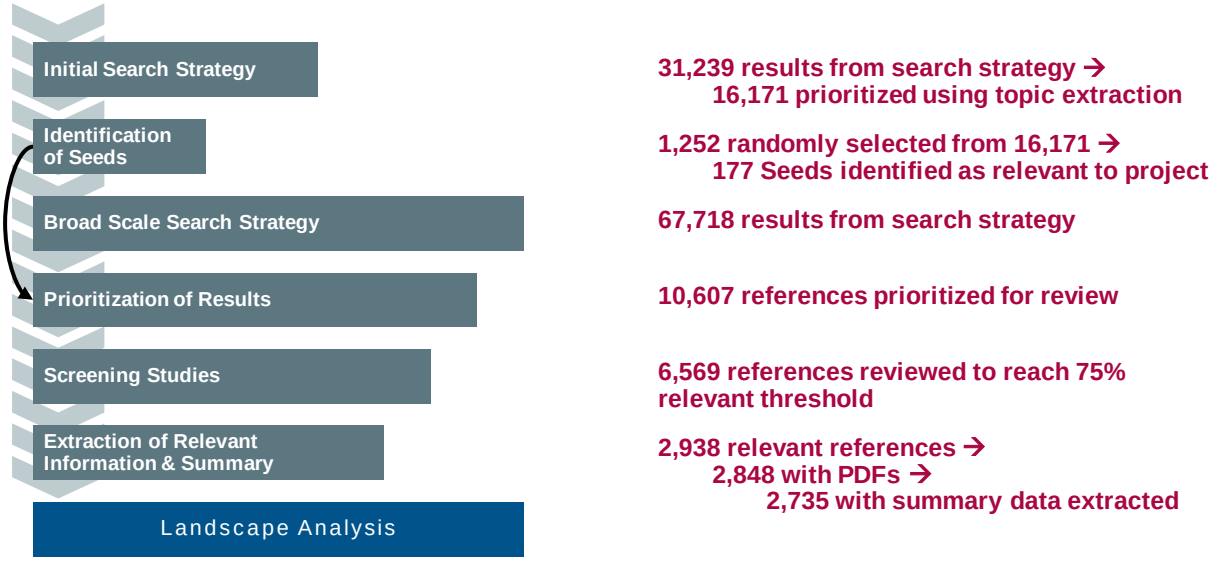


Figure 1. Overall Project Methods

Initial Search Strategy

We developed an initial search strategy to identify likely-to-be relevant review papers. We developed this strategy by creating a list of keywords for specific topics and then converting them to literature database syntax (specifically for the National Library of Medicine's PubMed database). Our initial search strategy with broad terms returned millions of hits, and so we tested the inclusion and exclusion of specific terms on the number of hits to identify the final list of terms. We also limited the scope of the years (2018 to Present) and to reviews (as indexed by PubMed) only. See Table 1 for a snapshot of this strategy and Appendix A for full details.

Table 1. Snapshot of Initial Search Strategy Methods

Sets of Terms	Syntax	Other Methods
Set 1: Alternative methods terms	Set 1 AND (Set 2 OR Set 3 OR Set 4)	Limited to 2018 – Present Reviews only (PubMed filter)
Set 2: In vitro terms		
Set 3: In silico terms		
Set 4: In chemico terms		
Total: 31,237 hits **(Ran on Oct 10, 2023)**		

Identification of Seeds

Topic Extraction

We applied the approach of "topic extraction" to prioritize the 31k references retrieved in the previous step. As shown in Figure 2, this method includes clustering references using their titles and abstracts so like references are grouped with like (and each reference only appears in a single cluster). The method also provides the most common topic keywords for each cluster, allowing us to quickly identify clusters likely to contain relevant information. We utilized ICF's Litstream® to perform this analysis.

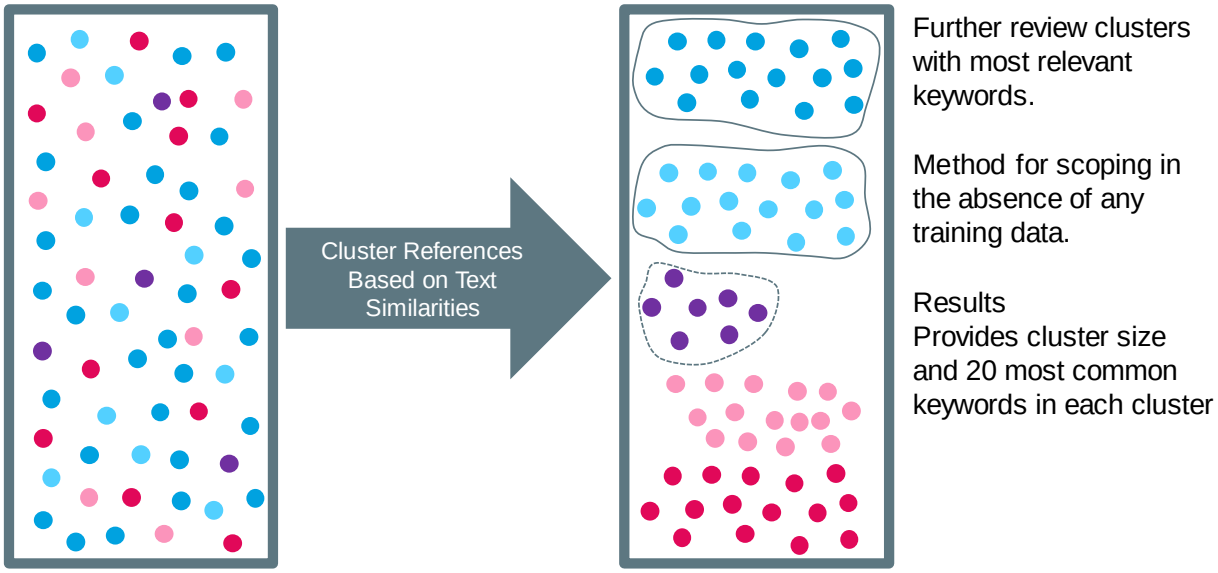


Figure 2. Illustration of Topic Extraction Methodology

We derived 10 clusters, shown in Table 2, with associated keywords. Using the results of topic extraction, we included 16,171 references from clusters 4, 8, 9, and 10 from Table 2 in subsequent screening and review steps.

Table 2. Topic Extraction Results

Cluster	# of Studies	Keywords/Topic Signature
1	6542	biomarkers, patients, clinical, disease, treatment, risk, diagnosis, studies, diagnostic, management, early, patient, based, therapy, current, outcomes, biomarker, results, new, methods
2	846	95, ci, meta, meta analysis, 95 ci, analysis, review meta, review meta analysis, systematic, systematic review meta, pooled, systematic review, studies, included, patients, results, hr, ratio, embase, value

Cluster	# of Studies	Keywords/Topic Signature
3	1901	cancer, patients, tumor, immunotherapy, checkpoint, immune, inhibitors, treatment, biomarkers, immune checkpoint, clinical, pd, response, therapy, predictive, checkpoint inhibitors, lung cancer, pd l1, l1, lung
4	2077	learning, machine, machine learning, ai, intelligence, artificial, artificial intelligence, data, ml, deep, deep learning, models, prediction, methods, algorithms, based, predictive, applications, intelligence ai, artificial intelligence ai
5	713	covid, covid 19, 19, cov, sars, sars cov, coronavirus, pandemic, respiratory, acute respiratory, severe, respiratory syndrome, 2019, severe acute respiratory, severe acute, infection, acute respiratory syndrome, 19 pandemic, covid 19 pandemic, syndrome
6	2501	gene, arna, expression, gene expression, rnas, coding, sequencing, genes, non coding, regulation, genome, cell, epigenetic, single, dna, non, mechanisms, throughput, genetic, coding rnas
7	2563	cancer, tumor, cells, cell, therapeutic, treatment, therapy, resistance, microenvironment, drug, tumors, progression, metastasis, cancer cells, cancers, models, clinical, targeting, development, potential
8	2480	stem, cells, stem cells, cell, stem cell, pluripotent, tissue, pluripotent stem, regenerative, derived, regeneration, human, mesenchymal, mscs, therapy, pluripotent stem cells, mesenchymal stem, induced pluripotent, induced pluripotent stem, disease
9	7202	based, methods, high, applications, approaches, systems, development, throughput, new, data, research, high throughput, recent, technologies, used, drug, techniques, analysis, advances, tools
10	4412	vitro, models, drug, studies, vivo, effects, disease, diseases, therapeutic, development, mechanisms, drugs, human, potential, treatment, inflammatory, anti, activity, compounds, clinical

Title/Abstract Screening and Extraction

We created random subsets of the 16,171 references for expert staff to review according to the methods outlined in Appendix B. Specifically, staff reviewed 1252 references (~8% of titles/abstracts randomly selected from the 16,171) for relevance to the project. Each reference was reviewed by a primary reviewer and a QA reviewer confirmed the accuracy of the primary review. References could be categorized as



Include, Supplemental, or Exclude as shown in Table 3 below, and high-level study details were extracted as shown in Appendix B.

Table 3. Categories for Classification of References According to Title and Abstract

Relevance	Categories	Definition
Include	In vitro In silico In chemico General Methods	Method has the potential to complement or replace the use of animals in biomedical research
Supplemental	FAIR	Relevant to findable, accessible, interoperable, and reusable concepts, including discussion of data availability or databases or other repositories
	Animal-based NAM	NAM but animal-based (using animal tissue – whole organisms are excluded)
	Potential application in biomedical research	Catch-all bin for when the paper has the potential for being relevant to a biomedical context and replacing animal tests, but the authors do not necessarily make that point explicitly
Exclude	n/a	The study does not describe an alternative test method/model that meets the requirements above, where the context is related to recapitulating a relevant human physiological process or increasing our understanding of a biological process

Broad Scale Search Strategy

We then developed a broader search strategy to complement the initial strategy. Specifically, we expanded to additional databases (Web of Science and Scopus) and did not limit to reviews only, but we looked at only the most recently published papers (2023 – 2024 references published early). This resulted in 67,718 potentially relevant references after deduplicating across databases. Expanding to 2018 – Present would have resulted in about 450k references, which was deemed beyond the project's scope given the focus on obtaining the most current perspective of NAMs in the scientific literature. Taken together, we assumed that the reviews identified during the initial search strategy and the most recent papers from this broad search strategy represent the landscape of the most cutting-edge developments able to be evaluated within the project's time constraints. See Table 4 for a snapshot of this strategy and Appendix C for full details.

Table 4. Snapshot of Broad Search Strategy Methods

Sets of Terms	Syntax	Other Methods
Set 1: Alternative methods terms	Set 1 AND (Set 2 OR Set 3 OR Set 4)	Limited to 2023 – Present
Set 2: In vitro terms		
Set 3: In silico terms		
Set 4: In chemico terms		
PubMed: 53,846 hits **Scopus: 25,470 hits** **Web of Science:33,434 hits** **Total: 67,718 hits** **(Removing duplicates across PubMed, Web of Science, and Scopus; ran on Oct 23, 2023)**		

Prioritization of Results

To increase the likelihood of identifying key papers in the field, a modular prioritization method was applied. Specifically, supervised ensemble clustering was performed using seed studies identified as relevant in Step 2.2. An ensemble of six clustering approaches using two clustering algorithms (K-means and non-negative matrix factorization) and three bin sizes (10, 20, and 30) were applied and each study was assigned a score based the number of times the study was found in a selected cluster containing a high proportion of seeds. Studies with an ensemble cluster score of greater than or equal to 4 (n=10,607, see Table 5) were prioritized for human screening based on a prior study that demonstrated the validity of the scoring method (Cawley et al., 2020)

Table 5. Ensemble Clustering Results

	# of Refs							
Seed	6	5	4	3	2	1	0	Grand Total
Unclassified	3720	2645	3637	5887	8824	10412	32593	67718
Seeds tagged as “exclude”	223	146	84	58	37	45	66	659
Seeds tagged as “include”	93	38	21	11	2	5	7	177
Grand Total	4036	2829	3742	5956	8863	10462	32666	68554

Screening of Studies

To expedite the process of title/abstract screening, the Active Machine Learning (AML) component of Litstream was employed to accelerate review of the 10,607 studies identified as being of potential interest. AML requires a user to annotate an initial set of references as relevant or not relevant to first train the model. Litstream provides additional references for the user to annotate, then iterates the model to optimize on recall. Using additional screening data from every 50 studies screened, a support vector machine algorithm builds a predictive model to re-rank not-yet-screened studies in terms of their likely relevance, based on natural language patterns to identify the most likely to be relevant studies (see Figure 3).

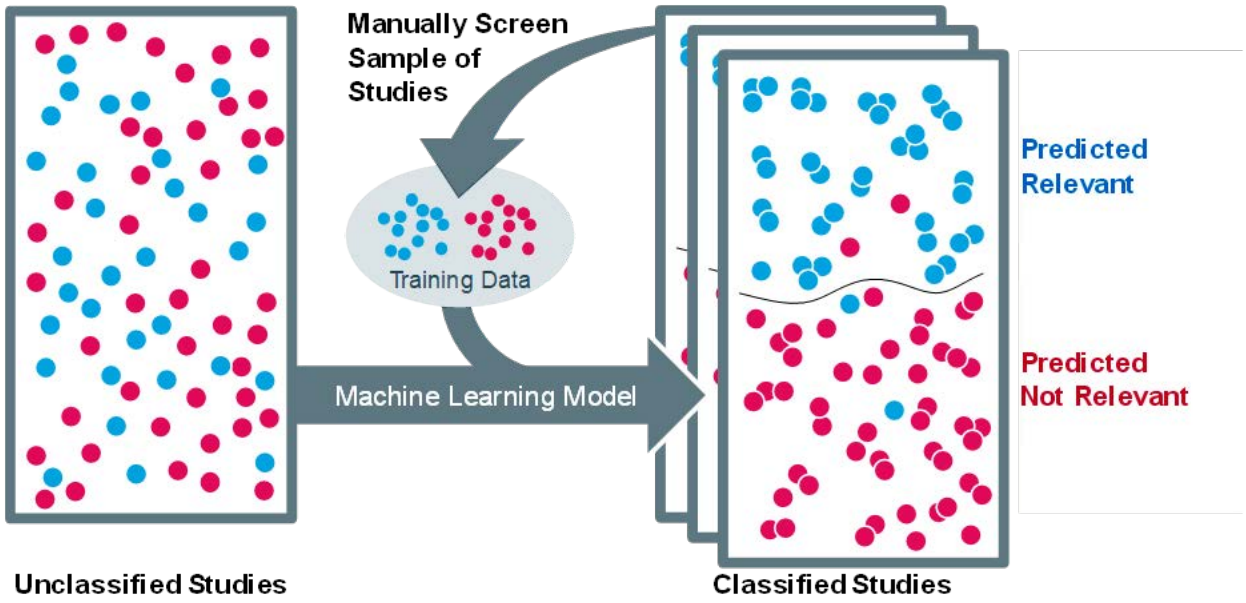


Figure 3. Illustration of Active Machine Learning Methodology

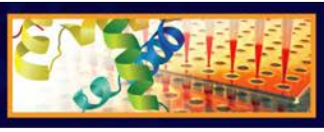
This ranking process sifts the unreviewed references and prioritizes those predicted to be relevant for human review. This allowed for the screening of 6,569 titles and abstracts and the identification of 2,938 relevant studies, which was estimated to be more than 75% of the total relevant studies. In discussion with the Complement-ARIE leadership, this threshold was decided to be enough relevant studies to proceed to analysis in order to meet the expedited timeline.

Extraction of Relevant Information and Summary

PDF Retrieval and Generation of Text Files

For references deemed relevant in previous steps, we retrieved PDFs from NIH Library and other web sources. Of the 3,091 studies to enter the pipeline (2,938 identified during AML + 261 seed studies, with 108 duplicates removed), we successfully





retrieved 2,848 (243 were not available without inter-library loans or purchasing or were not in English). See Appendix D for a reference list.

Available PDFs were converted to text files using Azure AI Document Intelligence for use in future steps of the project (see below).

Keyword Analysis

We developed a list of keywords to categorize relevant references. The Keyword Analysis Tool (KAT) searched for the occurrence of one or more keywords within either a title or abstract of a bibliographic reference. The tool generated a CSV file with a frequency array by keyword for each reference, which we used to understand the scope of the relevant literature. We developed the keyword list used in this step (Appendix E) in collaboration with Complement-ARIE leadership.

Generative Artificial Intelligence (AI)

For extraction of relevant information, generative AI technology was leveraged. Anthropic's Claude V2 Foundation Model LLM was chosen for this analysis as it was a secure technology designed for summarization with a high enough token limit to read a full PDF and return a satisfactory answer. To deploy this across all possible studies, we developed a Claude workspace in the AWS Bedrock environment. This allowed for compiling of responses across multiple papers and uploads of multiple PDFs in a systematic and streamlined way.

Two methods were proposed and piloted. The first was to feed a corpus of studies to an AI and then ask specific questions related to the overall state of the field/topic area. The second was to summarize studies individually and then aggregate the data across the bolus of literature based on individual level summaries. After piloting, it was determined that the first approach was not feasible as the generative AI tended to "hallucinate" information beyond that which was provided, and it would be difficult to do this approach in a systematic way. We therefore decided the best approach would be to summarize studies on a study-by-study basis and then aggregate the data afterwards.

For the generative AI, we developed questions in conjunction with Complement-ARIE leadership with the aim of (1) understanding the currently existing landscape of literature, (2) making funding decisions to move the state of the science forward and (3) thinking ahead to understand the current ethical, workforce, economic and social issues that may surround the use of NAMs. These questions were then converted into prompts that prevented the AI interface from making up answers (aka "hallucinating"), and quotes had to be given to justify the response generated by the AI for validation purposes. The full list of questions and prompts can be found in Appendix F.

Of the 2,848 studies, 47 were non-responsive in Claude and 66 PDFs were longer than 40 pages (exceeding the token limit). This resulted in a total of 2,735 successful outputs that were summarized by generative AI.

Post-Processing of Generative AI Outputs

After collecting the responses from the Claude outputs, we applied post-processing of the text to convert statements noting a lack of data to a standardized response (e.g., changing "I don't see…" or "The authors did not…" to "No"). Additionally, for a subset of questions, we re-entered the responses into Claude and asked it to extract the "named entities" of each statement so that the information could be returned in the format of a categorical list, to use for further classification of the studies and response data. These prompts can also be found in Appendix F.

FAIR Database Review

Twenty-eight out of 260 identified biomedical databases were evaluated for Findability, Accessibility, Interoperability and Reuse (FAIR) using a modified rubric originally developed by the U.S. Geological Survey (Hutchison et al., 2023; Wilkinson et al., 2016). This rubric consisted of 29 questions (Appendix H) aimed to assess computational readiness and was applied to each database by two evaluators: one initial screen/scoring and one review of assessment. If the evaluator was able to find the information for a given question, the question received a score of "1" and if the information was not identifiable, the question received a score of "0". These scores were summed and each evaluated database and FAIR question was given a composite score. Databases were also categorized using keyword lists (Appendix E) to determine NAM representation.

