

ACCEPTED PAPER · MAIN TEXT

New approach methodologies (NAMs): The enabling role of bioprinting

Paper version: Accepted Paper

Accepted Papers are manuscripts accepted for publication, encompassing all changes made following the peer review process, along with a standard cover page indicating the paper version and an “Accepted Paper” watermark, but excluding any other editing, typesetting or other changes made by AccScience Publishing and/or authors post-acceptance.

Article ID: IJB026230239

Citation: Meinert C, Cometta S, Levett P, *et al.* New approach methodologies (NAMs): The enabling role of bioprinting. *Int J Bioprint*. 2026. doi: 10.36922/IJB026230239

Copyright: © 2026 Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher’s Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

New approach methodologies (NAMs): The enabling role of bioprinting

Running title: NAMs – The role of bioprinting

Christoph Meinert^{1,2†}, Silvia Cometta^{3,4†}, Peter Levett¹, Jacqui McGovern^{2,3,5,6,7}, Terrance Thiel⁸, Anjali Jaiprakash^{1,9}, Ross Burdis¹⁰, Travis Klein^{2,3,4,7}, Gail Risbridger^{7,11,12,13,14}, Molly M. Stevens^{10,15*}, and Dietmar W. Hutmacher^{3,4,5,7,16*}

¹ Gelomics Pty Ltd, 60 Musk Avenue, Kelvin Grove, Queensland 4059, Australia

² Centre for Biomedical Technologies, Queensland University of Technology (QUT), Brisbane, Queensland, Australia

³ Max Planck Queensland Centre for the Materials Science of Extracellular Matrices, Queensland University of Technology, Brisbane, Queensland, Australia

⁴ School of Mechanical, Medical and Process Engineering, Faculty of Engineering, Queensland University of Technology (QUT), Brisbane, Queensland, Australia

⁵ School of Biomedical Sciences, Faculty of Health, Queensland University of Technology (QUT), Brisbane, Queensland, Australia

⁶ Translational Research Institute (TRI), Woolloongabba, Queensland, Australia

⁷ ARC Training Centre for Cell and Tissue Engineering Technologies, Queensland University of Technology (QUT), Brisbane, Queensland, Australia

⁸ TMT Consulting Australia, Umina Beach, NSW 2257, Australia

⁹ Faculty of Engineering, The University of Queensland, Brisbane, Queensland, Australia

¹⁰ Department of Materials, Department of Bioengineering, Institute of Biomedical Engineering, Imperial College London, London, SW7 2AZ, United Kingdom

¹¹ Melbourne Urological Research Alliance, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

¹² Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

¹³ Sir Peter MacCallum Department of Oncology, The University of Melbourne, Victoria, Australia

¹⁴ Cabrini Institute, Cabrini Health, Malvern, Victoria, Australia

¹⁵ Department of Physiology, Anatomy and Genetics, Department of Engineering Science, Kavli Institute for Nanoscience Discovery, University of Oxford, Oxford, United Kingdom

¹⁶ Australian Research Council (ARC) Training Centre for Multiscale 3D Imaging, Modelling, and Manufacturing (M3D Innovation), Queensland University of Technology, Brisbane, Queensland, Australia

[†]These authors contributed equally to this work.

***Corresponding authors:**

Molly M. Stevens (molly.stevens@dpag.ox.ac.uk)

Dietmar W. Hutmacher (dietmar.hutmacher@qut.edu.au)

Citation: Meinert C, Cometta S, Levett P, *et al.* New approach methodologies (NAMs): The enabling role of bioprinting. *Int J Bioprint*. 2026. doi: 10.36922/IJB026230239

Received: June 5, 2026

Revised: September 1, 2026

Accepted: September 4, 2026

Published online: September 4, 2026

Copyright: © 2026 Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Animal-based preclinical testing is increasingly misaligned with the biological and regulatory demands of modern drug development. Persistent failure of drug candidates underscores the limitations of using animal models for human drug development. In oncology and neurodegeneration, where disease mechanisms and therapeutic responses are largely human-specific, clinical success remains low despite decades of refinement of rodent and xenograft models. Meanwhile, the regulatory framework for human-relevant New Approach Methodologies (NAMs) has become significantly more defined. In addition to the U. S. Food and Drug Administration (FDA) Modernization Act 2.0 and 3.0 and the FDA's 2025 roadmap, the FDA has issued draft guidance on the general use of NAMs in drug development and on streamlined nonclinical programmes for monoclonal antibodies, while the UK Medicines and Healthcare products Regulatory Agency (MHRA) and European Medicines Agency (EMA) have each signalled more formal entry points for non-animal data. Here, we synthesise the emerging NAM ecosystem - engineered 3D cultures, patient-derived organoids, microphysiological systems (organ-on-a-chip), bioprinting, and AI-enabled *in silico* modelling - and evaluate each platform's position within the drug development pipeline. We argue that bioprinting should be viewed not as a standalone model class but as an enabling biofabrication strategy that can improve the reproducibility, architectural control, and manufacturability of organoids and organ-on-chip systems. Critically, we frame adoption as an engineering-regulatory translation problem: scalable manufacturing, assay robustness, defined biomaterials, and cross-site reproducibility must advance in parallel with biological fidelity. We propose a practical readiness framework and a tiered deployment strategy that enable immediate reductions in animal use in high-impact domains, notably safety testing and oncology triage, while building toward replacing more complex and longitudinal endpoints through integrated multi-organ and computational approaches.