Project Results

Literature Landscape Results

Overall breakdown of representation

Following assessment of the comprehensive literature review by generative AI, we summarized and mapped the relevant references into interactive visuals according to the results of AI, answers to relevant questions and categorical assignments, and reference characteristics using Tableau data visualization software (Seattle, WA) (see Complement-ARIE Landscape Analysis). Individual dashboards focus on different aspects of the data, with questions and categories featuring prominently. Interactive features include:

- Filtering by variables of interest
- Hovering over data elements for additional information and links to references
- Search for specific keywords of interest

The Tableau comprises three tabs:

- Explore Questions: Generative AI responses to questions for each reference, organized by question.
- Explore Categories: All references organized by keyword.
- Reference List: All references including bibliographic details.

As shown in Figure 4, data within Tableau can be sorted and filtered to identify, for example, the volume of studies associated with a specific type of NAM, identify papers that include multiple types of NAMs for potentially evaluating combinatorial approaches, or note trends associated with generative AI responses (see also Fig 5 and supplementary material).

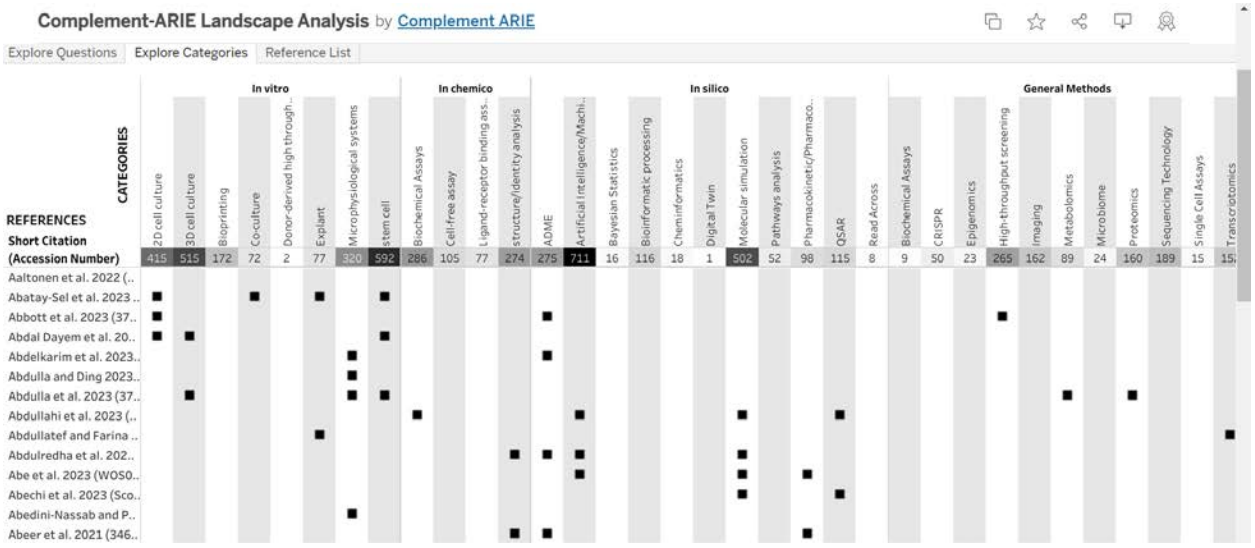


Figure 4a. Tableau screenshot demonstrating how to explore categories by organizing references organized by keyword.

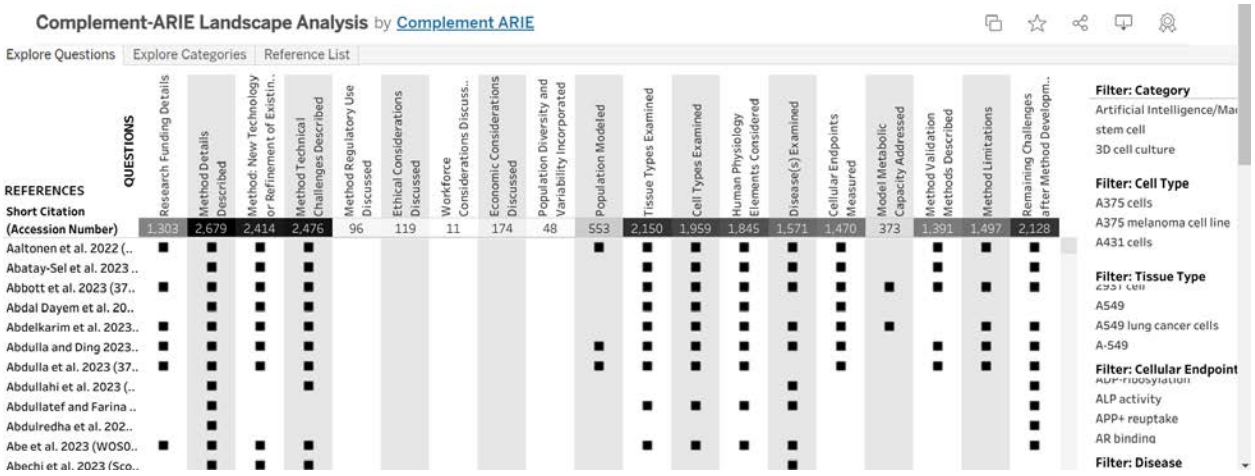
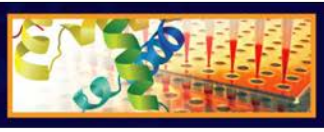


Figure 4b. Tableau screenshot demonstrating how to explore generative AI responses to questions for each reference.

The results demonstrate that NAMs work has mainly focused on in vitro (n=1284) and in silico (n=1300) studies with fewer in chemico (n=657) studies or general methods development such as high throughput screening (n=750). Within the in vitro category, stem cell (n=556) and 3D cell culture (n=487) are the best represented categories. In chemico analyses have focused on biochemical assays (n=273) or structure/identity analysis methods (n=262). In silico studies are generally focused on the development of AI/machine learning models (n=676) and molecular simulation (n=483). Review of the topical breakdowns following generative AI did note that the assignment of categories was not always completely accurate. It is likely that some manuscripts were overlooked within certain subject areas (or incorrectly included based on keyword analysis).

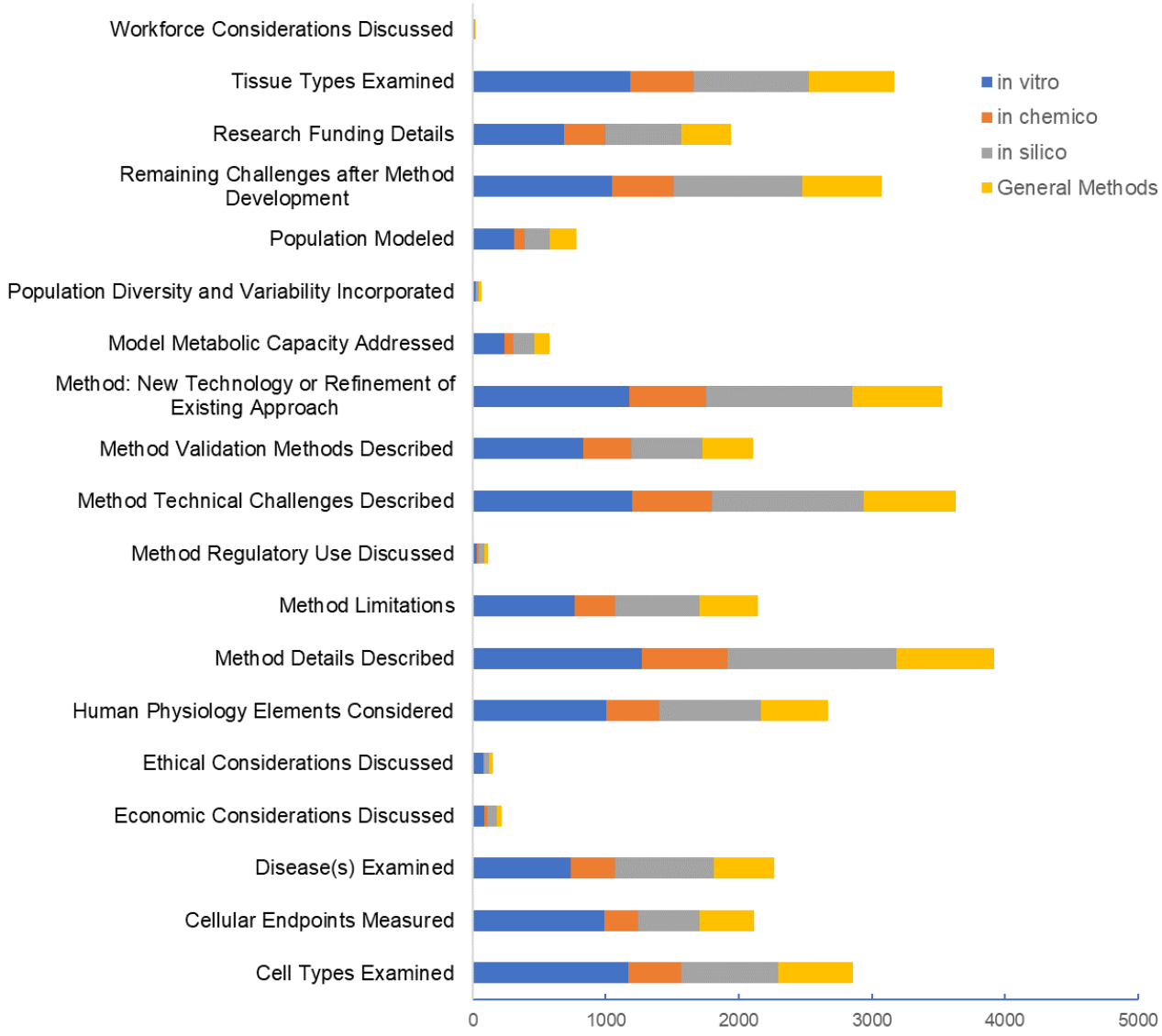




However, the overall outlook of the current state of NAMs in biomedical research is still appropriately reflected in this assessment based on the wide range of input received relating to the literature review approach implemented, and the alignment of observed trends with comprehensive NAMs review papers.

While there were differences in the amount of NAM publications gathered for each focal area, similar trends were noted in all (Figure 5, Supplemental Materials). The topics with the highest representation examined both cell and tissue types, human physiology considerations and method information including detailed description of new or improved technologies, and technological challenges. Diseases, metabolic capabilities, and method limitations were also addressed, but to a lesser extent. Areas that were seldom reported or addressed in these publications included regulatory application, workforce or ethical considerations, population diversity and variability, and economic considerations. However, it should be noted that publications often included related aspects such as future endeavors and ethical considerations regarding multiple cell culture techniques including biobanking, community engagement and informed patient consent. An assessment of these gaps is found in the Gap Analysis section. A common future direction noted in publications included the need for standardization and validation of NAMs.

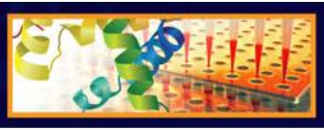
Figure 5. Number of Publications Gathered per General Topic Using Generative AI Approach.



In vitro biomedical NAMs

Across the literature on in vitro models, 2D and 3D cell cultures were highly represented, including models based on both induced pluripotent and embryonic stem cells (Supplemental Materials). Many of these publications are focused on the use of cardiomyocyte, neuronal and endothelial cell types and liver, bone, breast, lung, and skin tissues. Cancer (e.g., liver, ovarian, breast and pancreatic), diabetes, Alzheimer's and Parkinson's diseases were a particular focus. In vitro methodologies were the largest proportion of literature hits compared to in silico and in chemico approaches.





Within the in vitro space, bioprinting and microphysiological based systems were often used in conjunction with 2D and 3D cell cultures to better recapitulate organ and tissue human physiology for disease research, supporting the high representation of 2D and 3D methods. Examples include patient derived cells to create organoids for ovarian cancer and development of prostate organoids for preclinical cancer research (Buskin et al., 2023; Chan et al., 2023). Technological advances continue to foster the development and use of these more complex models, but challenges still remain to represent complex tumor microenvironments and metastasis processes. Other non-cancer related NAMs include stem cell cardiomyocyte “heart-on-a-chip” and hepatocyte microfluidic “lab-on-a-chip” platforms in addition to ocular organoids for drug development and diseases (Bai and Wang, 2020; Criscione et al., 2023; Di, 2023).

Keyword analysis revealed gaps in knowledge including lack of donor derived high throughput culture panels, co-culture and explant in vitro NAMs. Developmental neurological disorders including ADHD and autism spectrum disorders, asthma, and immune and inflammatory diseases were also seldomly represented. Multiple aspects of methods were reported including method limitations and challenges such as high cost with limited resolution of bioprinting along with the potential toxic waste materials generated through these processes and inclusion of multiple different cell types to help identify roles of genetic variants. While many of these approaches were not specifically noted for regulatory purposes, there were a few review papers highlighting the potential for alternative approaches (e.g., specialized cell lines and organ-on-chips) in the food safety and endocrine disruption space (Audouze et al., 2020; Reddy et al., 2023). Various ethical considerations were highlighted in two main areas: those related to the origin of human embryonic stem cells and the study of psychiatric disorders, which commonly combine both in vitro and in vivo techniques such as complex human brain organoid and mammalian behavioral observations, both of which may have some level of consciousness (Cota-Coronado et al., 2019; Dixon and Muotri, 2023). Workforce considerations are a noted gap for all biomedical NAMs, which takes into consideration aspects of an approach, such as training that would be needed in order to implement it. There was one instance of rapid bioprinting for medical supplies development (e.g., human tissues and bioactive bandages) for military use in austere locations and the need for training in both use and advancement of these technologies (Barnhill et al., 2023). Ongoing research of these bioprinting technologies can lead to the development of civilian/consumer level accessibility in medicine, providing a potentially biocompatible option for burns and traumatic injury, treatment of dermal diseases, etc. Lastly, areas that were not considered but mentioned as future directions include translation for clinical relevance, and lack of incorporation of immune and microvascular components, and drug development for rare diseases including precision medicine.

In silico biomedical NAMs

The main focal areas of in silico approaches were AI and machine learning; molecular simulation; and predictive models for absorption, distribution, metabolism, and excretion (see Supplemental Materials). Within these the largest percentage of NAMs focused on AI and machine learning. Areas with the least representation within the biomedical space include digital twins and read across approaches which may have been influenced by the focus of this review since the latter approach is more commonly utilized in toxicology fields, and the former is an emerging topic in precision medicine.

Due to the vast amount of literature within the AI/ML category, it was necessary to explore the “detailed answers,” i.e., 5,351 records for this category, produced by the generative AI. To do so, we have generated bar plots to indicate the frequency of phrases within the “detailed answer” records, with the assumption that word frequency distributions would indicate areas that are less or more explored.

As disease-treatment and drug-development are two broad areas within biomedical research, the phrase frequency distributions focus on these two topics. Cancer is the most highly explored disease type, with mental health, cardiovascular diseases and diabetes following closely. Figure 6 indicates the distribution of the different types of cancer and cancer therapies that were most highly represented within the literature. Removing the term "cancer” to look more specifically at cancer type shows that lung, breast, colorectal, prostate, glioma and glioblastoma are the most represented, in line with cancer rates and research funding (Figure 7). Regarding drug development, docking, molecular dynamic simulations, and virtual screening are among the frequently used methods (Figure 8). This analysis indicates that there is a lack of effort in utilizing or developing read across, pharmacophore, and homology methods for drug development.

Figure 6. Frequency Distributions of Cancer and Therapy Types

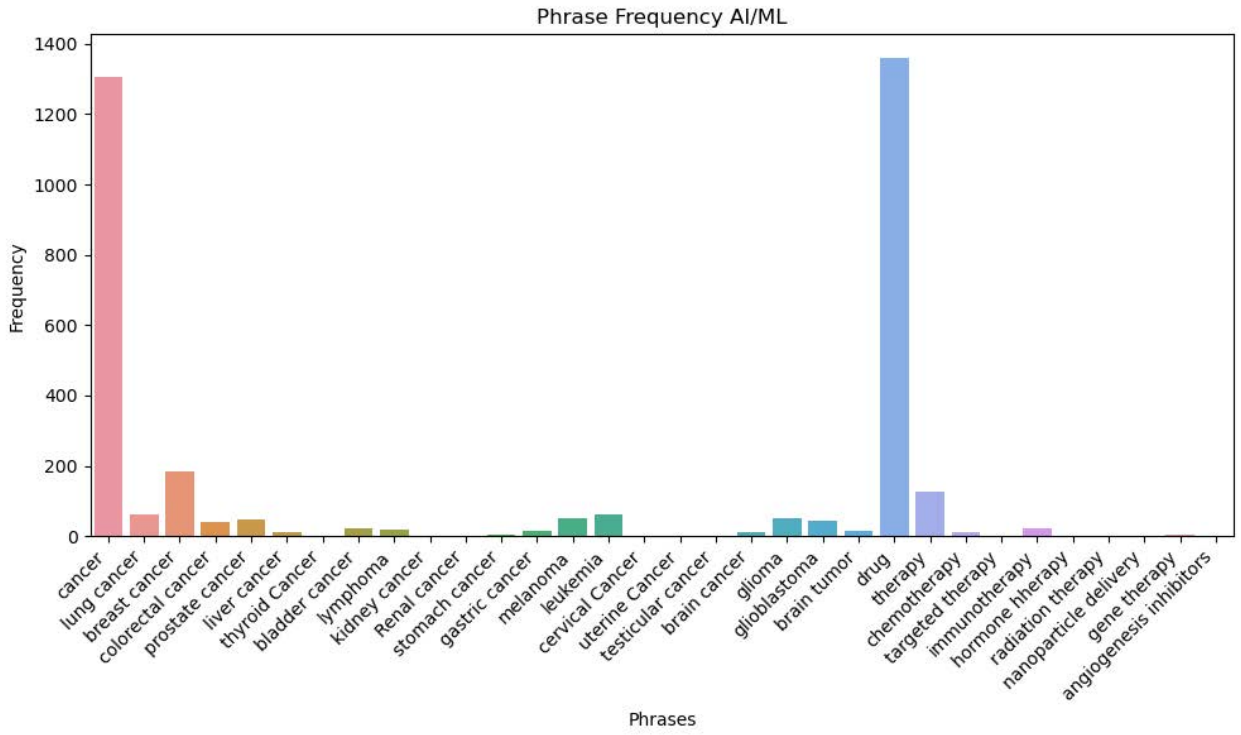


Figure 7. Frequency Distribution of Cancer Types

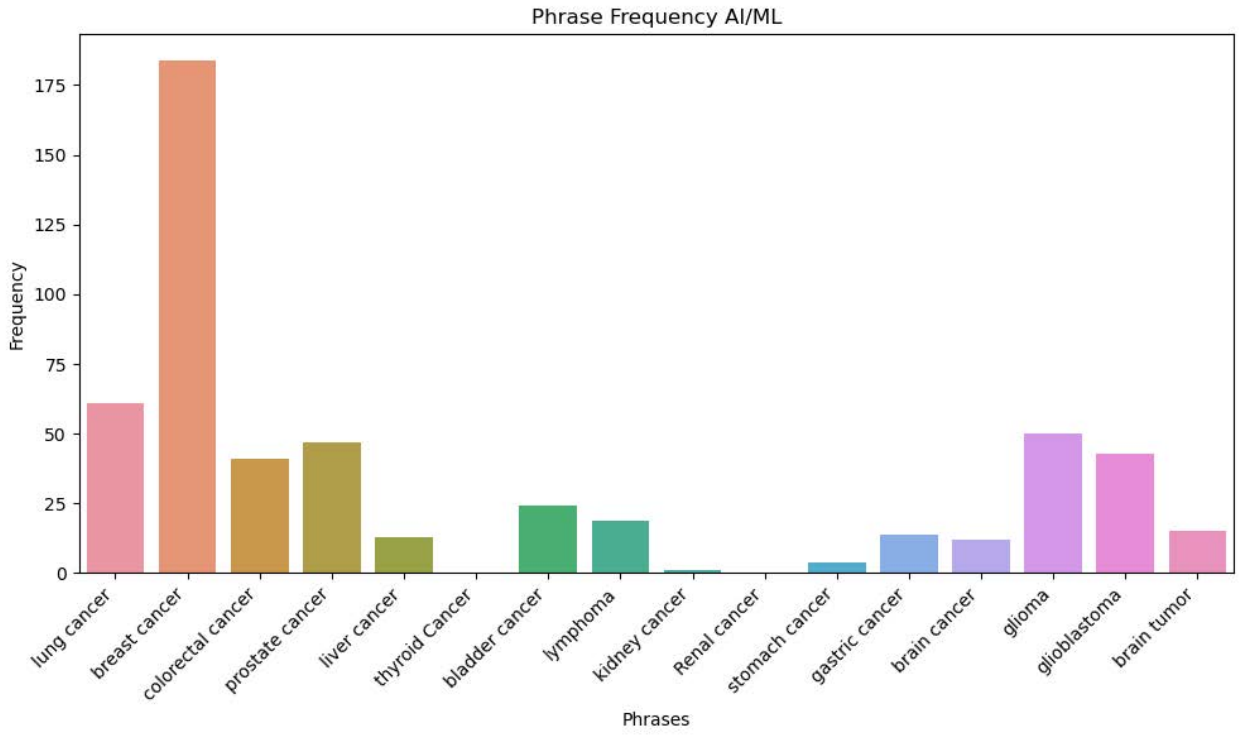
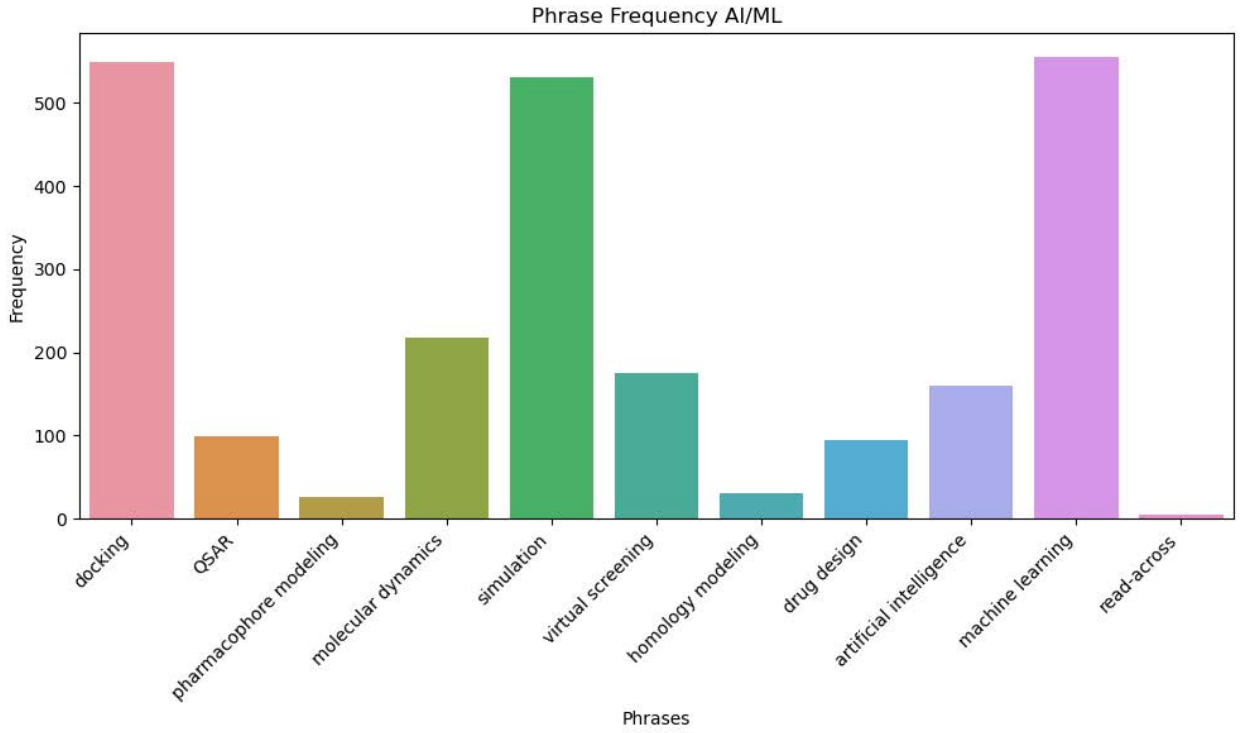


Figure 8. Frequency Distribution of Drug Development Computational Methods



In general, large language models (LLM) is one of the least studied AI/ML categories where only one relevant paper was identified (Zagirova et al., 2023). The model was developed on a large corpus of biomedical literature and generates targets predictions. The authors have shown its application on aging and age-related disease targets. However, as the practical availability of LLMs has exploded in the past year, it is expected that their use in biomedical research will grow significantly in the near future.

In chemico biomedical NAMs

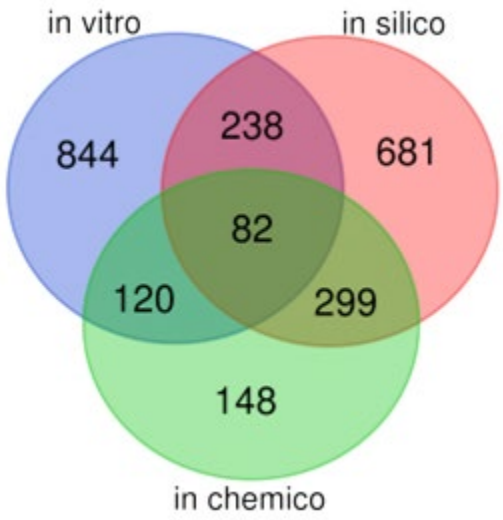
The two largest aspects of in chemico NAMs were biochemical assays and structure/identity analysis (Supplemental Materials). Many of the “in chemico” articles should have been categorized as “in silico” upon further assessment. Cell free and ligand-receptor binding assays were included as part of the keyword categorization but were rarely noted in these publications. Of the correctly categorized publications, these mainly included enzymatic assays and mass spectrometry and chromatography with a focus on brain and liver tissues. Similar to in vitro NAMs, cancer (particularly breast cancer), along with diabetes and Alzheimer’s disease, were some of the most common disease research topics. One of the most promising areas for application of in chemico biomedical NAMs will be in the identification of novel biomarkers. For instance, proteomics has helped to identify prognostic, predictive, and therapeutic biomarkers for various sarcoma subtypes (Connolly et al., 2023). Many screening approaches have

also been facilitated by in chemico NAMs, including a variety of reporter assays to assess endocrine disruption. For instance, the AR2 assay uses a luciferase enzyme fused to the human androgen receptor to assess androgen receptor homodimerization and allows for incorporation of enzymatic metabolism (Brown et al., 2023). The assay showed a high Z'-factor and balanced accuracy, and an interlaboratory investigation is now being conducted. Supramolecular chemistry (the chemistry of noncovalent bonding between molecules) is another exciting field opening up for screening. A supramolecular tandem assay in conjunction with a reporter assay for intracellular imaging of HDAC1 to identify HDAC1 inhibitors was recently developed (Li et al., 2023). This approach is 10X more sensitive than prior HDAC1 assays and has already identified one novel HDAC1 down-regulator. The primary challenge noted for in chemico biomedical NAMs is translation to in vivo models and then later into human clinical medicine. Additional challenges include single cell mass spectrometry, enabling real time analysis, and full automation. For example, the identification of metabolite biomarkers still faces a variety of challenges. These include the high variability of such data, which can be influenced by diet, exercise, environment and experimental handling; generally low sensitivity and specificity; and a lack of standardized protocols for statistical analysis. In sum, in chemico biomedical NAMs are poised to play an important role in biomarker identification and screening (Anwardeen et al., 2023).

Combinatorial NAM strategies

While this landscape analysis has provided information on the individual NAM groups highlighted above, it has also shed light on combinatorial approaches across the targeted NAM spaces. Main topics include cancer, drug discovery and digital pathology with most of the approaches focusing on molecular docking and spectral imaging. However, only roughly 1% of the gathered publications spanned all four targeted NAM categories (i.e., in vitro, in silico, in chemico and general methods) with the majority of these not integrating these approaches but instead reviewing their respective potential utility (see Figure 9).

Figure 9. Overlap of NAMs Categories in Literature Reviewed



Literature landscape challenges and limitations

Approaches were utilized to help identify key areas and gaps in knowledge by applying specific keywords or questions to the gathered publications. Review of publications revealed that there were some challenges with the algorithm in which topics or keywords were mentioned in publications (e.g. in the background or introduction sections) but weren't relevant to the particular study. While this is a noted challenge, this aided in identifying future applications for these NAMs in biomedical research as many of these instances were included as future directions or current limitations.

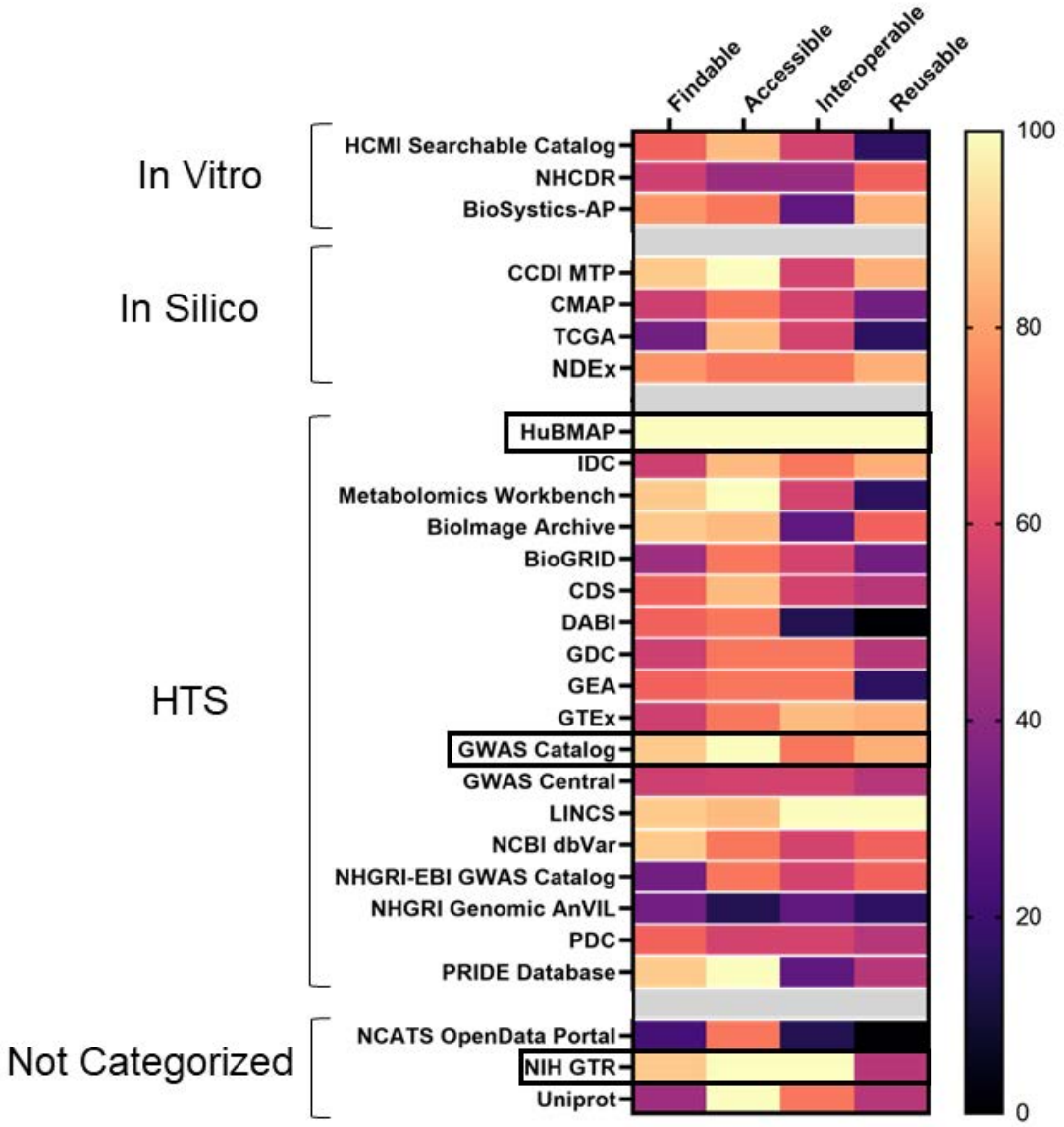
FAIR Database Results

FAIR assessment of the 28 databases reviewed differed with only 9 databases including at least 70% or more of the information needed to answer rubric questions. Only one database, the Human BioMolecular Atlas Program (HuBMAP), included all information targeted by our review. Other databases that included 93% and 86% of FAIR information were the Library of Integrated Network-Based Cellular Signatures (LINCS) and NIH Genetic Testing Registry (GTR) and Genome-wide Association Studies (GWAS) Catalog, respectively. The categories of reviewed databases also varied with most falling within General Methods (e.g., high throughput screening, sequencing technology, and omics approaches), suggesting that databases are centered more on the technological aspects of the assays as opposed to NAM space (e.g., cell culture type, microphysiological systems, AI, and machine learning).

Focusing on the four main principles of FAIR, there was not a single rubric question for which all databases provided information. The highest response ranges were for Accessible (12 – 27 databases, median of 19) and Findable (4 – 25 databases, median of 23) indicating the ability to identify and retrieve unique labeled data/metadata, through human and automated actions, is not a limiting factor among the evaluated biomedical databases. Areas that had the lowest reporting tended to fall within the Interoperable (7 – 27 databases, median of 15) and Reusable (8 – 22 databases, median of 13) domains which address integrating, replicating or combing data for processing, analyzing and storage applications. Information pertaining to seven out of the 29 questions were not able to be identified for more than half of the databases (Figure 10). The question with the lowest response addressed detailed author information (ORCID identifier), which was likely too specific for this exercise. There was also an additional question pertaining to citations for input datasets that was likely not relevant for those that did not include these and therefore this information could not be identified. Other questions that were less FAIR included metadata documentation such as information on data consistency/precision/accuracy and including suggestions for data reuse both in terms of data constraints and user groups that could reuse data.

Further review of documentation provided by databases revealed that while most encourage detailed reporting of metadata, sole reliance on the data originator or submitter for the level of detail resulted in a lower FAIR score. Additional aspects that led to less FAIR databases include limited access to data/metadata (e.g. required account credentials) or obtained data, without metadata, from existing databases and utilized internal data processing pipelines. For this reason, some of the above-mentioned areas that scored the lowest were related to method details including information about technological aspects of assay or data quality practices. Conversely, databases that incorporated curation/harmonization steps, detailed reporting criteria or forms tended to be more FAIR, indicating that establishment of metadata reporting guidelines or standardization will enable more FAIR resources.

Figure 10. FAIR Principle Ranking per Database[1]. Warmer colors indicate a higher FAIR score while cooler colors indicate a lower FAIR score. Databases with the overall highest FAIR score are highlighted. Detailed view of each FAIR principle can be viewed in Appendix G and FAIR questions are listed in Appendix H.



[1] NHCDR: NINDS Human Cell and Data Repository; BioSystics-AP: BioSystics Analytics Platform; CCDI MTP: Molecular Targets Platform; CMAP: Connectivity Map; IDC: Imaging Data Commons; TCGA: The Cancer Genome Atlas; NDEx: The Network Data Exchange; HuBMAP: The Human BioMolecular Atlas Program; IDC: Imaging Data Commons; BioGRID: Biological General Repository for Interaction Datasets; CDS: Cancer Data Service; DABI: Data Archive for the BRAIN Initiative; GDC: Genomic Data Commons; GEA: Genomic Expression Archive; GTEx: Genotype-Tissue Expression; GWAS: Genome-wide Association Studies Catalog; GWAS Central; LINCS: Library of Integrated Network-Based Cellular Signatures; NCBI dbVar: Structural Variation Database; NHGRI-EBI GWAS Catalog; The NHGRI Genomic AnVIL: Data Science Analysis, Visualization, and Informatics Lab-space; PDC: Proteomic Data Commons; PRIDE: Proteomics Identifications Database; NIH GTR: Genetic Testing Registry



Gap Analysis and Overall Recommendations

While this landscape analysis provides a general overview of the current status of biomedical NAMs, there are several areas to refine and explore in this space. Indeed, this report focuses on a snapshot in time of biomedical NAMs and recent NAM applications; evaluating these spaces over a broader range of time will enable our understanding of how certain NAM use has changed over time or how certain areas have evolved over the period being analyzed. The citations provided are by no means an exhaustive list of relevant studies, and the papers discussed below are simply examples of the observed trends. Additional post processing of generative AI including quality control of AI outputs, in depth review of AI summaries of assay group limitations and biological coverage of each technology type, and review of documents that failed generative AI are future directions to explore. Various other literature approaches can be applied to further assess the biomedical NAM space. Some of these include review of titles/abstracts that were not screened using keyword/computational approaches, evaluation of number of citations for references/methods to understand replicability, and review of other sources of information outside of peer-review.

With those caveats, the results suggest that cardiomyocytes, neuronal and endothelial cell types and liver, bone, breast, lung, and skin tissues are extensively represented among the in vitro NAMs identified, and thus perhaps other cell types/tissues should be prioritized for further development. Likewise, cancer, diabetes, Alzheimer's and Parkinson's diseases have all been a particular focus of recent efforts, although very few (or no) articles included the application of NAMs in translation for clinical relevance, and there was a general lack of **incorporation of immune and microvascular components**. Such issues could be the subject of targeted future efforts.