1. Introduction

Modern drug discovery is experiencing a paradigm shift as New Approach Methodologies (NAMs) and, more specifically, bioengineered human tissue models start to complement, and in some cases, replace animal testing.¹ The limitations of traditional rodent models are increasingly apparent: more than 90% of drug candidates entering clinical development do not reach approval, with failures dominated by lack of efficacy and unexpected human toxicities.^{2,3} This poor translation is especially evident in complex diseases such as cancer and Alzheimer's disease, where human-specific mechanisms are not adequately captured in animal models. In oncology, only 3.4% of drugs entering Phase I ultimately achieve regulatory approval, while Alzheimer's disease has remained marked by repeated clinical failure despite promising animal data.^{2,4,5} Ethical concerns, soaring costs, lengthy study durations, and limited availability of relevant animal models add further pressure to identify alternatives.⁶ In late 2022, the United States took a landmark step with the Food and Drug Administration (FDA) Modernization Act 2.0, explicitly stating that non-animal methods may be used to support Investigational New Drug (IND)-applications, and in April 2025 the FDA issued a strategic roadmap to make animal studies “the exception rather than the norm” in preclinical safety and toxicity testing over the next three to five years.^{1,7} This policy trajectory has since become more specific. In December 2025, the FDA issued draft non-binding guidance on streamlined nonclinical safety studies for certain monoclonal antibodies, and in March 2026 it issued draft guidance on general considerations for using NAMs in drug development, positioning NAM data within a “weight-of-evidence” approach.^{8,9} In the same month, the UK Medicines and Healthcare products Regulatory Agency (MHRA) published guidance allowing advance review of non-animal data in Module 4, while the European Medicines Agency (EMA) opened consultation on virtual control groups as the first qualification opinion for a NAM used in toxicity assessment.^{10,11} A related piece of legislation, the FDA Modernization Act 3.0, which requires the FDA to establish a process to qualify NAMs, has passed both the U.S. Senate and the House of Representatives.¹² These regulatory changes, along with major public, philanthropic, and ecosystem-building initiatives in the U.S., Europe, the UK, Australia, and the UAE, have set the stage for systematic adoption of bioengineered models in preclinical research.^{13–20}

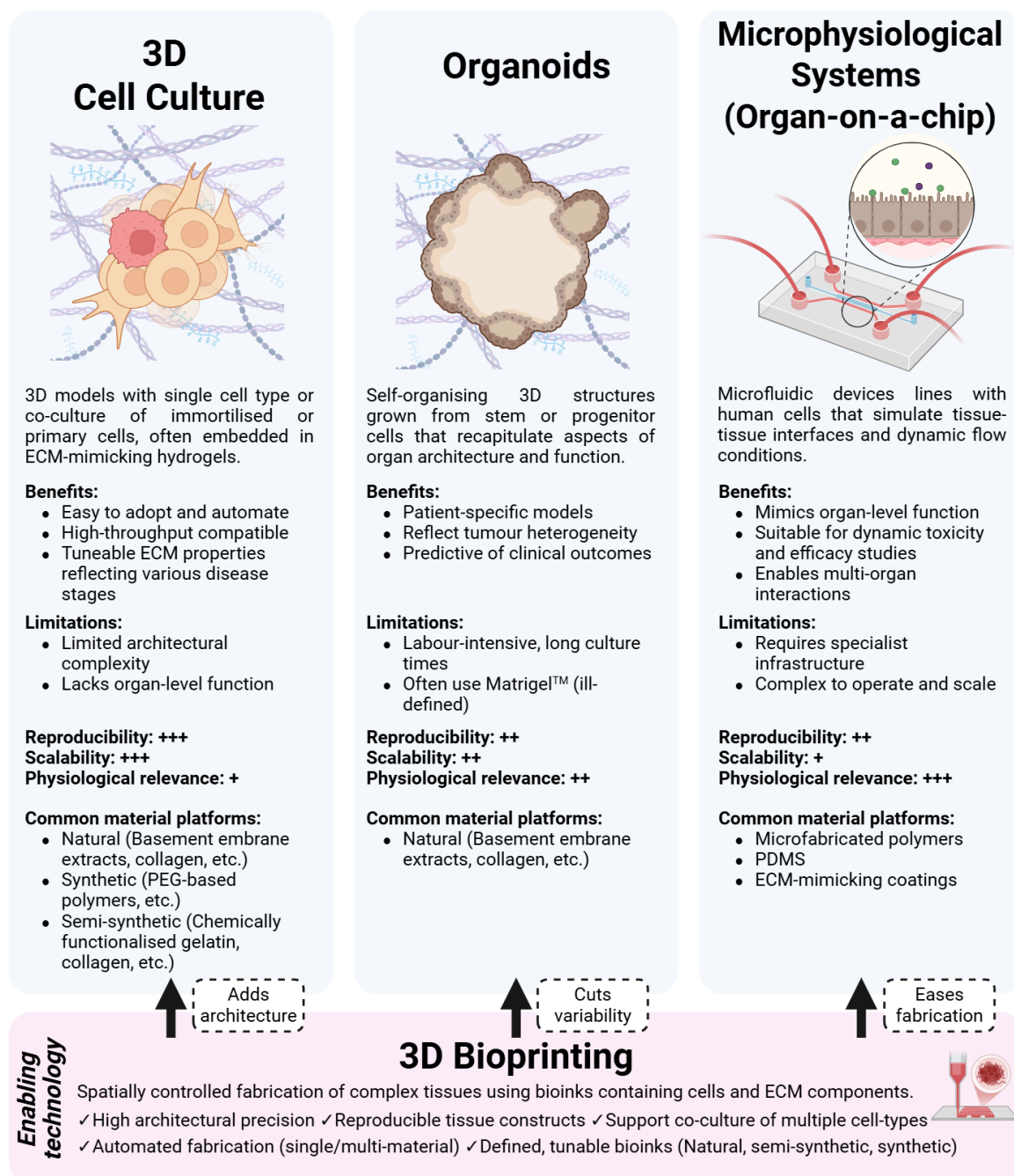


Figure 1. Bioengineered human model platforms for preclinical drug discovery and the converging role of 3D bioprinting. Schematic overview of three key *in vitro* platforms: 3D cell cultures, organoids and microphysiological systems (organ-on-a-chip). These models offer complementary strengths but face limitations in architectural complexity, reproducibility, and fabrication. 3D bioprinting can address these constraints by enabling spatial control and reproducible, scalable fabrication. Together, these platforms form a modular toolkit for reducing animal use in preclinical drug discovery. Created with BioRender.com.

2. Bioengineered human models come of age

With advances in tissue engineering, microfluidics and computational modelling, human-relevant models can now replicate key aspects of human biology with high fidelity.^{21–24} These NAMs include human-derived 3D cultures (organoids, spheroids, “tumouroids”), microfluidic organ-on-a-chip devices (Figure 1), and sophisticated *in silico* modeling.