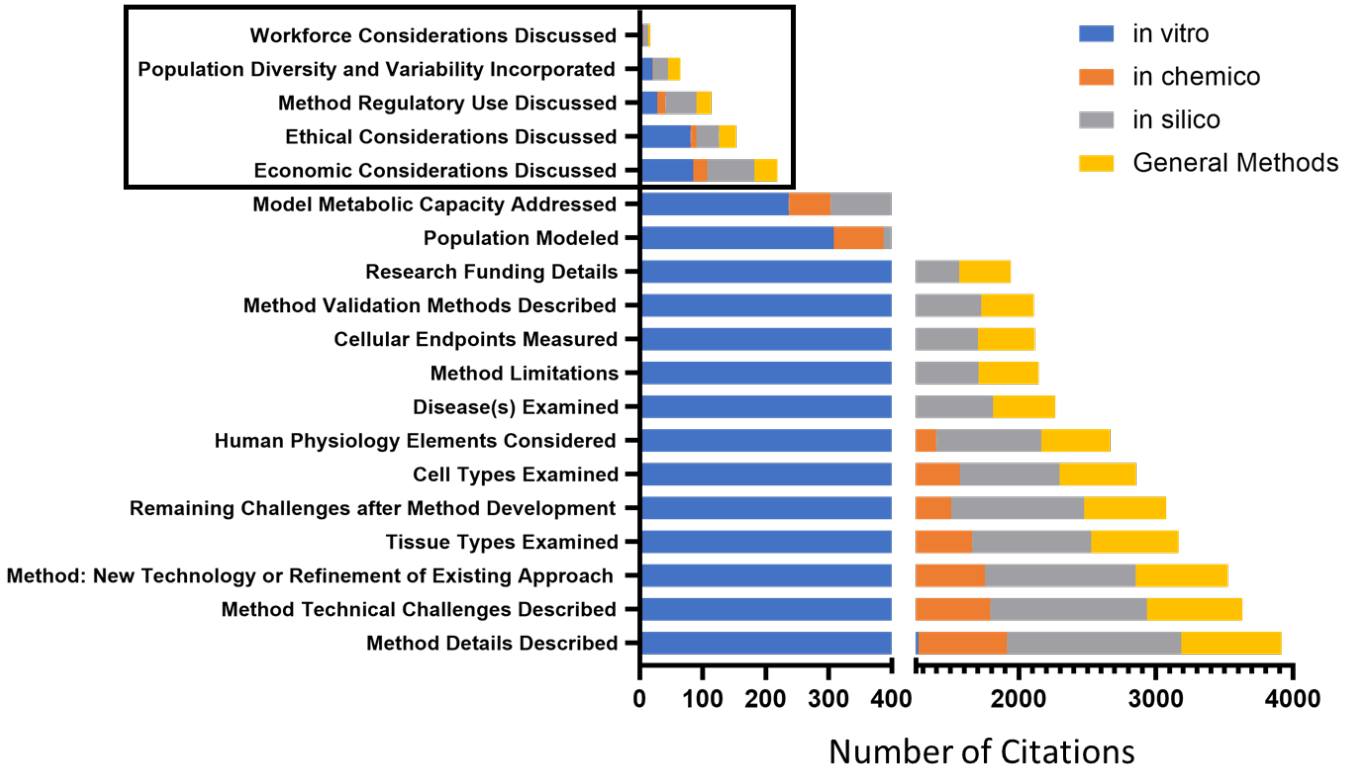
Regarding in chemico methods, there is modest representation across all four of the principal categories defined for this analysis, with biochemical and structure/identity analysis methods most highly represented among those studies. We did find that many in chemico studies were misclassified, suggesting further refinement of the search and interpretation algorithms is necessary. However, overall, such methods have the capacity to efficiently screen compounds of interest with exquisite sensitivity and thus could be considered in future efforts to **design combinatorial approaches** in conjunction with other types of NAMs to address a wide range of research questions.

Given the rapid growth of computational approaches, it is not surprising that AI and machine learning represent the majority of silico NAMs that were identified. There is little debate of the power of such approaches in biomedical research, limited only by the quality and quantity of input data with which they are initially developed and trained. Future efforts should harness the data generated by NAMs and therefore an essential

future effort is the development of a readily accessible data repository. Considering the observation that databases incorporating curation/harmonization steps, detailed reporting criteria or forms tended to be more FAIR, **establishment of metadata reporting guidelines or standardization** to enable more FAIR resources should be a priority.

Regardless of the category of method considered (i.e., in vitro, in chemico, in silico, or general method), **economic and workforce considerations, ethical considerations, regulatory use, and population diversity and variability** were consistently underrepresented, highlighting key areas where additional research efforts should be directed (Figure 11 and S2, S4, S6, S8). Given their potential throughput, human relevance, and opportunity for representative heterogeneity, it would seem prudent to more extensively evaluate the utility of NAMs for addressing these research questions.

Figure 11. Identification of Topical Areas Least Identified by Generative A.I.



This analysis also indicates that future efforts need to be focused on using combinatorial or multidisciplinary NAMs to investigate and address human health science. Only 1% of the reviewed literature described research encompassing in vitro, in silico, in chemico and high dimensionality research methods. Research programs



centered on specific aspects of human health or disease states could be used to develop concerted, multidisciplinary efforts that complement each other to provide insight and advances that would be impossible to achieve using single NAM modalities.

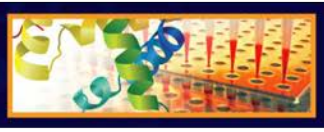
Review of Population Diversity and Variability Incorporated Findings

Forty-eight manuscripts were identified as having potential information on population diversity and variability incorporated. Not surprisingly, the greatest number of hits (14) were found in stem cell studies, followed by 12 on AI/machine learning within in chemico approaches, and 10 each for high-throughput screening and sequencing technology within general methods. Screening the identified manuscripts, it appears most make only a cursory mention of donor-related diversity. For example, X. Gao et al. published a manuscript entitled *Toxicological applications of human induced pluripotent stem cell-derived hepatocyte-like cells: an updated review* (Gao et al., 2023). The manuscript presents data on hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (iPSCs) as in vitro hepatotoxicity models and makes mention of the fact that HLCs maintain their original donor genotype and allow for donor diversity to be studied.

Within the references on AI, Y. Gao et al. published a manuscript entitled A*ddressing the Challenge of Biomedical Data Inequality: An AI Perspective*" (Y. Gao et al., 2023) which focus on issues of population diversity. Central to their manuscript, Gao et al. state "existing biomedical data, which are a vital resource and foundation for developing medical AI models, do not reflect the diversity of the human population. The low representation in biomedical data has become a significant health risk for non-European populations…".

A small number of additional manuscripts, including Pike et al. (2023) and Hnatiuk et al. (2021), discuss donor diversity and its influence on test results in more detail (Hnatiuk et al., 2021; Pike et al., 2023). Pike et al. published a manuscript entitled *Characterization and optimization of variability in a human colonic epithelium culture model.* This manuscript analyzes donor-specific differences and presents the influence of these on test results. Hnatiuk et al. (2021) published a manuscript entitled *Human iPSC modeling of heart disease for drug development.* Hnatiuk et al. state "hiPSCs retain the genetic makeup of their human donor, so they have the potential to recapitulate essential aspects of genetic diseases or mimic drug responses in vitro". Based on this, Hnatiuk et al. suggest several points, including the fact that large numbers of patient-derived hiPSC lines might be needed to infer the effect of a rare variant on a clinical phenotype or drug response, that studies using hiPSC lines may experience confounding effects caused by the donors' genetics, and the use of hiPSC's offers the advantage that there is no limit on the number of different "people" that can be tested. An important take home point from Hnatiuk et al. is that to date, all published large-scale drug screens





have used hiPSCs from a small number of healthy donors and future studies should look to include stem cells from patients with mutations relevant to the disease being studied.

Review of Workforce Considerations Findings

Eleven manuscripts were identified as having potential information on workforce considerations with five of these covering in silico approaches under AI/machine learning. A brief review of abstracts and a subset of the manuscripts revealed limited relevant information and suggests the search strategy to identify references with information on workforce considerations may require revision. While not focused on workforce considerations for NAMs, Miller et al. 2023 published a manuscript entitled *Machine Learning in Clinical Trials: A Primer with Applications to Neurology* (Miller et al., 2023). The authors present ways in which AI and machine learning can be used to facilitate successful clinical trials and discuss technical and regulatory challenges.

Manual Internet Search on Population Diversity and Workforce Considerations

Given the limited material identified using the automated search we conducted a cursory, manual internet search for material on both population diversity or workforce considerations. This search reveals manuscripts that discuss considerations for NAM application (Petersen et al., 2022; Stucki et al., 2022); however, these manuscripts generally appear to have little information on workforce considerations. While an automated search with revised search terms may be helpful, it's possible there is simply a lack of information on workforce considerations with NAMs.

In terms of specific material on population diversity, this quick search did reveal the 2023 SACATM report entitled *Using New Approach Methodologies to Address Variability and Susceptibility Across Populations – Report from the October 2022 Symposium/Workshop* (Hogberg, 2023) which included a call for relevant papers that would be expected to provide useful material on population diversity.

In Depth Exploration of Specific Areas of Interest

Using this landscape analysis as a starting point, there is ample opportunity to retrieve and review current research activity on specific areas of interest. For example, EPA expressed a strong interest in NAMs for breast and prostate cancer during the Complement-ARIE strategic planning process. As an example, we looked for breast cancer manuscripts within the retrieved literature. To accomplish this, the retrieved manuscripts were filtered for the term "breast cancer" within the tag for "disease". This text search produced 112 manuscripts, including 39 manuscripts identified as using AI. A quick manual screen of this subset of manuscripts revealed materials that may be of interest including a recent review by Orsini et al (2023 entitled *Omics Technologies*



Improving Breast Cancer Research and Diagnostics ((Orsini et al., 2023). To prepare their review, Orsini et al screened PubMed and Medline for manuscripts discussing omics research for breast cancer evolution and progression published between the years 2010 and 2023. The authors summarize their findings as follows "This review focuses on the findings of recent multi-omics-based research that has been applied to BC research, with an introduction to every omics technique and their applications for the different BC phenotypes, biomarkers, target therapies, diagnosis, treatment and prognosis, to provide a comprehensive overview of the possibilities of BC research." A thorough review of retrieved literature would likely identify other manuscripts of interest that could be used, in this case, to review the state of the science for breast cancer research but could be applied to numerous other areas of interest.

For on-going or future efforts, the following recommendations/action items are proposed to provide further insight into the current landscape of NAMs for biomedical use and proposals for future programmatic efforts to develop the biomedical NAMs field beyond its current capacity. As described previously, several areas lack robust efforts, particularly with respects to individual variability, workplace effects, etc. Efforts directed toward these areas would be highly recommended.

Potential Future Directions and Opportunities for Additional Analyses

- Conduct more extensive post-processing and curation of the generative AI outputs to confirm quality and establish additional trends in the available data for further investigation.
- Conduct additional in-depth review of generative AI outputs, such as:
    - Summarize common limitations by assay group
    - Summarize most commonly used methods
    - Review coverage of each cell and tissue type within each technology type
- Conduct supplemental literature searches focused on population variability
- Conduct supplemental literature searches focused on the workforces needed for implementation of NAMs.
- Review the full range of studies retrieved to evaluate trends indicative of NAMs increasing (or declining) in the extent to which they are being used in biomedical research.
- Develop an action plan for the implementation of FAIR data practices
- Implement research programs to develop and utilize combinatorial NAM approaches
- Conduct reviews to understand epigenetic reprogramming in iPSC and iPSC-derived organoid models and determine how reflective the epigenetic profile is of a mature in vivo cell.

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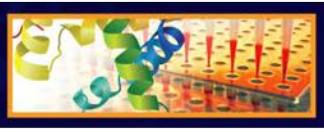
Appendix A. Initial Search Strategy

Table 6. Search Strategy for PubMed*

Set	Topic	Search Strategy for PubMed	Initial Results (No Limits)
1	**NAMs**	"Animal Testing Alternative"[tiab] OR "Animal Testing Alternatives"[tiab] OR "Animal Use Alternative"[tiab] OR "Animal Use Alternatives"[tiab] OR "computational toxicology"[tiab] OR "high content"[tiab] OR "high throughput"[tiab] OR "high-throughput"[tiab] OR "HTS"[tiab] OR "HTTr"[tiab] OR "integrated testing strategies"[tiab] OR "integrated testing strategy"[tiab] OR "NAMs"[tiab] OR 3R[tiab] OR 3Rs[tiab] OR “Animal alternative*”[tiab] OR "assessment batteries"[tiab] OR "assessment battery"[tiab] OR "test batteries"[tiab] OR "test battery"[tiab] OR “Test system*”[tiab] OR ((“alternative*”[tiab] OR “predictive”[tiab] OR “non-animal”[tiab] OR “new approach*”[tiab] OR novel[tiab]) AND (method*[tiab] OR approach*[tiab] OR model*[tiab] OR test*[tiab] OR assay*[tiab]))	1,988,623
2	**In vitro models**	(("3D tissue model"[tiab] OR "3D tissue models"[tiab] OR "biomarker"[tiab] OR "biomarkers"[tiab] OR "embryonic stem cell"[tiab] OR "embryonic stem cells"[tiab] OR "Engineered organoid"[tiab] OR "Engineered organoids"[tiab] OR "Functionally integrate"[tiab] OR "Functionally integrated"[tiab] OR "gene expression"[tiab] OR genomic*[tiab] OR "hESC"[tiab] OR "human embryonic stem cell"[tiab] OR "human embryonic stem cells"[tiab] OR "in vitro"[tiab] OR "induced pluripotent"[tiab] OR "micromass"[tiab] OR "Microfabrication"[tiab] OR Microfluidic*[tiab] OR "Microphysiological Systems"[tiab] OR "Microphysiological System"[tiab] OR microdevice*[tiab] OR "Organ chip"[tiab] OR "Organ chips"[tiab] OR "organ-on-a-chip"[tiab] OR organoid*[tiab] OR "pharmacokinetic"[tiab] OR "Phenotype disappearance"[tiab] OR "Physiological relevance"[tiab] OR "Pluripotent Stem Cell"[tiab] OR "pluripotent stem	3,049,541





Set	Topic	Search Strategy for PubMed	Initial Results (No Limits)
		cells"[tiab] OR "Quantitative systems pharmacology"[tiab] OR "Reporter gene"[tiab] OR "Reporter genes"[tiab] OR "Single cell analysis"[tiab] OR "Stem Cell"[tiab] OR "Stem Cells"[tiab] OR "Tissue-engineered organ construct"[tiab] OR "Tissue-engineered organ constructs"[tiab] OR "Tissue-on-chip"[tiab] OR "Tissue-on-a-chip"[tiab] OR "Tissue chip"[tiab] OR "toxicogenomic"[tiab] OR "toxicogenomics"[tiab] OR "toxicokinetic"[tiab] OR "toxicokinetics"[tiab] OR "transcriptome"[tiab] OR "transcriptomics"[tiab] OR "Vascularization"[tiab]) OR (“Test side effects”[tiab] AND “non-target tissues”[tiab]) OR spheroid*[tiab] OR coculture[tiab] OR “co-culture”[tiab] OR “cell culture”[tiab] OR “primary culture”[tiab])	
3	**In silico models**	(Algorithm*[tiab] OR bioinformat*[tiab] OR "Chemical space"[tiab] OR "Chemical space"[tiab] OR "Chemical spaces"[tiab] OR "Chemical structure"[tiab] OR "Chemical structures"[tiab] OR cheminformat*[tiab] OR "Chemogenomic"[tiab] OR "Chemogenomics"[tiab] OR "Computational model"[tiab] OR "Computational models"[tiab] OR "computational study"[tiab] OR "computational analysis"[tiab] OR “computer model”[tiab] OR "mathematical models"[tiab] OR "mathematical model"[tiab] OR Simulation*[tiab] OR "Deep learning"[tiab] OR "Disease database"[tiab] OR "Disease databases"[tiab] OR "Docking software"[tiab] OR "Drug database"[tiab] OR "Drug databases"[tiab] OR "in silico"[tiab] OR "In vitro to in vivo extrapolation"[tiab] OR "informatics"[tiab] OR "IVIVE"[tiab] OR "Machine learning"[tiab] OR “artificial intelligence”[tiab] OR "Molecular Docking"[tiab] OR "Molecular Dynamics"[tiab] OR "PBPK"[tiab] OR "Pharmacokinetic"[tiab] OR "Pharmacodynamic"[tiab] OR "toxicokinetic"[tiab] OR "toxicodynamic"[tiab] OR "Pharmacology database"[tiab] OR "Pharmacology databases"[tiab] OR "Protein space"[tiab] OR "QSAR"[tiab] OR "quantitative structure activity relationship"[tiab] OR "Read-across"[tiab] OR "Small molecule database"[tiab]	1,326,522

Set	Topic	Search Strategy for PubMed	Initial Results (No Limits)
		OR "Small molecule databases"[tiab] OR "Structural database"[tiab] OR "Structural databases"[tiab] OR "structure activity relationship"[tiab] OR “Digital Twins”[tiab] OR “multiscale models”[tiab])	
4	**In chemico models**	("In chemico"[tiab] OR "Virtual tissue model"[tiab] OR "Virtual tissue models"[tiab] OR "Exposure model"[tiab] OR "Exposure models"[tiab] OR "Cell-free assays"[tiab] OR "Cell-free assay"[tiab] OR "Synthetic biochemistry"[tiab] OR "Receptor binding"[tiab] OR "Synthetic biology"[tiab] OR "Ligand binding"[tiab] OR “biochemical assay”[tiab])	98,383
	Other limits	Reviews: ((english[Filter] AND 2018:2024[pdat] AND hasabstract AND (Review[pt] OR Systematic Review[pt]))	

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Appendix B. Title/Abstract Screening and Extraction

Overall Methods

Each reference was reviewed and screened for relevance by a primary staff person who noted high level information about each reference (see instructions, below). A study was considered relevant if it met the inclusion criteria outlined below. Each reference was also reviewed by a second person for QA purposes, to confirm the original categorization and extraction was accurate.

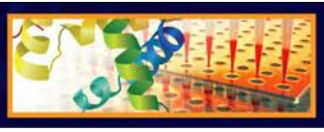
Inclusion Criteria – if any of the following criteria were met, it was considered relevant

- Contains information related to the development or application of a NAM for a human-relevant endpoint or biological process
- Could putatively be a replacement for an in vivo animal test
- NAM must not rely solely on animal tissue or data
    - For in vitro and in chemico studies, should be exclusively human
    - For in silico models, it is relevant if it was built using animal data if it is predicting a human relevant outcome
- Data are collected from a relevant model system/using a relevant technology

Exclusion Criteria

- The study does not describe an alternative test method/model that meets the requirements above, where the context is related to recapitulating a relevant human physiological process or increasing our understanding of a biological process
- Model or approach could not be used in a biomedical context
- Model or approach makes use of whole organisms (including mice, humans, whole embryos)
- Model is designed for application in an ecological context (making decisions about health of organisms in the environment, not humans)
- Diagnostic method related to diagnosing outcomes in humans
- Replacing a method that already does not use animals (e.g., micromass culture derived from a chick embryo, mouse cell culture lines)
- Biomedical devices
- Data collected for the purpose of studying plants, pathogenic bacteria, microorganisms
- Other exclusion criteria:
    - Secondary literature (letters to the editor, notes, meeting abstract, etc.)
    - Not in English
    - Retracted publication





Supplemental Categories

FAIR: Discussion of data availability, any FAIR concept, database or other repository

Animal-based NAM: NAM but animal-based (using animal tissue – whole organisms are excluded)

Potential application in biomedical research: Catch-all bin for when the paper has the potential for being relevant to a biomedical context and replacing animal tests, but the authors do not necessarily make that point explicitly

Instructions for Litstream® form

Copy-Paste from the abstract wherever possible

Type of model *[multi select]*

In vitro
In chemico
In silico
General method

Use the multitool to select the correct system(s). A given abstract might discuss more than one.

Method details *[free text]*

Copy-paste straight from abstract a description of the method (the information that indicated which type of model you selected above); if you selected more than one model type, include information for all of them.

Endpoint details (e.g., tissue type) *[free text]*

(if available) copy-paste straight from abstract a description of the endpoint of interest (what are they evaluating using the specific technology/method?)

Context/application *[free text]*

Brief description of the big picture of the paper; this can be copied from abstract directly if available
What question are the authors answering? Why did they write the review?

Supplemental

Relevance to the reduction of animals *[checklist]*

FAIR
Animal-based NAM
Potential application in biomedical research

- *A given paper can be both include and supplemental*
- *Select all relevant tags*

Description *[free text]*

Include database info, method details

Include details related to the specific supplemental category selected: for FAIR, copy-paste the name of the system; if animal-based NAM, include details on the NAM method; if potential application, include the details that make you think it should be in that bin

Exclude

☐ This study has no relevance to the replacement of animals in biomedical research

Select for excluded papers – should not be checked off if anything is included in Include, Supplemental, or Unclear

☐ Unclear how to categorize this study

Use when you have spent a few min reviewing the abstract and do not know how to classify it to pass it to a more senior person

Include any other details

Use this field as a catch-all for anything relevant not otherwise captured, including rationale for exclusion or unclear (provide the QC'r with a bit of information about what you are confused by)

National Institutes of Health
Office of Strategic Coordination–The Common Fund

Appendix C. Broad Search Strategy

Table 7. Search Strategy for PubMed*

Set	Topic	Search Strategy for PubMed	Initial Results (No Limits)
1	**NAMs**	"Animal Testing Alternative"[tiab] OR "Animal Testing Alternatives"[tiab] OR "Animal Use Alternative"[tiab] OR "Animal Use Alternatives"[tiab] OR "computational toxicology"[tiab] OR "high content"[tiab] OR "high throughput"[tiab] OR "high-throughput"[tiab] OR "HTS"[tiab] OR "HTTr"[tiab] OR "integrated testing strategies"[tiab] OR "integrated testing strategy"[tiab] OR "NAMs"[tiab] OR 3R[tiab] OR 3Rs[tiab] OR “Animal alternative*”[tiab] OR "assessment batteries"[tiab] OR "assessment battery"[tiab] OR "test batteries"[tiab] OR "test battery"[tiab] OR “Test system*”[tiab] OR ((“alternative*”[tiab] OR “predictive”[tiab] OR “non-animal”[tiab] OR “new approach*”[tiab] OR novel[tiab]) AND (method*[tiab] OR approach*[tiab] OR model*[tiab] OR test*[tiab] OR assay*[tiab]))	1,988,623
2	**In vitro models**	(("3D tissue model"[tiab] OR "3D tissue models"[tiab] OR "biomarker"[tiab] OR "biomarkers"[tiab] OR "embryonic stem cell"[tiab] OR "embryonic stem cells"[tiab] OR "Engineered organoid"[tiab] OR "Engineered organoids"[tiab] OR "Functionally integrate"[tiab] OR "Functionally integrated"[tiab] OR "gene expression"[tiab] OR genomic*[tiab] OR "hESC"[tiab] OR "human embryonic stem cell"[tiab] OR "human embryonic stem cells"[tiab] OR "in vitro"[tiab] OR "induced pluripotent"[tiab] OR "micromass"[tiab] OR "Microfabrication"[tiab] OR Microfluidic*[tiab] OR "Microphysiological Systems"[tiab] OR "Microphysiological System"[tiab] OR microdevice*[tiab] OR "Organ chip"[tiab] OR "Organ chips"[tiab] OR "organ-on-a-chip"[tiab] OR organoid*[tiab] OR "pharmacokinetic"[tiab] OR "Phenotype disappearance"[tiab] OR "Physiological relevance"[tiab] OR "Pluripotent Stem Cell"[tiab] OR "pluripotent stem cells"[tiab] OR "Quantitative systems pharmacology"[tiab] OR "Reporter gene"[tiab] OR "Reporter genes"[tiab] OR	3,049,541

Set	Topic	Search Strategy for PubMed	Initial Results (No Limits)
		"Single cell analysis"[tiab] OR "Stem Cell"[tiab] OR "Stem Cells"[tiab] OR "Tissue-engineered organ construct"[tiab] OR "Tissue-engineered organ constructs"[tiab] OR "Tissue-on-chip"[tiab] OR "Tissue-on-a-chip"[tiab] OR "Tissue chip"[tiab] OR "toxicogenomic"[tiab] OR "toxicogenomics"[tiab] OR "toxicokinetic"[tiab] OR "toxicokinetics"[tiab] OR "transcriptome"[tiab] OR "transcriptomics"[tiab] OR "Vascularization"[tiab]) OR (“Test side effects”[tiab] AND “non-target tissues”[tiab]) OR spheroid*[tiab] OR coculture[tiab] OR “co-culture”[tiab] OR “cell culture”[tiab] OR “primary culture”[tiab])	
3	**In silico models**	(Algorithm*[tiab] OR bioinformat*[tiab] OR "Chemical space"[tiab] OR "Chemical space"[tiab] OR "Chemical spaces"[tiab] OR "Chemical structure"[tiab] OR "Chemical structures"[tiab] OR cheminformat*[tiab] OR "Chemogenomic"[tiab] OR "Chemogenomics"[tiab] OR "Computational model"[tiab] OR "Computational models"[tiab] OR "computational study"[tiab] OR "computational analysis"[tiab] OR “computer model”[tiab] OR "mathematical models"[tiab] OR "mathematical model"[tiab] OR Simulation*[tiab] OR "Deep learning"[tiab] OR "Disease database"[tiab] OR "Disease databases"[tiab] OR "Docking software"[tiab] OR "Drug database"[tiab] OR "Drug databases"[tiab] OR "in silico"[tiab] OR "In vitro to in vivo extrapolation"[tiab] OR "informatics"[tiab] OR "IVIVE"[tiab] OR "Machine learning"[tiab] OR “artificial intelligence”[tiab] OR "Molecular Docking"[tiab] OR "Molecular Dynamics"[tiab] OR "PBPK"[tiab] OR "Pharmacokinetic"[tiab] OR "Pharmacodynamic"[tiab] OR "toxicokinetic"[tiab] OR "toxicodynamic"[tiab] OR "Pharmacology database"[tiab] OR "Pharmacology databases"[tiab] OR "Protein space"[tiab] OR "QSAR"[tiab] OR "quantitative structure activity relationship"[tiab] OR "Read-across"[tiab] OR "Small molecule database"[tiab] OR "Small molecule databases"[tiab] OR "Structural database"[tiab] OR	1,326,522

Set	Topic	Search Strategy for PubMed	Initial Results (No Limits)
		"Structural databases"[tiab] OR "structure activity relationship"[tiab] OR “Digital Twins”[tiab] OR “multiscale models”[tiab])	
4	**In chemico models**	("In chemico"[tiab] OR "Virtual tissue model"[tiab] OR "Virtual tissue models"[tiab] OR "Exposure model"[tiab] OR "Exposure models"[tiab] OR "Cell-free assays"[tiab] OR "Cell-free assay"[tiab] OR "Synthetic biochemistry"[tiab] OR "Receptor binding"[tiab] OR "Synthetic biology"[tiab] OR "Ligand binding"[tiab] OR “biochemical assay”[tiab])	98,383
	Other limits	Reviews: ((english[Filter] AND 2023:2024[pdat] AND hasabstract AND (Review[pt] OR Systematic Review[pt]))	

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Table 8. Search Strategy for Web of Science*

Set	Topic	Search Strategy for Web of Science	Initial Results (No Limits)
1	**NAMs**	"Animal Testing Alternative" OR "Animal Testing Alternatives" OR "Animal Use Alternative" OR "Animal Use Alternatives" OR "computational toxicology" OR "high content" OR "high throughput" OR "high-throughput" OR "HTS" OR "HTTr" OR "integrated testing strategies" OR "integrated testing strategy" OR "NAMs" OR 3R OR 3Rs OR “Animal alternative*” OR "assessment batteries" OR "assessment battery" OR "test batteries" OR "test battery" OR “Test system*” OR ((“alternative*” OR “predictive” OR “non-animal” OR “new approach*” OR novel) AND (method* OR approach* OR model* OR test* OR assay*))	3,972,818
2	**In vitro models**	(("3D tissue model" OR "3D tissue models" OR "biomarker" OR "biomarkers" OR "embryonic stem cell" OR "embryonic stem cells" OR "Engineered organoid" OR "Engineered organoids" OR "Functionally integrate" OR "Functionally integrated" OR "gene expression" OR genomic* OR "hESC" OR "human embryonic stem cell"	4,161,732

Set	Topic	Search Strategy for Web of Science	Initial Results (No Limits)
		OR "human embryonic stem cells" OR "in vitro" OR "induced pluripotent" OR "micromass" OR "Microfabrication" OR Microfluidic* OR "Microphysiological Systems" OR "Microphysiological System" OR microdevice* OR "Organ chip" OR "Organ chips" OR "organ-on-a-chip" OR organoid* OR "pharmacokinetic" OR "Phenotype disappearance" OR "Physiological relevance" OR "Pluripotent Stem Cell" OR "pluripotent stem cells" OR "Quantitative systems pharmacology" OR "Reporter gene" OR "Reporter genes" OR "Single cell analysis" OR "Stem Cell" OR "Stem Cells" OR "Tissue-engineered organ construct" OR "Tissue-engineered organ constructs" OR "Tissue-on-chip" OR "Tissue-on-a-chip" OR "Tissue chip" OR "toxicogenomic" OR "toxicogenomics" OR "toxicokinetic" OR "toxicokinetics" OR "transcriptome" OR "transcriptomics" OR "Vascularization") OR (“Test side effects” AND “non-target tissues”) OR spheroid* OR coculture OR “co-culture” OR “cell culture” OR “primary culture”)	
3	**In silico models**	(Algorithm* OR bioinformat* OR "Chemical space" OR "Chemical spaces" OR "Chemical structure" OR "Chemical structures" OR cheminformat* OR "Chemogenomic" OR "Chemogenomics" OR "Computational model" OR "Computational models" OR "computational study" OR "computational analysis" OR “computer model” OR "mathematical models" OR "mathematical model" OR Simulation* OR "Deep learning" OR "Disease database" OR "Disease databases" OR "Docking software" OR "Drug database" OR "Drug databases" OR "in silico" OR "In vitro to in vivo extrapolation" OR "informatics" OR "IVIVE" OR "Machine learning" OR “artificial intelligence” OR "Molecular Docking" OR "Molecular Dynamics" OR "PBPK" OR "Pharmacokinetic" OR "Pharmacodynamic" OR "toxicokinetic" OR "toxicodynamic" OR "Pharmacology database" OR "Pharmacology databases" OR "Protein Aspace" OR "QSAR" OR "quantitative structure activity	6,710,316

Set	Topic	Search Strategy for Web of Science	Initial Results (No Limits)
		relationship" OR "Read-across" OR "Small molecule database" OR "Small molecule databases" OR "Structural database" OR "Structural databases" OR "structure activity relationship" OR “Digital Twins”)	
4	**In chemico models**	("In chemico" OR "Virtual tissue model" OR "Virtual tissue models" OR "Exposure model" OR "Exposure models" OR "Cell-free assays" OR "Cell-free assay" OR "Synthetic biochemistry" OR "Receptor binding" OR "Synthetic biology" OR "Ligand binding" OR “biochemical assay”)	128,827
	Other limits	2023 or 2024 (Publication Years) and Article or Review Article (Document Types) and English (Languages) and Biochemistry Molecular Biology or Materials Science Multidisciplinary or Oncology or Pharmacology Pharmacy or Medicine Research Experimental or Genetics Heredity or Cell Biology or Neurosciences or Mathematical Computational Biology or Biochemical Research Methods or Biology or Materials Science Biomaterials or Toxicology or Respiratory System or Cell Tissue Engineering or Reproductive Biology or Materials Science Composites or Developmental Biology (Web of Science Categories)	

**Ran on October 23, 2023 using https://www.webofscience.com/wos*

Table 9. Search Strategy for Scopus*

Set	Topic	Search Strategy for Scopus	Initial Results (No Limits)
1	**NAMs**	TITLE-ABS ("Animal Testing Alternative" OR "Animal Testing Alternatives" OR "Animal Use Alternative" OR "Animal Use Alternatives" OR "computational toxicology" OR "high content" OR "high throughput" OR "high-throughput" OR "HTS" OR "HTTr" OR "integrated testing strategies" OR "integrated testing strategy" OR "NAMs" OR 3r OR 3rs OR "Animal alternative*" OR "assessment batteries" OR "assessment battery" OR "test batteries" OR	4,753,857

Set	Topic	Search Strategy for Scopus	Initial Results (No Limits)
		"test battery" OR "Test system*" OR (("alternative*" OR "predictive" OR "non-animal" OR "new approach*" OR novel) AND (method* OR approach* OR model* OR test* OR assay*)))	
2	**In vitro models**	TITLE-ABS ("3D tissue model" OR "3D tissue models" OR "biomarker" OR "biomarkers" OR "embryonic stem cell" OR "embryonic stem cells" OR "Engineered organoid" OR "Engineered organoids" OR "Functionally integrate" OR "Functionally integrated" OR "gene expression" OR genomic* OR "hESC" OR "human embryonic stem cell" OR "human embryonic stem cells" OR "in vitro" OR "induced pluripotent" OR "micromass" OR "Microfabrication" OR microfluidic* OR "Microphysiological Systems" OR "Microphysiological System" OR microdevice* OR "Organ chip" OR "Organ chips" OR "organ-on-a-chip" OR organoid* OR "pharmacokinetic" OR "Phenotype disappearance" OR "Physiological relevance" OR "Pluripotent Stem Cell" OR "pluripotent stem cells" OR "Quantitative systems pharmacology" OR "Reporter gene" OR "Reporter genes" OR "Single cell analysis" OR "Stem Cell" OR "Stem Cells" OR "Tissue-engineered organ construct" OR "Tissue-engineered organ constructs" OR "Tissue-on-chip" OR "Tissue-on-a-chip" OR "Tissue chip" OR "toxicogenomic" OR "toxicogenomics" OR "toxicokinetic" OR "toxicokinetics" OR "transcriptome" OR "transcriptomics" OR "Vascularization") OR ("Test side effects" AND "non-target tissues") OR spheroid* OR coculture OR "co-culture" OR "cell culture" OR "primary culture"	4,955,213
3	**In silico models**	TITLE-ABS (algorithm* OR bioinformat* OR "Chemical space" OR "Chemical space" OR "Chemical spaces" OR "Chemical structure" OR "Chemical structures" OR cheminformat* OR "Chemogenomic" OR "Chemogenomics" OR "Computational model" OR "Computational models" OR "computational study" OR "computational analysis" OR "computer model" OR	8,434,230

Set	Topic	Search Strategy for Scopus	Initial Results (No Limits)
		"mathematical models" OR "mathematical model" OR simulation* OR "Deep learning" OR "Disease database" OR "Disease databases" OR "Docking software" OR "Drug database" OR "Drug databases" OR "in silico" OR "In vitro to in vivo extrapolation" OR "informatics" OR "IVIVE" OR "Machine learning" OR "artificial intelligence" OR "Molecular Docking" OR "Molecular Dynamics" OR "PBPK" OR "Pharmacokinetic" OR "Pharmacodynamic" OR "toxicokinetic" OR "toxicodynamic" OR "Pharmacology database" OR "Pharmacology databases" OR "Protein space" OR "QSAR" OR "quantitative structure activity relationship" OR "Read-across" OR "Small molecule database" OR "Small molecule databases" OR "Structural database" OR "Structural databases" OR "structure activity relationship" OR "Digital Twins")	
4	**In chemico models**	TITLE-ABS ("In chemico" OR "Virtual tissue model" OR "Virtual tissue models" OR "Exposure model" OR "Exposure models" OR "Cell-free assays" OR "Cell-free assay" OR "Synthetic biochemistry" OR "Receptor binding" OR "Synthetic biology" OR "Ligand binding" OR "biochemical assay")	119,284
	Other limits	Subject areas: Pharmacology, Toxicology and Pharmaceutics; Biochemistry, Genetics and Molecular Biology 22,365 and Multidisciplinary 2,740, Immunology and Microbiology 5,294 PUBYEAR > 2022 AND PUBYEAR < 2025 AND (LIMIT-TO (LANGUAGE , "English"))	

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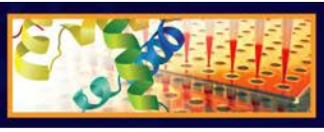


Appendix D. Reference List

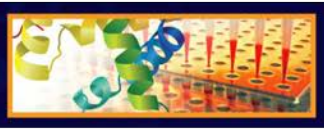
Note: The reference list is provided separately for this project. (See third tab of Tableau dashboard “Reference List”: Complement-ARIE Landscape Analysis).