Advanced 3D cell culture models, rooted in cutting-edge biomedical research and tissue engineering, are foundational to preclinical drug discovery. These platforms often embed cells in biomimetic engineered hydrogels that replicate key aspects of the human extracellular matrix (ECM), providing essential biochemical and mechanical signals that influence cell organisation, morphology, polarity, gene expression, and drug response. Compared with traditional 2D monolayers, these 3D cultures more accurately mimic the microenvironment of native tissues and are increasingly used in toxicity screening, epithelial barrier modelling, and tumour biology.^{25,26} These 3D models are underpinned by tuneable semi-synthetic hydrogels, enabling precise adjustment of their physical and biochemical properties while maintaining a balance between complexity and scalability. For example, matrix stiffness, degradability, and biofunctionality (e.g., inclusion of specific peptides or growth factors) can be tailored to mimic specific tissue environments or disease states. Because they are easy to implement, these engineered hydrogel-based 3D cultures scale well and are readily deployed in upstream applications that require high-throughput microwell plates. As such, they integrate with automated liquid-handling and imaging systems without requiring specialised equipment or protocol overhauls. Their compatibility with robotic and automated workflows makes them well suited for high-throughput drug screening, early-stage toxicity testing, and phenotypic profiling, in which compound libraries of 100,000 to over 1 million molecules are routinely screened in 384-well plates, yielding approximately 3,000 plates and over 1 million data points per screening campaign.²⁷ Such 3D cell culture platforms are particularly valuable when full tissue/organ complexity is not required, but a representative microenvironment is essential to improve predictive accuracy. As such, they offer a practical, scalable alternative to animal models in early drug development workflows and serve as a gateway to more complex *in vitro* systems. Because these platforms rely on defined materials, simple geometries, and plate-based workflows, they also align well with the current regulatory emphasis on specified inputs, quantitative performance metrics, and cross-site reproducibility for fit-for-purpose NAM deployment.^{8,10}

Organoids build on this foundation and are the next level of biological fidelity. These “miniature organs”, grown from human stem or progenitor cells, self-organise into realistic micro-anatomy and recapitulate key structural and functional aspects of native tissues. Pioneered in 2009 for intestinal tissue²⁸, organoid technology has since expanded to numerous organs and tumour types.²⁹ Patient-derived tumour organoids (PDTOs) can mirror the histology and genetic landscape of patient tumours. Other patient-derived organoid (PDO) systems incorporate relevant mechanical stresses: cardiac organoids can beat, and airway organoids can be pressurised to simulate breathing.^{30–32} PDTOs derived from patient cancers have demonstrated remarkable predictive power. In a systematic review of 17 studies, PDTOs demonstrated 81% sensitivity and 74% specificity for predicting patient treatment responses, outperforming traditional cell lines.^{29,33} Indeed, accumulating evidence suggests that PDTOs can be more predictive of cancer drug efficacy than animal xenograft models.²⁹ This human-like complexity may enable organoids to better capture patient-specific responses to therapeutic drugs, highlighting their potential as a more reliable preclinical tool for assessing anticancer drug efficacy.

The establishment of PDO biobanks has become pivotal in advancing preclinical drug discovery. These biobanks, encompassing diverse cancer types, provide well-characterized, renewable models that enable high-throughput drug screening and personalized medicine. For instance, living organoid biobanks have enabled large-scale studies, allowing assessment of drug efficacy across genetically diverse tumour models.³⁴⁻³⁶ Furthermore, integrating multi-omic analyses with organoid biobanking enhances the precision of therapeutic targeting. By thoroughly characterizing each PDO through genomic, transcriptomic, and proteomic profiling, pre-clinical scientists can match patient tumours to specific organoid lines with closely aligned molecular features. This approach, potentially assisted by machine learning algorithms, augments the predictive power of preclinical models and supports the development of individualized treatment regimens.³⁷ However, because of their spontaneous self-organisation, biological complexity, and culture requirements, organoids face challenges in scalability, standardisation, and batch-to-batch reproducibility compared with simpler 3D models. These limitations make it difficult to incorporate organoids into high-throughput screening workflows typical of early-stage drug discovery, in which compound libraries of hundreds of thousands of molecules are tested in parallel. As a result, organoids are most useful in the later stages of the drug development pipeline, such as hit validation, lead optimisation, and patient stratification, where biological fidelity is prioritised over throughput. From a regulatory perspective, this positions organoids best for later-stage translational decision-making, patient stratification, and mechanistically anchored efficacy testing rather than the earliest phases of ultra-high-throughput screening. Extrusion bioprinting can increase automation, reduce variability, and control shape, which has proven beneficial for kidney organoid maturation.²⁴

“Organ-on-a-chip,” or microphysiological systems (MPS), which are relevant to translational research, have emerged globally from many bioengineering laboratories.²² These bioengineered platforms use microfluidic chips lined with living human cells, cultured under flow to mimic the mechanical and chemical environment of real organs. By incorporating tissue-tissue interfaces and shear stress, organ-chips recapitulate organ-level physiology (and pathology) that static cell cultures cannot.²² Multiple organ chips can be connected to create a “body-on-a-chip” that simulates inter-organ interactions. For instance, linking a liver chip to a heart chip enables concurrent study of drug metabolism and cardiotoxicity, and connecting a liver chip to downstream tissue or tumour models enables assessment of the effects of liver-activated prodrugs. Notably, these chips now exist for most major organs, including the liver, heart, lung, kidney, gut, and brain, and even in integrated multi-organ setups.^{1,38} They can be as predictive as, or more so than, animal models in assessing human responses. For instance, a human Liver-Chip was shown to predict drug-induced liver injury with 87% sensitivity and 100% specificity in a blinded study of 27 hepatotoxic and non-toxic compounds.³⁹ This far exceeds the predictive power of standard animal toxicology, which often omits consideration of human liver vulnerability. Notably, the so-called Liver-Chip study - the largest of its kind with 870 chips - demonstrated that human organ-chips can better predict drug safety than *in vivo* animal models, thus preventing false assurances from animal data. Researchers have also used these chips to model human disease states, from cancer to rare genetic disorders, and to reproduce clinical responses to drugs and pathogens *in vitro*.²² Despite the potential of this

cutting-edge technology, MPS often require dedicated infrastructure and microfluidic expertise, creating barriers to rapid, large-scale adoption in industrial high-throughput environments. Among current NAM classes, MPS are therefore the most obviously aligned with near-term, endpoint-specific qualification in safety assessment, provided their engineering performance is sufficiently stable, and their context of use is tightly defined.^{8,11,40–42}