Appendix E. Keyword List

Table 10. Keyword List

Model Type	Category	Keyword
0_in vitro	2D cell culture	2D
0_in vitro	2D cell culture	2D cell culture
0_in vitro	2D cell culture	2D in vitro cell culture
0_in vitro	2D cell culture	2D traditional cell cultures
0_in vitro	2D cell culture	cell culture
0_in vitro	2D cell culture	cell cultures
0_in vitro	2D cell culture	cell line
0_in vitro	2D cell culture	cell lines
0_in vitro	2D cell culture	immortalized cell line
0_in vitro	2D cell culture	immortalized cell lines
0_in vitro	3D cell culture	3D
0_in vitro	3D cell culture	3D cell culture
0_in vitro	3D cell culture	3D cell cultures
0_in vitro	3D cell culture	colonoid
0_in vitro	3D cell culture	embryo body
0_in vitro	3D cell culture	embryoid
0_in vitro	3D cell culture	organoid
0_in vitro	3D cell culture	Organoids
0_in vitro	3D cell culture	spheroid
0_in vitro	3D cell culture	Spheroids
0_in vitro	Bioprinting	biofabrication
0_in vitro	Bioprinting	bioink
0_in vitro	Bioprinting	bioprint
0_in vitro	Bioprinting	Bioprinting
0_in vitro	Bioprinting	tissue engineering
0_in vitro	Bioprinting	tissue scaffold
0_in vitro	Co-culture	coculture
0_in vitro	Co-culture	Co-culture
0_in vitro	Co-culture	cocultures
0_in vitro	Co-culture	co-cultures
0_in vitro	Donor-derived high throughput culture panels	Donor Derived Cell Panels
0_in vitro	Donor-derived high throughput culture panels	Donor derived high throughput cultures
0_in vitro	Donor-derived high throughput culture panels	Donor derived panel



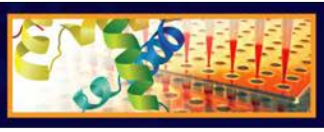


Model Type	Category	Keyword
0_in vitro	Donor-derived high throughput culture panels	donor panel
0_in vitro	Donor-derived high throughput culture panels	donor-derived
0_in vitro	Explant	ex vivo
0_in vitro	Explant	explant
0_in vitro	Explant	explants
0_in vitro	Explant	Tissue slice
0_in vitro	Microphysiological systems	chip
0_in vitro	Microphysiological systems	microfluidic
0_in vitro	Microphysiological systems	microfluidics
0_in vitro	Microphysiological systems	microphysiologic
0_in vitro	Microphysiological systems	microphysiologic system
0_in vitro	Microphysiological systems	microphysiologic systems
0_in vitro	Microphysiological systems	Microphysiological
0_in vitro	Microphysiological systems	Microphysiological system
0_in vitro	Microphysiological systems	Microphysiological systems
0_in vitro	Microphysiological systems	on a chip
0_in vitro	Microphysiological systems	on chip
0_in vitro	Microphysiological systems	organ chip
0_in vitro	Microphysiological systems	organ on a chip
0_in vitro	Microphysiological systems	tissue chip
0_in vitro	Microphysiological systems	tissue on a chip
0_in vitro	stem cell	adult stem cell
0_in vitro	stem cell	adult stem cells
0_in vitro	stem cell	differentiation
0_in vitro	stem cell	embryonic stem cells
0_in vitro	stem cell	hipscs
0_in vitro	stem cell	human induced pluripotent stem cell
0_in vitro	stem cell	human induced pluripotent stem cells
0_in vitro	stem cell	human pluripotent stem cell
0_in vitro	stem cell	human pluripotent stem cells
0_in vitro	stem cell	induced pluripotent stem cell
0_in vitro	stem cell	induced pluripotent stem cells
0_in vitro	stem cell	ipsc
0_in vitro	stem cell	ipscs
0_in vitro	Stem Cell	mixed culture



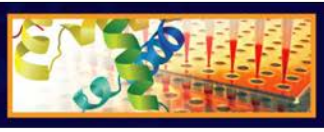
Model Type	Category	Keyword
0_in vitro	stem cell	pluripotent
0_in vitro	stem cell	Stem Cell
0_in vitro	stem cell	Stem Cells
1_In chemico	Biochemical Assays	biochemical
1_In chemico	Biochemical Assays	biochemical assay
1_In chemico	Biochemical Assays	Biochemical Assays
1_In chemico	Biochemical Assays	enzyme
1_In chemico	Biochemical Assays	G protein-coupled receptors
1_In chemico	Biochemical Assays	GPCR
1_In chemico	Biochemical Assays	Ion Channels
1_In chemico	Biochemical Assays	lipid bilayer
1_In chemico	Cell-free assay	area under curve
1_In chemico	Cell-free assay	area under the curve
1_In chemico	Cell-free assay	AUC
1_In chemico	Cell-free assay	cell free
1_In chemico	Cell-free assay	cell membrane
1_In chemico	Cell-free assay	Cell-free assay
1_In chemico	Cell-free assay	conformation
1_In chemico	Cell-free assay	membrane protein
1_In chemico	Cell-free assay	micelle
1_In chemico	Cell-free assay	microsome
1_In chemico	Cell-free assay	transport assay
1_In chemico	Ligand-receptor binding assay	ligand binding
1_In chemico	Ligand-receptor binding assay	ligand binding assay
1_In chemico	Ligand-receptor binding assay	ligand binding assays
1_In chemico	Ligand-receptor binding assay	Ligand receptor binding assay
1_In chemico	Ligand-receptor binding assay	receptor binding
1_In chemico	Ligand-receptor binding assay	receptor binding assay
1_In chemico	Ligand-receptor binding assay	receptor binding assays
1_In chemico	structure/identity analysis	chromatography
1_In chemico	structure/identity analysis	CryoEM
1_In chemico	structure/identity analysis	crystal structure
1_In chemico	structure/identity analysis	Fourier transform infrared
1_In chemico	structure/identity analysis	ft _ ir
1_In chemico	structure/identity analysis	Gas Chromatography Mass Spectrometry
1_In chemico	structure/identity analysis	GC MS





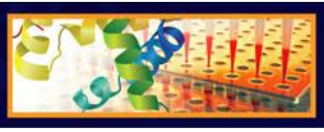
Model Type	Category	Keyword
1_In chemico	structure/identity analysis	high performance liquid chromatography
1_In chemico	structure/identity analysis	HPLC
1_In chemico	structure/identity analysis	infrared spectroscopy
1_In chemico	structure/identity analysis	LC MS
1_In chemico	structure/identity analysis	Liquid Chromatography Mass Spectrometry
1_In chemico	structure/identity analysis	Mass Spectrometry
1_In chemico	structure/identity analysis	NMR
1_In chemico	structure/identity analysis	NMR structure
1_In chemico	structure/identity analysis	Nuclear magnetic resonance
1_In chemico	structure/identity analysis	Raman
1_In chemico	structure/identity analysis	Spectroscopies
1_In chemico	structure/identity analysis	Spectroscopy
1_In chemico	structure/identity analysis	SPR
1_In chemico	structure/identity analysis	Surface Plasmon Resonance
1_In chemico	structure/identity analysis	x ray crystallography
1_In chemico	structure/identity analysis	X ray structure
1_In chemico	Synthetic organelles/cells	synesthetic cell
1_In chemico	Synthetic organelles/cells	synesthetic membranes
1_In chemico	Synthetic organelles/cells	synthetic cell membrane
1_In chemico	Synthetic organelles/cells	synthetic membrane
1_In chemico	Synthetic organelles/cells	synthetic mitochondria
1_In chemico	Synthetic organelles/cells	synthetic mitochondrias
1_In chemico	Synthetic organelles/cells	synthetic nucleus
1_In chemico	Synthetic organelles/cells	synthetic organelle
1_In chemico	Synthetic organelles/cells	synthetic organelles / cells
2_In silico	ADME	Absorption
2_In silico	ADME	ADME
2_In silico	ADME	Distribution
2_In silico	ADME	Excretion
2_In silico	ADME	kinetics
2_In silico	ADME	Metabolism
2_In silico	Artificial Intelligence/Machine Learning	algorithm
2_In silico	Artificial Intelligence/Machine Learning	artificial intelligence
2_In silico	Artificial Intelligence/Machine Learning	computational





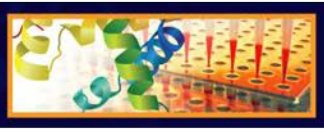
Model Type	Category	Keyword
2_In silico	Artificial Intelligence/Machine Learning	deep learning
2_In silico	Artificial Intelligence/Machine Learning	GAN
2_In silico	Artificial Intelligence/Machine Learning	Generative adversarial networks
2_In silico	Artificial Intelligence/Machine Learning	Generative AI
2_In silico	Artificial Intelligence/Machine Learning	high performance computation
2_In silico	Artificial Intelligence/Machine Learning	Large language model
2_In silico	Artificial Intelligence/Machine Learning	LLM
2_In silico	Artificial Intelligence/Machine Learning	machine learning
2_In silico	Artificial Intelligence/Machine Learning	Neural networks
2_In silico	Bayesian Statistics	advanced sampling
2_In silico	Bayesian Statistics	Bayesian
2_In silico	Bayesian Statistics	Bayesian Statistics
2_In silico	Bayesian Statistics	metadynamics
2_In silico	Bayesian Statistics	umbrella sampling
2_In silico	Bioinformatic processing	bioinformatic
2_In silico	Bioinformatic processing	Bioinformatic processing
2_In silico	Bioinformatic processing	bioinformatics
2_In silico	Bioinformatic processing	force field
2_In silico	Cheminformatics	cheminformatic
2_In silico	Cheminformatics	Cheminformatics
2_In silico	Cheminformatics	chemogenomic
2_In silico	Cheminformatics	Chemogenomics
2_In silico	Digital Twin	Digital Twin
2_In silico	Molecular simulation	binding affinity
2_In silico	Molecular simulation	Coarse grained simulation
2_In silico	Molecular simulation	free energy perturbation
2_In silico	Molecular simulation	GROMACS
2_In silico	Molecular simulation	molecular docking
2_In silico	Molecular simulation	molecular dynamic simulations
2_In silico	Molecular simulation	molecular dynamics
2_In silico	Molecular simulation	Molecular simulation





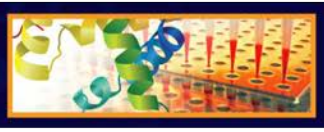
Model Type	Category	Keyword
2_In silico	Molecular simulation	NAMD
2_In silico	Molecular simulation	optimized structure
2_In silico	Molecular simulation	quantum mechanical calculation
2_In silico	Molecular simulation	RMSD
2_In silico	Molecular simulation	scalable molecular dynamics
2_In silico	Molecular simulation	virtual screening
2_In silico	Molecular simulation	visual molecular dynamics
2_In silico	Molecular simulation	VMD
2_In silico	Pathways analysis	Adverse Outcome Pathway
2_In silico	Pathways analysis	AOP
2_In silico	Pathways analysis	Connectivity Map
2_In silico	Pathways analysis	Dynamic modeling
2_In silico	Pathways analysis	IATA
2_In silico	Pathways analysis	integrated approaches to testing and assessment
2_In silico	Pathways analysis	Kinetic modeling
2_In silico	Pathways analysis	Pathways analysis
2_In silico	Pathways analysis	Systems biology
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	In vitro to in vivo extrapolation
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	ivive
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	PB / PK
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	PBPK
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	PBPK model
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	pharmacodynamic
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	pharmacokinetic
2_In silico	Pharmacokinetic/Pharmacodynamic modeling	physiologically based
2_In silico	QSAR	Chemical structure
2_In silico	QSAR	QSAR
2_In silico	QSAR	quantitative structure activity relationship
2_In silico	QSAR	structure activity relationship
2_In silico	Read Across	Read Across





Model Type	Category	Keyword
3_General Methods	Biochemical Assays	Förster resonance energy transfer
3_General Methods	Biochemical Assays	FRET
3_General Methods	CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
3_General Methods	CRISPR	CRISPR
3_General Methods	Droplet-based assays	droplet based assay
3_General Methods	Droplet-based assays	Droplet based assays
3_General Methods	Epigenomics	epigenome
3_General Methods	Epigenomics	epigenomic
3_General Methods	Epigenomics	epigenomics
3_General Methods	High-throughput screening	cell painting
3_General Methods	High-throughput screening	High throughput screening
3_General Methods	High-throughput screening	multi omics
3_General Methods	High-throughput screening	omics
3_General Methods	Imaging	image based
3_General Methods	Imaging	imaging
3_General Methods	Imaging	Live cell imaging
3_General Methods	Metabolomics	metabolome
3_General Methods	Metabolomics	metabolomic
3_General Methods	Metabolomics	Metabolomics
3_General Methods	Microbiome	microbiome
3_General Methods	Proteomics	proteome





Model Type	Category	Keyword
3_General Methods	Proteomics	proteomic
3_General Methods	Proteomics	Proteomics
3_General Methods	Sequencing Technology	DNA Seq
3_General Methods	Sequencing Technology	genomic
3_General Methods	Sequencing Technology	genomics
3_General Methods	Sequencing Technology	miRNA Seq
3_General Methods	Sequencing Technology	Next Generation Sequencing
3_General Methods	Sequencing Technology	NGS
3_General Methods	Sequencing Technology	oxford nanopore
3_General Methods	Sequencing Technology	RNA Seq
3_General Methods	Sequencing Technology	second generation sequencing
3_General Methods	Sequencing Technology	Sequencing Technology
3_General Methods	Sequencing Technology	third generation sequencing
3_General Methods	Single Cell Assays	single cell assay
3_General Methods	Single Cell Assays	Single Cell Assays
3_General Methods	Single Cell Assays	single cell imaging
3_General Methods	Single Cell Assays	single cell sequencing
3_General Methods	Single Cell Assays	single cell sorting
3_General Methods	Transcriptomics	transcriptome
3_General Methods	Transcriptomics	transcriptomic
3_General Methods	Transcriptomics	Transcriptomics

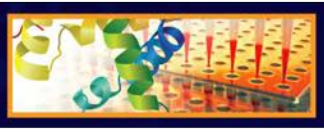
Appendix F. Prompts Used in Generative AI Approach

Generative AI Summary Questions

The following is text from an article: <json here>
Please act as a biomedical researcher exploring alternatives to animal testing. Wrap each response in an XML tag based on the prefix 'response' concatenated with the question number. Replace '&' characters with '&' in your responses. Replace '<' characters with '<' in your responses. Replace '>' characters with '>' in your responses.

1. What is the title of this paper?
1. Give a brief response to the question 'What are the details on the funding of this research, including any grants or sponsors?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
2. Give a brief response to the question 'Provide details on the method described.'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
3. Give a brief response to the question 'Is the method novel (new technology) or is it an improvement on an existing approach (refinement)?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
4. Give a brief response to the question 'What were the main technical challenges described in the paper that this method was trying to overcome?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
5. Give a brief response to the question 'Did the authors discuss the method in terms of a regulatory use? If so, what did the authors say?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
6. Give a brief response to the question 'Did the authors discuss ethical considerations? If so, what did they say?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
7. Give a brief response to the question 'Did the authors discuss workforce considerations? If so, what did they say?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
8. Give a brief response to the question 'Did the authors discuss economic considerations? If so, what did they say?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
9. Give a brief response to the question 'Did the authors incorporate considerations of population diversity or interindividual variability into the technology? If so, how?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
10. Give a brief response to the question 'Did this paper model outcomes in a specific population? If so, what population?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'





11. Give a brief response to the question 'What tissue types does this paper examine?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
12. Give a brief response to the question 'What cell types does this paper examine?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
13. Give a brief response to the question 'What elements of human physiology does this paper consider?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
14. Give a brief response to the question 'Is a disease examined in this paper? If so what disease?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
15. Give a brief response to the question 'Are cellular endpoints being measured in this paper? If so, what are the endpoints?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
16. Give a brief response to the question 'Did this model seek to address metabolic capacity of the tissue? If so, in what way?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
17. Give a brief response to the question 'Did the authors describe the validation methods, such as the use of controls, taken for this method? If so, what are they?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
18. Give a brief response to the question 'What are the limitations of the method as described in this paper?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'
19. Give a brief response to the question 'What challenges remain even after the development of this method?'. Justify your answer with direct quotes. If you can't tell, say 'I don't know'

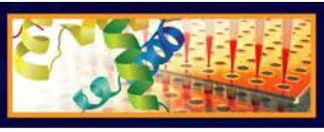
Entity Extraction Questions

For cell types: In the attached CSV extract the cell type entities or cell type(s) in the "cell.type" column and produce a tab delimited output with the extracted values separated by commas in a column adjacent to the "cite" column. Please do not output any other columns from the original file. Additionally, please iterate through every row in the CSV so each row has an output.

For tissue types: In the attached CSV extract the tissue entities or tissue type(s) in the "tissue" column and produce a tab delimited output with the extracted values separated by commas in a column adjacent to the "cite" column. Please do not output any other columns from the original file. Additionally, please iterate through every row in the CSV so each row has an output.

For diseases: In the attached CSV extract the disease entities or disease(s) in the "disease" column and produce a tab delimited output with the extracted values

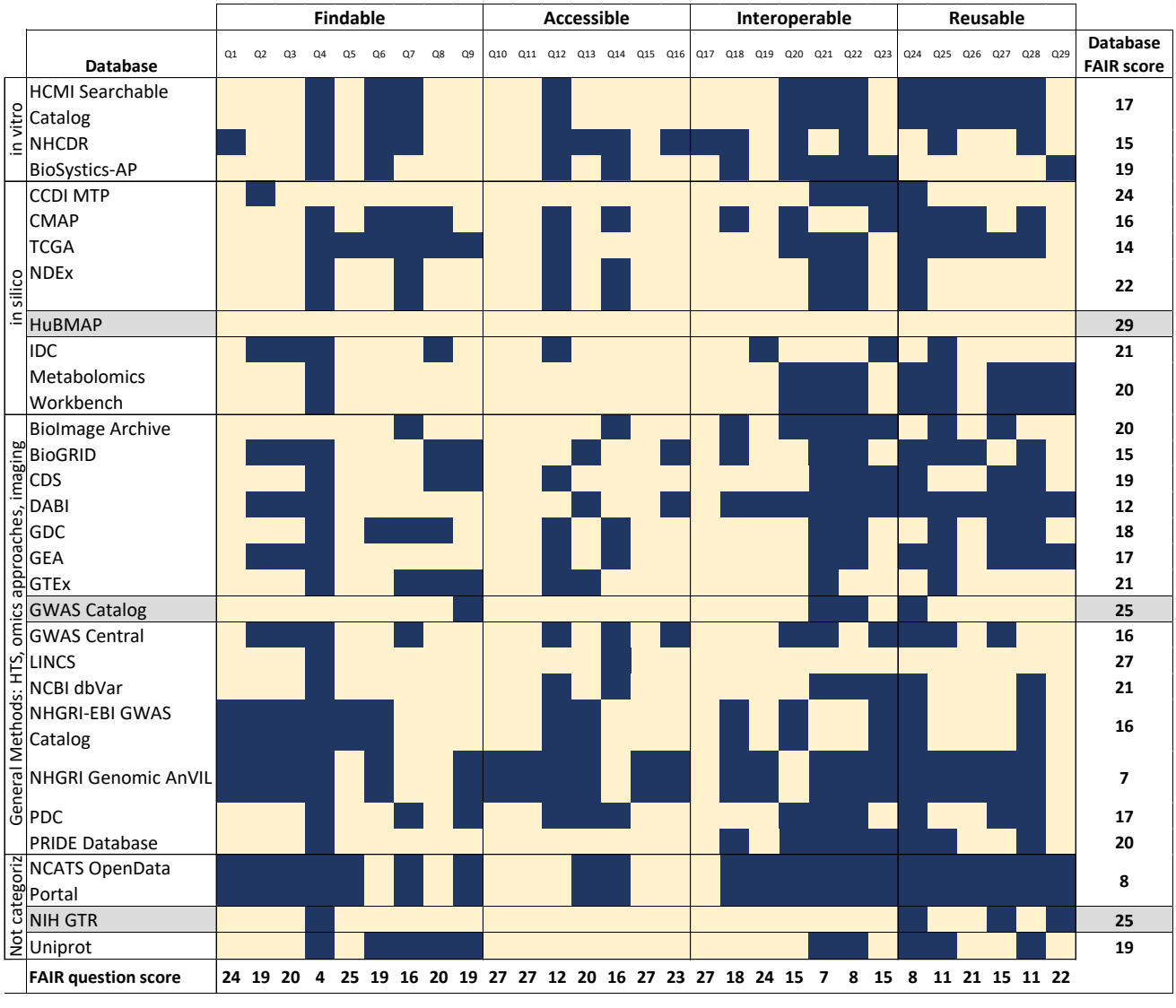




separated by commas in a column adjacent to the "cite" column. Please do not output any other columns from the original file. Additionally, please iterate through every row in the CSV so each row has an output.
For cell endpoints: In the attached CSV extract the cell endpoint entities or cell endpoints in the "cellular.endpoints" column and produce a tab delimited output with the extracted values separated by commas in a column adjacent to the "cite" column. Please do not output any other columns from the original file. Additionally, please iterate through every row in the CSV so each row has an output
For funding statement: In the attached CSV map the "funding_statement" column to the funding organizations affiliate country, if it is a private organization or company then map to "private", if the row contains "no", or "NO_RESPONSE" then respond "none", and produce a tab delimited output with the extracted values separated by commas in a column called "country_org" contained in quotes adjacent to the "cite" column. Please do not output any other columns from the original file. Additionally, please iterate through every row in the CSV so each row has an output.