Bioprinting supports all three pillars in the initial phase of biofabrication of complex, potentially vascularized tissue constructs and miniature organ-like structures on demand. Using bioinks containing human cells, organoid-forming cells, or supportive matrices, 3D bioprinters can create bioengineered tissues with precise geometries, targeted cell placement, and consistent architecture. Unlike traditional organoids that form randomly, bioprinting enables standardised organoid size, spacing, and multicellular composition, improving high-content screening and mechanistic research.^{43,44}

Bioprinting can manufacture organoid arrays and incorporate multiple cell types directly into organ-on-chip formats, making it increasingly relevant as a manufacturing layer for other NAMs rather than a separate model category.^{43,45,46} In this sense, the main translational value of bioprinting lies in its ability to improve the manufacturability, traceability, and lot-to-lot consistency of advanced human models, which are precisely the attributes that industry and regulators require for broader uptake. Nevertheless, print fidelity, bioink standardisation, post-print maturation, costs, and integration with automated assay workflows remain active engineering bottlenecks.^{44–46}

Artificial intelligence (AI) and *in silico* modelling, alongside bioprinting, are emerging as powerful tools in preclinical research, particularly when integrated with innovative NAMs. AI-driven simulations and digital twins, which are virtual models of organs or whole-body physiology, are being developed to combine multi-omic data from cell-culture studies, organoids, organ-on-chip systems, and 3D-bioprinted tissues to predict human drug responses.⁴⁷ For example, machine learning models trained on transcriptomic, genomic, and high-content imaging data from patient-derived tumour organoids have been used to predict drug response and stratify responders in colorectal and pancreatic cancer studies.^{23,48} Digital twin approaches are being piloted to simulate patient-specific drug metabolism, immune response, and toxicity using MPS outputs.^{47,49} A notable recent example is Rentosertib (formerly ISM001-055), a TNIK inhibitor discovered through generative AI, which has now reported results from a randomized phase 2a trial in idiopathic pulmonary fibrosis.⁵⁰ AI also improves reproducibility by identifying optimal assay parameters across NAM platforms. Convolutional neural networks have been used to classify morphological outcomes in 3D cultures,⁵¹ while AI-supported analysis is increasingly combined with automated image capture, perfusion management, and multi-parameter decision-making. As trust in these methods increases, AI-enhanced NAMs are expected to become essential to a more predictive, reproducible, and ethical approach to drug development.

Recent policy signals suggest that not all NAMs are equally ready for immediate adoption. In engineered 3D cultures, the main opportunity is in standardized, automation-friendly assays with clearly defined use cases. For organoids, their immediate translational benefits include patient stratification, late-stage efficacy assessment, and disease modeling, but wider

regulatory acceptance will require defined metrics, better manufacturing control, and more consistent results. MPS applications mainly focus on safety endpoints, such as liver toxicity, biologics safety, and dose ranges, as indicated by current FDA, MHRA, and EMA actions. For AI and virtual control methods, their near-term purpose is to enhance interpretation, reduce animal testing, and support qualification, rather than replace testing systems entirely. 3D bioprinting overlaps with these areas by applying spatial regulation and manufacturing discipline to otherwise variable tissues, making these systems more auditable and regulator-ready platforms.^{8–11,23,41,44–46}

3. Applications in oncology and immuno-oncology

Oncology has been at the forefront of adopting these bioengineered models. Traditional preclinical cancer research has relied heavily on animal models, typically murine xenografts of human cell lines or patient-derived xenograft tumours (PDX models). Indeed, in 2022, the European Union reported using 8.39 million animals for research, with 954,702 utilised in basic and translational cancer research, 95.5% of which were mice.⁵² While these models have provided valuable insights, they often fail to capture the true heterogeneity of human tumours and immune interactions. In contrast, PDOs can retain the histological architecture and genetic profile of the original patient tumour. They can be grown from small biopsy samples of colorectal, lung, pancreatic, breast, prostate and other cancers.²⁹ These tumoroids enable *in vitro* testing of multiple therapies on a patient's own cancer cells, offering a platform for personalised medicine. Moreover, when stratified into diverse panels representing different tumour subtypes and clinical histories, PDOs facilitate human-relevant drug screening, effectively serving as a “clinical trial in a dish”. This approach enables assessment of therapeutic responses across a broad spectrum of tumour profiles, thereby aiding clinical trial design. Indeed, studies in pancreatic and gastrointestinal cancers have shown that PDO drug sensitivities correlate strongly with patient outcomes.²⁹ In one report, *ex vivo* treatment responses in gastrointestinal PDOs predicted clinical response with 100% sensitivity and 93% specificity³³ - an incredible level of accuracy and predictive performance. This has spurred the establishment of organoid biobanks and clinical trials using organoids for personalised medicine. Worldwide, cancer centres and companies (e.g., HUB Organoids B.V., the Human Cancer Models Initiative, Crown Bioscience, and Tempus AI) now screen drug candidates on organoid panels representing diverse patient tumours to guide precision oncology and drug discovery. Importantly, organoids frequently reveal treatment failures at an early stage. A drug that may reduce a mouse xenograft but has no effect on human PDOs is unlikely to be effective in patients, potentially saving resources and reducing animal use. For 3D bioprinting, the most immediate contribution in oncology may be producing standardised tumour arrays in which organoid size, cell density, and stromal composition are controlled prospectively rather than emerging stochastically, improving assay comparability across compounds and sites.^{43,44}

Immuno-oncology faces a more complex challenge: modelling the human immune response to cancer. Mice lack a human immune system, and even “humanised” mouse models with engrafted human immune cells only partially recapitulate human immunology. Bioengineered models are rising to this challenge by incorporating immune components *in vitro*. Researchers have developed tumoroid-immune cell co-culture systems to recreate the tumour