Appendix G. Biomedical database with FAIR score per question

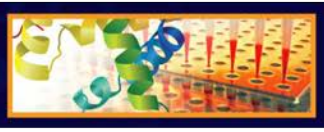
FAIR evaluation of 28 biomedical databases[2]. A score of 1 (yellow) indicates that the evaluator was able to explicitly identify the information pertaining to each question and a score of 0 (navy) indicates that the information could not be found. Scores were added for each question and database. Databases with the highest FAIR score are shaded. FAIR questions are listed in Appendix H.



		Findable									Accessible							Interoperable							Reusable						
	Database	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18	Q19	Q20	Q21	Q22	Q23	Q24	Q25	Q26	Q27	Q28	Q29	Database FAIR score
in vitro	HCMI Searchable Catalog																													17
	NHCDR																													15
	BioSystics-AP																													19
in silico	CCDI MTP																													24
	CMAP																													16
	TCGA																													14
	NDEx																													22
	HuBMAP																													29
	IDC																													21
	Metabolomics Workbench																													20
General Methods: HTS, omics approaches, imaging	BioImage Archive																													20
	BioGRID																													15
	CDS																													19
	DABI																													12
	GDC																													18
	GEA																													17
	GTEx																													21
	GWAS Catalog																													25
	GWAS Central																													16
	LINCS																													27
	NCBI dbVar																													21
	NHGRI-EBI GWAS Catalog																													16
	NHGRI Genomic AnVIL																													7
	PDC																													17
	PRIDE Database																													20
Not categoriz	NCATS OpenData Portal																													8
	NIH GTR																													25
	Uniprot																													19
	FAIR question score	**24**	**19**	**20**	**4**	**25**	**19**	**16**	**20**	**19**	**27**	**27**	**12**	**20**	**16**	**27**	**23**	**27**	**18**	**24**	**15**	**7**	**8**	**15**	**8**	**11**	**21**	**15**	**11**	**22**	

[2] NHCDR: NINDS Human Cell and Data Repository; BioSystics-AP: BioSystics Analytics Platform; CCDI MTP: Molecular Targets Platform; CMAP: Connectivity Map; IDC: Imaging Data Commons; TCGA: The Cancer Genome Atlas; NDEx: The Network Data Exchange; HuBMAP: The Human BioMolecular Atlas Program; IDC: Imaging Data Commons; BioGRID: Biological General Repository for Interaction Datasets; CDS: Cancer Data Service; DABI: Data Archive for the BRAIN Initiative; GDC: Genomic Data Commons; GEA: Genomic Expression Archive; GTEx: Genotype-Tissue Expression; GWAS: Genome-wide Association Studies Catalog; GWAS Central; LINCS: Library of Integrated Network-Based Cellular Signatures; NCBI dbVar: Structural Variation Database; NHGRI-EBI GWAS Catalog; The NHGRI Genomic AnVIL: Data Science Analysis, Visualization, and Informatics Lab-space; PDC: Proteomic Data Commons; PRIDE: Proteomics Identifications Database; NIH GTR: Genetic Testing Registry

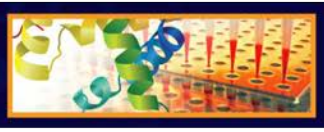




Appendix H. FAIR rubric questions

Findable	Q1:	Is a unique, persistent, viewable identifier assigned for the data release and documented in the data release's metadata record?
	Q2:	Is a separate identifier assigned for the data release's metadata record?
	Q3:	Is the assigned separate identifier unique and persistent?
	Q4:	Are the authors/originators' ORCID identifiers provided in the data release's landing page and data release metadata?
	Q5:	Is a description included in the data release's metadata?
	Q6:	Is the author/originator included in the data release's metadata?
	Q7:	Is a data point of contact included in the data release's metadata?
	Q8:	Is the data publication date included in the data release's metadata?
	Q9:	If applicable, is the data type, data version and revision dates included in the data release's metadata?
Accessible	Q10:	Does the data release have a human readable landing page that provides direct access to the data?
	Q11:	Is this landing page publicly accessible?
	Q12:	Is the data release's author/originator information available on the landing page?
	Q13:	Does the data release's identifier take users to the human readable landing page?
	Q14:	Is the data distributor, and its contact information, included with the data release's landing page?
	Q15:	Can users obtain the data release's data and metadata files by manual actions (human)?
	Q16:	Can users obtain the data release's data files and metadata files by automated actions?
Interoperable	Q17:	Are all data files available in an open format that is commonly used by the relevant research community?
	Q18:	Are all data files available in multiple formats, including those that are machine readable?
	Q19:	Does the data release's metadata contain unique names/labels?





	Q20:	Does the data release and metadata contain at least one name/label using a citable and publicly available source including community-recognized ontologies used in Resource Description Format (RDF)/linked data?
	Q21:	Is information about precision/accuracy documented in the metadata?
	Q22:	Is information about data value consistency documented in the metadata?
	Q23:	Is the relationship between the data and related data releases documented in the metadata?
Reusable	Q24:	Are recommended reuses included in the data release's metadata or landing page along with any reuse limits?
	Q25:	If input datasets are used, are the citations to the input datasets included with the data release's metadata?
	Q26:	Is the process/methodology summary included with the data release's metadata?
	Q27:	Is data quality information included with the data release's metadata?
	Q28:	Are citation(s) used to describe the process/methodology including the data quality information included with the data release's metadata?
	Q29:	Are related resources documented in the data release's metadata?

Supplemental Materials

The following figures represent the distribution of biomedical NAM categories, keywords and answers to questions applied to generative AI.

Counts represent the number of publications that included the keyword or generative AI found information relevant to each question. Note that scales are different per figure.

In vitro biomedical NAMs:

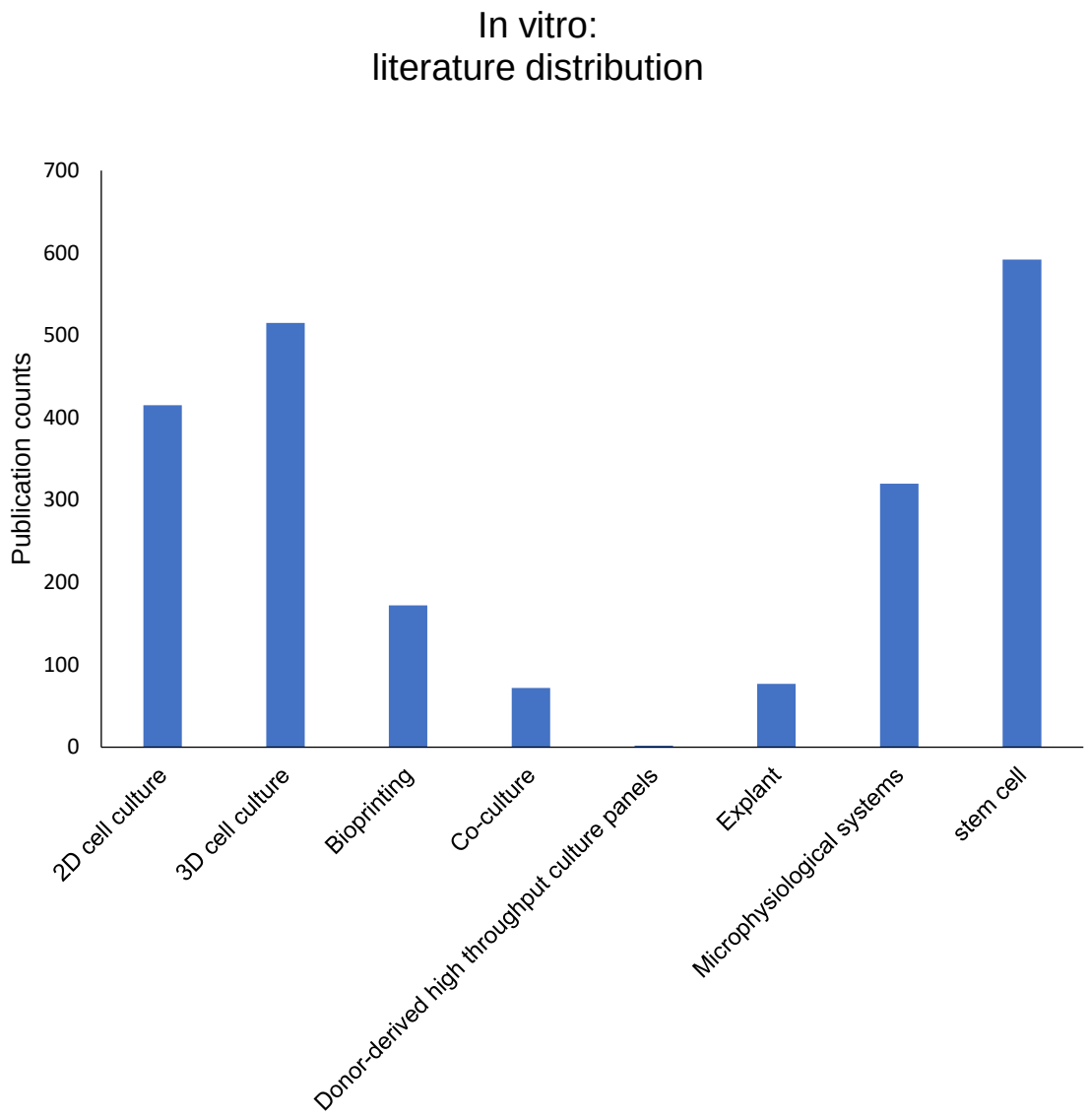


Figure S1: Literature distribution of in vitro biomedical NAMs per keyword.

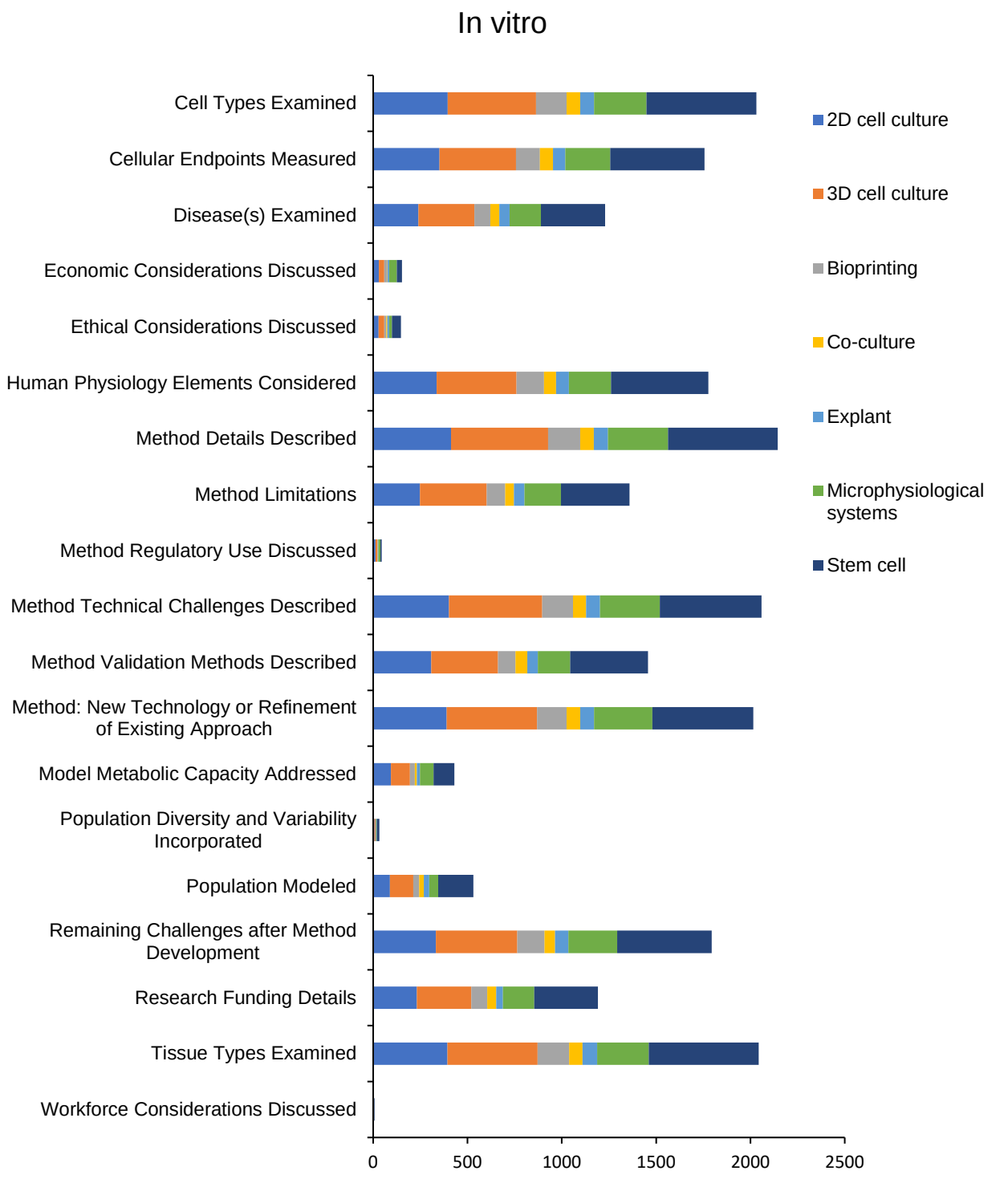


Figure S2: Number of publications (x-axis) per question across in vitro categories. Information on publication count can be viewed in Table S1.

Table S1: Publication count per in vitro category for each question area applied to Generative AI.

	2D cell culture	3D cell culture	Bioprinting	Co-culture	Explant	Microphysiological systems	Stem cell
Workforce Considerations Discussed	1	3	1	0	0	0	3
Tissue Types Examined	393	478	168	71	77	274	583
Research Funding Details	232	288	84	48	36	166	337
Remaining Challenges after Method Development	332	432	144	56	71	259	500
Population Modeled	88	125	31	24	29	47	187
Population Diversity and Variability Incorporated	7	5	1	0	2	4	14
Model Metabolic Capacity Addressed	94	101	25	11	17	72	111
Method: New Technology or Refinement of Existing Approach	390	479	157	72	73	309	535
Method Validation Methods Described	308	354	92	63	56	172	412
Method Technical Challenges Described	402	494	163	70	74	316	540
Method Regulatory Use Discussed	13	9	1	2	2	11	7
Method Limitations	248	354	98	46	56	194	363
Method Details Described	413	514	170	72	76	318	581
Human Physiology Elements Considered	336	424	145	65	68	223	516
Ethical Considerations Discussed	29	29	12	5	9	16	47
Economic Considerations Discussed	30	29	16	2	8	40	28
Disease(s) Examined	240	296	85	47	55	166	340
Cellular Endpoints Measured	351	406	127	69	66	238	499
Cell Types Examined	395	468	163	71	74	278	582

In chemico biomedical NAMs:

Figure S3: Literature distribution of in chemico biomedical NAMs per keyword.

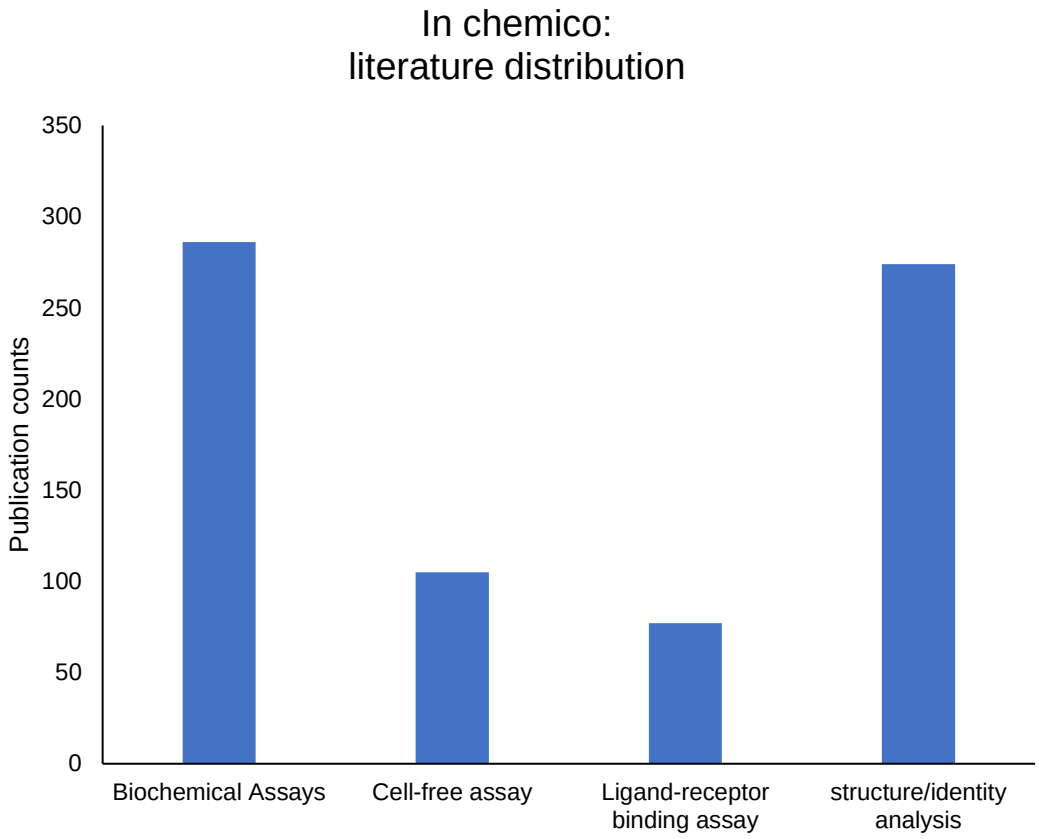


Figure S4: Number of publications (x-axis) per question across in chemico categories. Information on publication count can be viewed in Table S2.