microenvironment, including autologous immune cells. For instance, patient T lymphocytes can be co-cultured with PDOs, enabling real-time observation of immune cell infiltration and tumour cell killing.⁵³ In one study, T cells extracted from a patient's blood selectively attacked and destroyed matching colorectal cancer organoids, demonstrating the potential of organoid assays to evaluate immunotherapy efficacy at an individualised level.⁵⁴ Similarly, co-cultures of pancreatic cancer organoids with peripheral blood mononuclear cells revealed activation of cancer-associated fibroblasts and immune infiltration, mirroring the complex immune–tumour dynamics seen *in vivo*.⁵³ These systems enable testing of checkpoint inhibitors, CAR-T cells, or bispecific antibodies by monitoring how effectively human immune cells engage and eliminate the organoid tumour. Although still emerging, such immune-organoid platforms are rapidly improving. Innovations include organoids that incorporate lymphoid structures or that add engineered immune components to microfluidic chips. For instance, researchers have created “immune organoids” that resemble lymph nodes or spleen tissue to assess vaccines and immunotherapies in a human context.^{1,55} Microfluidic “blood-on-a-chip” devices can perfuse immune cells through organ constructs; one application is *in vitro* cytokine release assays for safety testing of immunotherapies.^{1,56} After the TGN1412 trial, in which a monoclonal antibody caused a cytokine storm in humans despite appearing safe in monkeys, drug developers routinely use human blood-cell assays to screen for such immunotoxicity.^{1,57} Another notable example is tebentafusp (Kimmtrak), a gp100 peptide-HPA-directed CD3 T-cell engager approved for HLA-A*02L01-positive unresectable or metastatic uveal melanoma. Since no pharmacologically relevant animal species was available for toxicity studies, human-based *in vitro* assays were used to assess cross-reactivity and potential organ-specific toxicity – and these NAM-derived data played a central role in the regulatory decision making.⁵⁸ *In vitro* immune models are thus increasingly capable of reducing reliance on certain animal tests, with the potential to replace them in specific contexts of use as their predictive validity is further established. For example, assessing a new immuno-oncology drug's ability to activate T cells or elicit cytokines can often be performed using human blood or immune organoid systems, rather than *in vivo* primate studies.¹ In the next few years, as these immuno-oncology models become more sophisticated (incorporating multiple immune cell types, 3D tumour architecture, and potentially patient microbiome factors), they will substantially reduce the need for animal models in testing cancer immunotherapies. Here again, bioprinting offers a practical route to defined multicellular geometry, enabling more reproducible placement of tumour, stromal, and immune compartments and potentially reducing the experimental noise that currently limits cross-study comparison of immune-organoid assays.^{44,45}

4. Applications in toxicology and safety testing

Perhaps the most immediate impact of bioengineered models lies in toxicology and drug safety assessment, areas where animal use has traditionally been substantial. Regulatory safety testing that evaluates a drug's potential to harm organs or cause side effects often requires multiple animal studies across various species. However, animal toxicology is notoriously unreliable: many compounds that appear safe in rodents or primates later prove toxic in humans.¹ Human-based *in vitro* systems can detect human-specific toxic effects earlier, improving safety and saving time and cost. For example, human liver organoids and liver-on-chip devices can model drug metabolism and liver injury with human-specific accuracy. The Emulate Liver-Chip,

which contains human hepatocytes and supporting cells under flow, has shown strong performance in detecting drug-induced liver injury in humans.³⁹ This kind of endpoint-specific performance is precisely the sort of evidence regulators are now beginning to consider within clearer validation frameworks, particularly where human relevance can be benchmarked against clinical or accepted reference data.^{8,11,40}

Human MPS for other organs, including kidney, lung, gut, heart, and brain, are also being utilised in safety and pharmacology studies. Integrated multi-organ platforms are under development to assess systemic toxicology; for instance, connecting gut, liver, and kidney compartments can simulate oral absorption, metabolism, and excretion within a single human-relevant system.^{22,40} Skin reconstructs and skin-on-chip systems have already displaced animal tests in several domains, illustrating that regulatory adoption of NAMs often begins with narrowly defined use cases rather than through wholesale substitution.^{1,40} Bioprinting is especially relevant here because it can fabricate perfusable channels, barrier layers, and compartmentalised multicellular architectures that are difficult to achieve consistently by manual assembly alone.⁴⁴⁻⁴⁶ For biologics and immunotherapies, the most promising near-term opportunity may be hybrid test batteries that combine blood-based cytokine release assays, immune-enabled liver or vascular chips, advanced 3D immune co-cultures, and computational models, thereby replacing the longest and least informative animal studies while preserving human relevance.^{8,9,53,57}

Regulators are increasingly receptive to these human-based toxicology methods, but the bar is now clearer and arguably higher: sponsors must define the regulatory question, the context of use, the performance standards, and the decision criteria for each NAM.^{8,10,11,58} In practice, this favours platforms that can generate reproducible quantitative data, integrate orthogonal evidence such as physiologically based pharmacokinetic (PBPK) modelling, and transfer across sites without major performance loss. For 3D cultures and organoids, that means defined matrices, standardised workflows, and robust imaging or molecular readouts. For MPS, it means engineering robustness, analytical quality control, and carefully selected reference compounds. For bioprinted systems, it means treating printing parameters themselves as critical quality attributes rather than purely technical settings.^{8,10,44}

Indeed, companies are finding that adopting these models earlier in their R&D strategies enables them to “fail fast” on unsafe drugs, focus resources on safer candidates, and reduce costly late-stage attrition.³⁹ The potential economic gains are immense: the regular use of a Liver Chip alone could save \$3 billion annually in R&D by preventing late failures, and up to \$24 billion if organ chips for multiple organs are widely implemented.³⁹ Reduced failure rates mean not only lower costs but also reduced the number of animals sacrificed for inaccurate tests.

5. A roadmap to replace animal models: Now, next, and future

Given rapid progress in bioengineered models and a supportive regulatory environment, a clear roadmap is emerging to minimise, and ultimately eliminate, animal use in preclinical research. Different domains of drug discovery are at different stages of readiness. We propose a segmented strategy:

Areas to replace animal use today: These are areas in which validated non-animal methods already offer clear value and, in some contexts, can provide sufficient predictive performance to replace certain animal tests. *In vitro* skin and eye irritation assays, pyrogen and endotoxin assays using non-animal reagents, and cytokine release assays using human blood already exemplify this principle.^{1,57,59} Routine hepatic liability screening can increasingly incorporate human liver spheroids or Liver-Chips, which have demonstrated advantages over conventional animal liver studies for selected liabilities.³⁹ In oncology, human organoid panels can provide a more human-relevant approach to prioritising candidate therapies, reducing the need for low-value mouse screening in specific drug-development contexts, allowing residual animal studies to be reserved for a much smaller number of highly prioritised candidates.^{29,33,35}

Recent FDA guidance makes this near-term opportunity more concrete. The 2025 draft guidance on streamlined nonclinical safety studies for certain monoclonal antibodies explicitly describes circumstances in which long-term animal toxicology studies are not warranted or may be limited to short-term studies, aiming to avoid unnecessary use of non-human primates.⁹ The 2026 FDA draft guidance on general NAM use, together with the MHRA's new non-animal pathway, means that sponsors can now discuss organ-chip, organoid, advanced 3D culture, and computational evidence within a clearer validation framework than was available even a year ago.^{8,10} For 3D bioprinted models, the immediate regulatory role is less likely to be a standalone replacement and more likely to be as a manufacturing enabler that turns complex organoid or MPS assays into more reproducible and transferable submission packages.⁴⁴⁻⁴⁶

Areas to replace animal use in 1–3 years: In the near term, the largest reductions will likely come from hybrid strategies that use human-relevant models to significantly reduce animal numbers rather than eliminate them outright. Oncology drug development falls squarely into this category: organoids, immune co-cultures, and bioprinted tumoroid and organoid arrays can triage larger candidate sets, interrogate mechanisms, and select only the most compelling agents for any residual *in vivo* confirmation.^{43,44,53,54} Similar logic applies to pharmacokinetics and safety pharmacology, where *in vitro* human liver models, MPS, and PBPK simulations can reduce the number, duration, and scope of rodent, dog, and primate studies.^{1,8-10,49} The next one to three years should therefore be viewed as a platform-qualification phase: animal studies will increasingly become targeted gap-fillers rather than the default backbone of every programme.

Areas where animal use can be replaced in the long term: Some animal studies remain difficult to fully recapitulate *in vitro*. Complex systemic interactions, chronic immune remodelling, whole-body metabolism, reproductive biology, and long-duration toxicities are not yet comprehensively modelled by current experimental or computational systems. Chronic toxicity and carcinogenicity studies, such as two-year rodent bioassays, still lack validated non-animal replacements.⁹ Likewise, modelling pregnancy, placental transfer, and embryo development remains highly complex, although embryoid, placenta-on-chip, and developmental systems are advancing.^{1,60} Immuno-oncology modalities that depend on the full adaptive immune repertoire, and neurodegenerative disease models that must reproduce long biological time scales, remain particularly challenging.

In these domains, the path forward is likely to depend on incremental integration rather than single-platform replacement: more mature organoids, multi-organ MPS, better digital twins, and biofabricated tissues with physiologically comparable vascular and immune architecture will need to converge before broader replacement of animal studies can be considered with confidence.^{23,44–47} In the meantime, refinement of any remaining animal studies, coupled with deliberate investment in validation, standardisation, and regulatory science, remains essential. The realistic near-term goal is therefore not absolute replacement across every endpoint, but a progressive shift in which animal studies become exceptional, targeted, and increasingly difficult to justify when robust human-relevant alternatives exist.

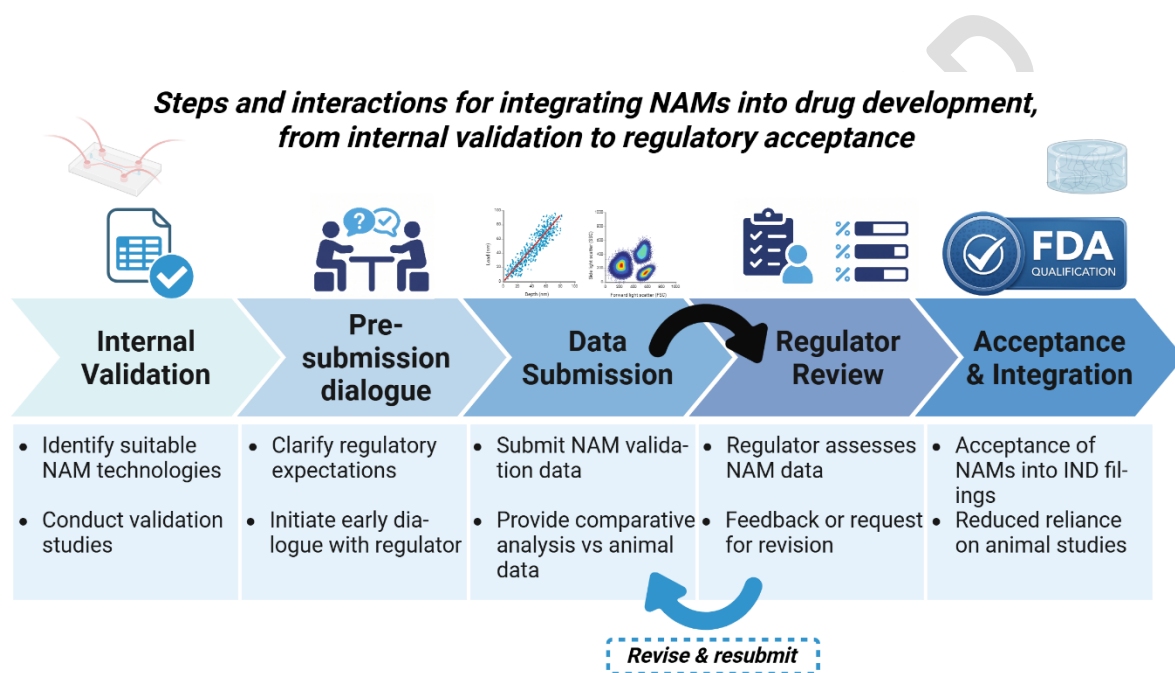


Figure 2. Key regulatory steps for integrating NAMs into drug development. Develop a clear strategy to navigate the FDA's Drug Development Tool (DDT) Qualification Programs, emphasising the approval of New Approach Methodologies (NAMs) for a specific Context of Use (COU). An effective regulatory NAM strategy should include parallel activities such as initiating FDA engagement and preparing the Letter of Intent (LOI) early, alongside drafting the Qualification Plan. Afterwards, follow a sequential process by executing the approved Qualification Plan and submitting a thorough Qualification Package to obtain the final Qualification Decision for your COU. Created with BioRender.com.