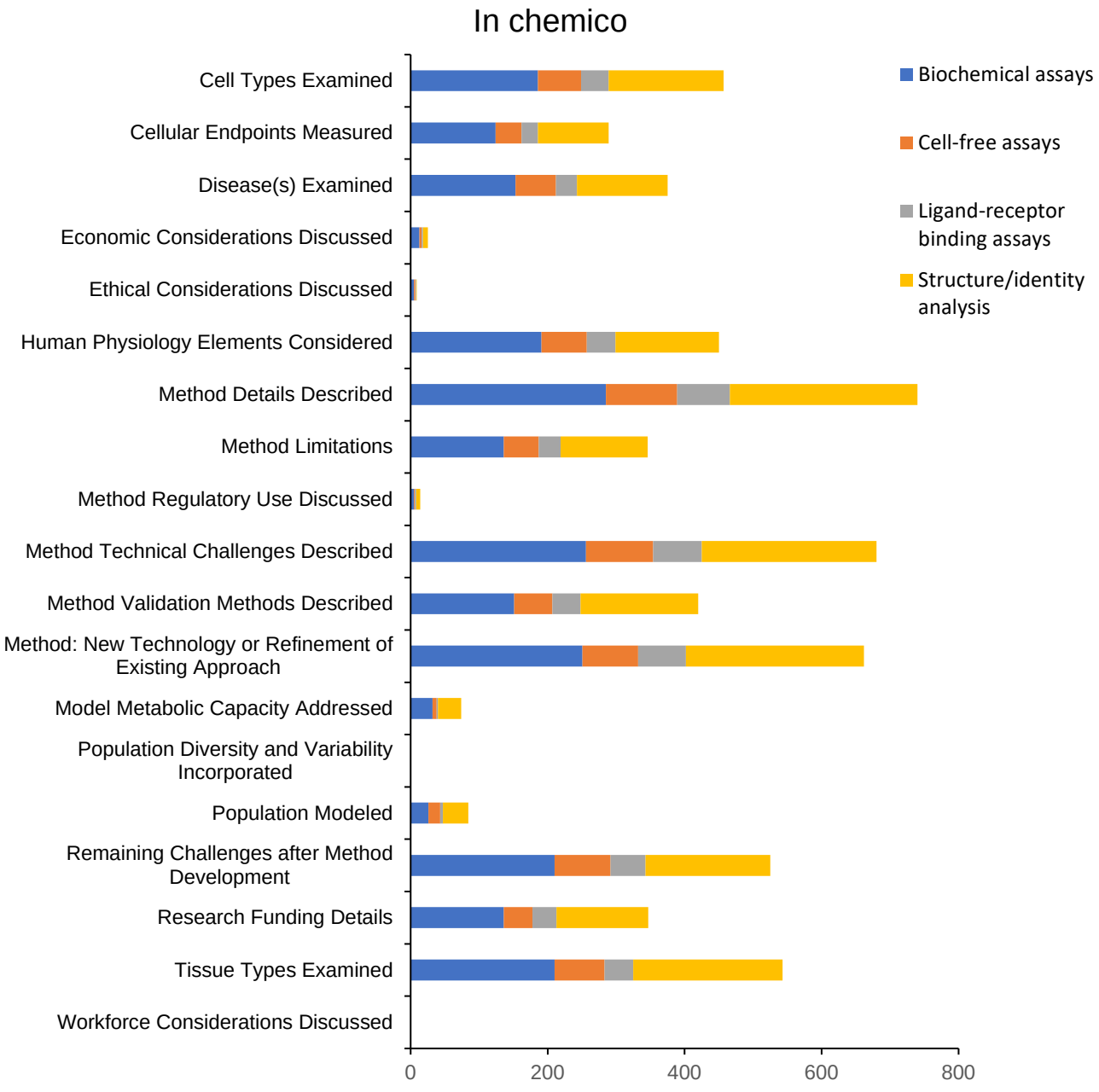


Table S2: Publication count per in chemico category for each question area applied to Generative AI.

	Biochemical assays	Cell-free assays	Ligand-receptor binding assays	Structure/identity analysis
Workforce Considerations Discussed	1	0	0	0
Tissue Types Examined	210	73	42	218
Research Funding Details	136	42	35	134
Remaining Challenges after Method Development	210	82	51	182
Population Modeled	26	17	4	37
Population Diversity and Variability Incorporated	1	0	0	0
Model Metabolic Capacity Addressed	32	6	2	34
Method: New Technology or Refinement of Existing Approach	251	81	70	260
Method Validation Methods Described	151	56	41	172
Method Technical Challenges Described	256	98	71	255
Method Regulatory Use Discussed	5	1	2	6
Method Limitations	136	51	32	127
Method Details Described	285	104	77	274
Human Physiology Elements Considered	191	66	42	151
Ethical Considerations Discussed	5	2	1	1
Economic Considerations Discussed	13	3	2	7
Disease(s) Examined	153	59	31	132
Cellular Endpoints Measured	124	38	24	103
Cell Types Examined	186	63	40	168

In silico biomedical NAMs:

Figure S5: Literature distribution of in silico biomedical NAMs per keyword.

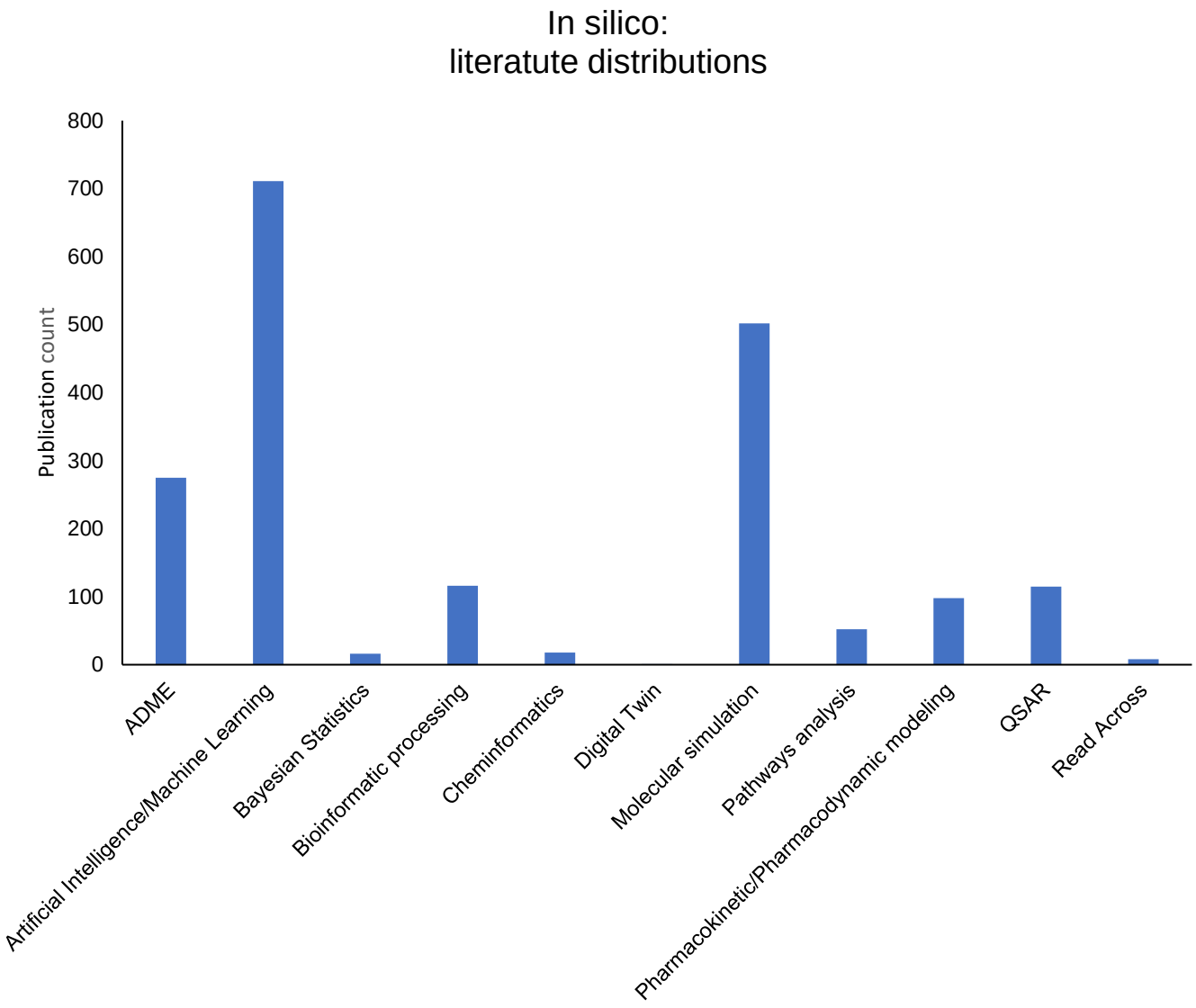


Figure S6: Number of publications (x-axis) per question across in silico categories. Information on publication count can be viewed in Table S3.

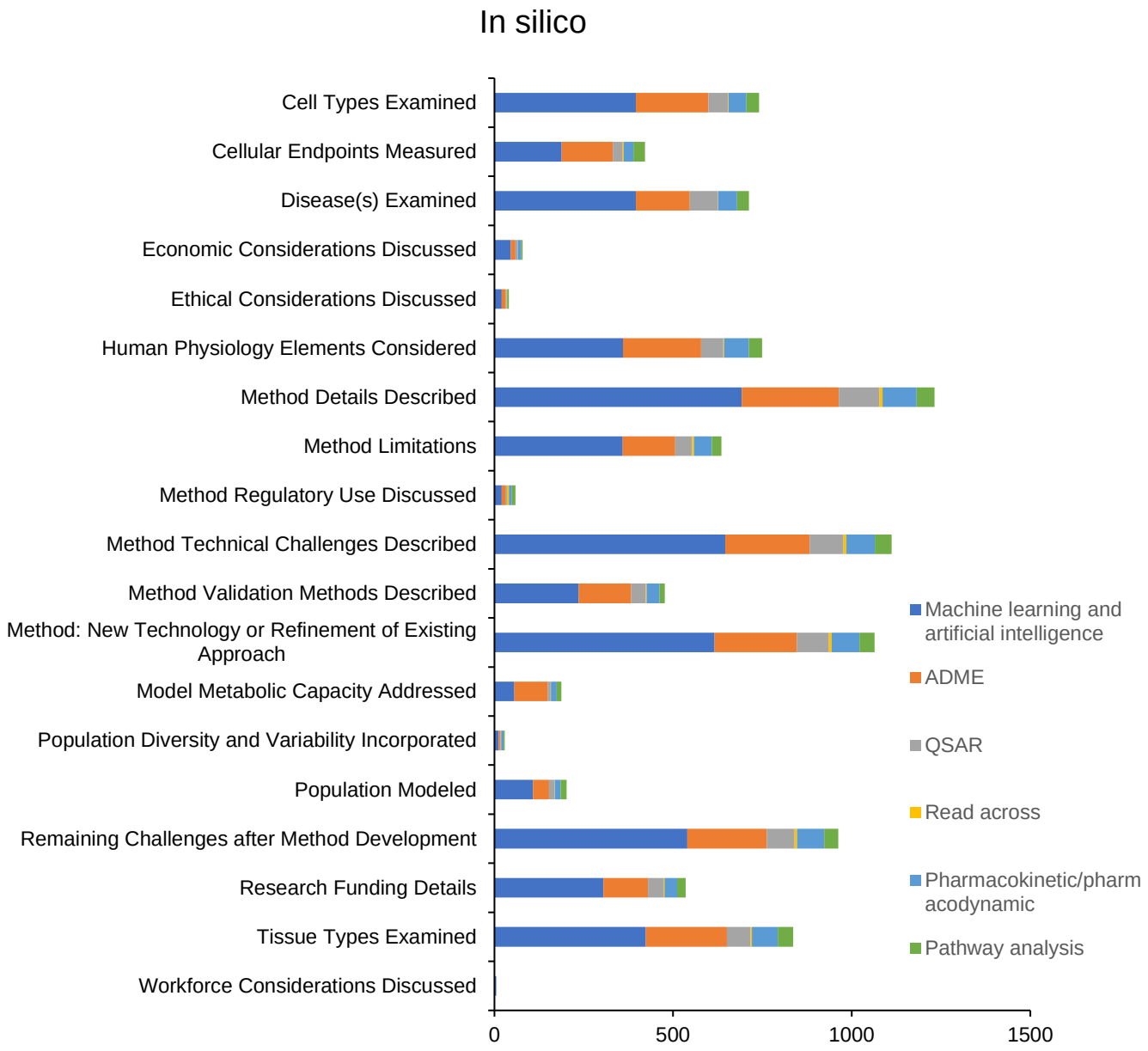


Table S3: Publication count per in silico category for each question area applied to Generative AI.

	Machine learning and artificial intelligence	ADME	QSAR	Read across	Pharmacokinetic/ pharmacodynamic	Pathway analysis
Workforce Considerations Discussed	5	0	1	0	0	0
Tissue Types Examined	424	227	66	4	72	43
Research Funding Details	305	125	43	3	35	24
Remaining Challenges after Method Development	540	222	78	7	76	39
Population Modeled	108	45	15	1	16	17
Population Diversity and Variability Incorporated	12	5	1	1	7	3
Model Metabolic Capacity Addressed	56	94	7	1	16	13
Method: New Technology or Refinement of Existing Approach	616	231	89	8	78	42
Method Validation Methods Described	236	147	41	2	36	15
Method Technical Challenges Described	646	236	95	8	80	47
Method Regulatory Use Discussed	20	10	5	5	9	10
Method Limitations	359	147	47	5	50	27
Method Details Described	692	272	114	8	96	50
Human Physiology Elements Considered	360	218	63	2	69	37
Ethical Considerations Discussed	20	12	1	1	2	4

Economic Considerations Discussed	46	13	5	1	10	4
Disease(s) Examined	396	151	78	1	53	33
Cellular Endpoints Measured	188	143	28	3	27	32
Cell Types Examined	396	203	55	1	50	36

General Methods biomedical NAMs:

Figure S7: Literature distribution of General Methods biomedical NAMs per keyword.

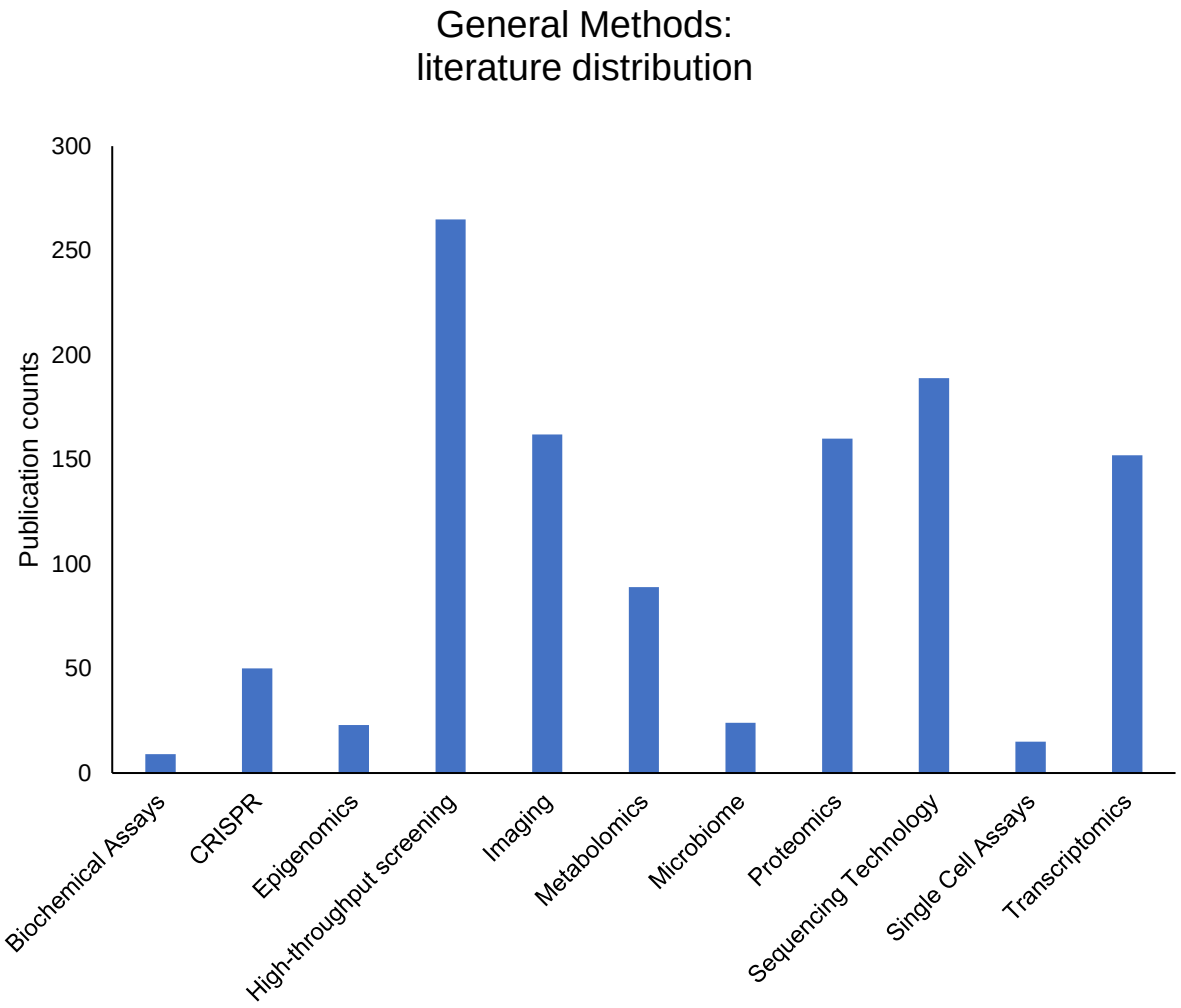


Figure S8: Number of publications (x-axis) per question across General Methods categories. Information on publication count can be viewed in Table S4.

Table S4: Publication count per General Method category for each question area applied to Generative

	Biochemical assays	CRISPER	Epigenomics	HTS	Imaging	Metabolomics	Microbiome	Proteomics	Sequencing technology	Single cell assays	Transcriptomics
Workforce Considerations Discussed	0	1	0	1	1	0	0	0	0	0	0
Tissue Types Examined	7	42	20	220	151	71	22	136	141	15	137
Research Funding Details	7	13	7	136	90	34	12	77	71	8	70
Remaining Challenges after Method Development	5	42	22	214	137	69	20	132	147	14	125
Population Modeled	0	19	10	65	42	35	7	47	53	3	51
Population Diversity and Variability Incorporated	0	2	3	10	2	4	1	6	10	0	2
Model Metabolic Capacity Addressed	0	5	5	44	21	36	3	18	22	0	28
Method: New Technology or Refinement of Existing Approach	8	45	20	234	149	75	16	147	164	13	129
Method Validation Methods Described	0	29	4	115	94	28	6	72	0	6	65
Method Technical Challenges Described	9	48	22	245	151	81	19	147	172	13	133
Method Regulatory Use Discussed	0	1	0	12	4	1	0	0	5	0	3
Method Limitations	2	38	14	155	110	50	14	86	96	10	19

Method Details Described	9	48	21	258	158	86	23	157	181	14	144
Human Physiology Elements Considered	4	36	16	178	113	65	20	100	114	10	105
Ethical Considerations Discussed	0	1	1	9	7	1	0	4	10	1	5
Economic Considerations Discussed	0	3	0	16	11	2	1	4	13	0	5
Disease(s) Examined	0	40	18	166	106	56	15	94	117	12	92
Cellular Endpoints Measured	7	33	12	143	108	40	7	70	0	8	84
Cell Types Examined	0	41	17	191	131	52	10	114	128	15	134