6. Complexity versus scale: The trade-off in 3D tissue models

As bioengineered human models enter mainstream drug discovery, a critical challenge has emerged: balancing biological complexity with manufacturability, scalability, and reproducibility. While advanced models such as patient-derived organoids and multi-organ chips offer unique physiological relevance, they are often labour-intensive, costly, and poorly suited to high-throughput screening workflows, which can require upwards of 1.5 million assay wells to screen 1 million compounds with appropriate controls.²⁷ Thus, their complexity often comes at the cost of reproducibility, throughput, and standardisation. In contrast, simpler 3D culture technology platforms may offer a scalable path forward and be ideally suited for early drug discovery. These systems can be readily integrated into high-throughput multi-well

formats, automated liquid-handling systems, and industrial workflows without requiring major capital investment or procedural overhauls. In this continuum, 3D bioprinting is valuable not because it automatically solves the complexity–scale trade-off, but because it offers a manufacturing language for controlling geometry, cell placement, and batch architecture across platforms.^{44,46}

Patient-derived organoids, for instance, are powerful tools for modelling tumour heterogeneity and drug response. However, they often exhibit batch-to-batch variability, require prolonged culture times, and depend on poorly defined matrices such as Matrigel®, derived from the Engelbreth–Holm–Swarm mouse sarcoma. Matrigel® shows substantial batch-to-batch variability, lacks tunable mechanical properties, and contains undefined growth factors that obscure experimental outcomes.⁶¹ Similarly, despite their sophistication, organ-on-a-chip systems often require manual fabrication, may incorporate multiple cell types that need different media compositions, pose scaling challenges, and require microfluidic expertise that limits adoption in high-throughput industrial environments. On the other end of the spectrum, simpler 3D spheroids or scaffold-based models, often relying on immortalised cell lines and standardised formats, offer greater reproducibility and throughput but lack the complex architecture and dynamic tissue interactions necessary to fully recapitulate *in vivo* human biology. These models may be sufficient for early toxicity or efficacy screens but are less useful for mechanistic studies or precision medicine applications. This complexity–scalability trade-off underscores a pressing need for standardised, scalable 3D platforms that retain key aspects of human physiology while being amenable to automation, quality control, and regulatory compliance. One key enabler of this balance is the choice of biomaterials used to fabricate these 3D models. Recent organoid bioprinting work reinforces this point: spatial patterning and controlled deposition can reduce stochasticity in organoid assembly, but only when paired with defined biomaterials and tightly controlled post-print maturation.^{44,45}

7. Biomaterial innovation: From natural and synthetic to semi-synthetic matrices

Historically, ECMs used in 3D cell culture have been naturally derived, including collagen, fibrin, hyaluronic acid, and basement membrane extracts such as Matrigel®. These materials are biologically active and typically offer cell-binding motifs and protease-sensitive degradation sites that support cell differentiation and tissue morphogenesis. However, they suffer from inherent limitations:

- Batch-to-batch variability, especially in tumour-derived products.
- Lack of tunability: mechanical stiffness, porosity, and degradation profiles are difficult to customise.
- Contamination risks, especially for clinical translation.
- High-viscosity, temperature-sensitive precursor solutions impede effective integration with high-throughput automated liquid handling.

In contrast, fully synthetic polymers (e.g., polyethylene glycol (PEG), polyacrylamide, PLGA) provide excellent control over mechanical and chemical properties, batch consistency, and manufacturability. However, they are often biologically inert unless extensively modified, and

even modified versions do not fully replicate the dynamic biological cues required for organotypic function.⁶² Although several fully synthetic ECM products have been available for decades, their adoption in complex tissue modelling has been limited by their inability to support cell differentiation, matrix remodelling, and dynamic tissue responses—features critical for *in vitro* modelling of native human biology.

To overcome these limitations, a new class of semi-synthetic materials has emerged. These hybrid matrices combine synthetic backbones with bioactive components (e.g., peptides, protein domains, ECM fragments) to achieve the precision of synthetic materials alongside the biological richness of natural ECMs. Semi-synthetic hydrogels can be engineered with defined stiffness, degradation profiles, and biofunctionality, enabling researchers to align the mechanical and biochemical properties of specific tissues. Notably, semi-synthetic matrices are tunable, reproducible, and compatible with automation and scaling, making them ideal for industrial applications in 3D cell culture and organoid manufacturing.

For instance, click-chemistry-based hydrogels enable precise cross-linking under physiological conditions, support both embedded and surface-seeded cultures, and provide photocrosslinkable control over stiffness, which is critical for mimicking soft tissues such as brain or tumour microenvironments, as well as the stiffer ECMs typically found in tumour microenvironments. These matrices can be integrated into standard multi-well formats and combined with machine learning tools to standardise matrix formulation based on cell type and desired phenotype. These same properties are also central to 3D bioprinting, where precursor rheological properties, cross-linking kinetics, stress shielding during deposition, and batch consistency directly determine print fidelity and cell survival; defined semi-synthetic matrices are therefore likely to become as important for regulatory-grade bioprinting as they already are for advanced 3D culture.^{44–46}

As the field evolves, we envision a tiered model ecosystem featuring high-throughput, standardised 3D models built on semi-synthetic matrices for early-stage screening, alongside more complex, patient-specific or organ-chip systems for downstream validation at a significantly smaller scale. This strategic layering of models will enable researchers to maximise biological relevance where necessary and efficiency where possible, thus accelerating the shift away from animal testing while maintaining scientific rigour, patient safety, and commercial viability. From a translational perspective, this also suggests that bioprinting should often be deployed as a downstream standardisation tool for the most informative assays, rather than forced indiscriminately into early ultra-high-throughput settings where simpler 3D cultures still offer the better engineering solution.^{44,46}

8. The Translation Challenge: Aligning Academic Innovation with Industrial and Regulatory Requirements

A persistent challenge in advancing NAMs is the fundamental misalignment between academic research practices and industrial operational realities. Academic research environments focus on discovery, mechanistic insight, and technological innovation. These objectives have driven remarkable progress in developing increasingly sophisticated human-relevant models.

However, this emphasis often results in systems that are highly customised, dependent on specialist expertise, tightly coupled to the laboratory in which they were developed, and often lack reproducibility. Experimental platforms may rely on poorly defined biological materials, bespoke protocols, or manual handling steps that limit reproducibility. As a consequence, many academically developed NAMs are difficult to scale, automate, and transfer across laboratories.

By contrast, industrial and regulatory stakeholders approach NAMs from a fundamentally different perspective: namely, biological relevance must be accompanied by operational robustness. NAM platforms must function with clearly defined inputs and outputs, demonstrate consistent performance across sites and over time, and integrate seamlessly into automated, high-throughput workflows. Moreover, the data generated must be suitable for regulatory evaluation, requiring traceability, documentation, and well-characterized sources of variability. When these criteria are not met, even scientifically compelling NAMs struggle to gain traction beyond exploratory or pilot use.

The absence of a shared framework to reconcile these divergent expectations has contributed significantly to stalled adoption. Academic researchers, industry practitioners, and regulators often operate with different implicit definitions of readiness and success, leading to misaligned development trajectories. Without a common language to describe the maturity of NAM platforms and their suitability for downstream use, promising innovations risk remaining confined to proof-of-concept demonstrations rather than progressing toward meaningful impact. The FDA iSTAND programme partially addresses this by providing a structured pathway and common terminology for qualifying novel drug development tools, including eligible NAMs, for a defined context of use. However, it does not provide a general framework for describing maturity from early proof of concept through technical evaluation, manufacturability, transferability through to routine adoption.

To address this gap, a structured approach to translational maturity is required. Traditional Technology Readiness Levels (TRLs), while effective for conventional engineering systems, are not fully applicable to NAMs. NAM platforms are inherently hybrid, integrating living biological systems with engineered materials, devices, and computational tools. Their translational challenges arise not only from technical feasibility but also from issues of reproducibility, standardization, scalability, and regulatory alignment—dimensions that are insufficiently captured by classical TRL frameworks.

In response, a Translational Readiness Level (TRL) framework tailored specifically to NAMs (TRL-NAM) provides a more appropriate lens for assessing and guiding the progression from academic research to industrial use (Figure 3). At the earliest stages, TRL-NAM levels 1 and 2 focus on biological feasibility. At this point, the primary objective is to demonstrate that a system captures a relevant aspect of human biology. Experimental setups are typically exploratory and bespoke, with limited consideration of scale, standardization, or transferability. These stages align closely with traditional academic research goals and are essential for establishing scientific credibility.

As technologies mature to TRL-NAM levels 3 and 4, the emphasis shifts toward reproducibility and experimental control. Materials and protocols become more clearly defined, quantitative

performance metrics are introduced, and initial benchmarking and repeatability assessments are conducted. Although scalability may not yet be fully realized, it is entering the design conversation, marking a transition from purely exploratory research toward translational intent.

TRL-NAM levels 5 and 6 mark a critical inflection point, where systems evolve from isolated experiments into reusable platforms. At this stage, compatibility with standard multi-well formats and automated workflows is a priority; components are selected based on manufacturability and characterization data. Reproducibility across laboratories is particularly important, as it signals that the platform no longer depends on tacit knowledge or local expertise. This phase is often where many NAMs falter, highlighting the importance of intentional platform-oriented design.

At higher levels, TRL-NAM stages 7 and 8 emphasise industrial and regulatory alignment. Technologies are deployed in pilot industrial settings and evaluated against clinically or regulatorily relevant endpoints. Considerations such as documentation, quality control, supply chain robustness, and cost become central, reflecting the realities of routine use in regulated environments. Finally, TRL-NAM level 9 corresponds to operational adoption, in which NAM platforms are used routinely in industrial decision-making, incorporated into regulatory submissions, and subject to continuous monitoring and improvement rather than experimental iteration.

Importantly, the TRL-NAM framework serves as a tool for transparency and alignment. By explicitly recognizing where a given NAM platform sits along the TRL-NAM spectrum, researchers, funders, industry partners, and regulators can engage more productively, set realistic expectations, and direct resources strategically. Awareness of translational readiness enables informed collaboration and targeted investment, helping ensure that academic innovation translates into practical, scalable solutions that support the broader transition away from animal-based testing.

The transition from animal testing to NAMs requires deliberate alignment between applied research, industrial implementation, and regulatory expectations. As shown in other industries such as aerospace and automotive, adopting a TRL framework offers a practical mechanism to accelerate this alignment. By integrating TRL-NAM thinking from the start of a project or grant proposal into university-driven NAM development, stakeholders can help ensure promising technologies advance beyond proof-of-concept and publications.

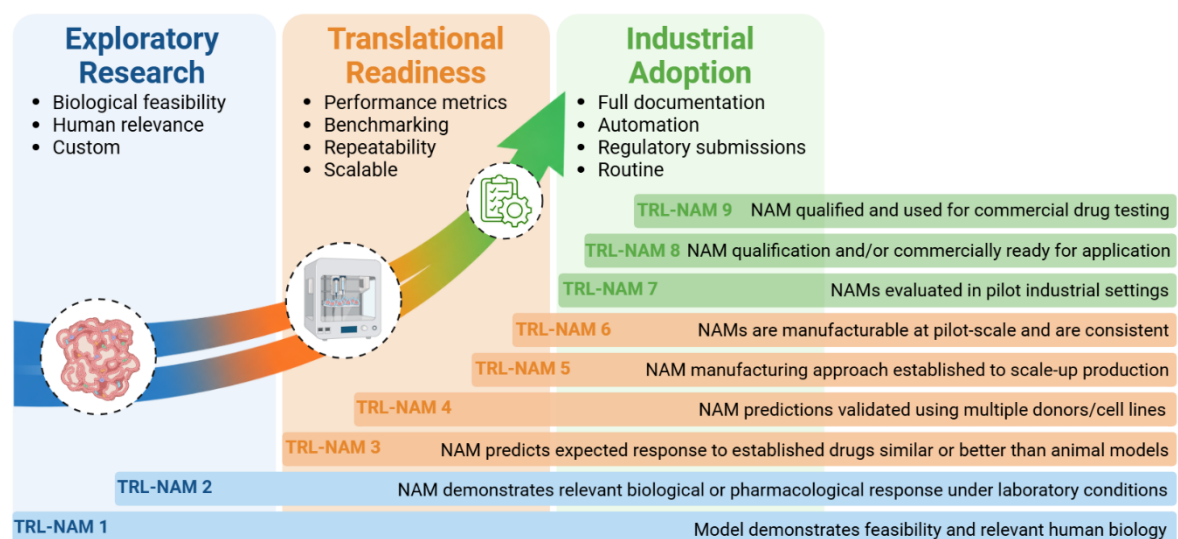


Figure 3. The TRL-NAM (Translational Readiness Level for New Approach Methodology platforms) framework. The framework defines nine stages that describe the progression from exploratory academic research to the industrial and regulatory adoption of NAM platforms, including organoids, microphysiological systems, and advanced 3D cultures. TRL-NAM emphasises scalability, reproducibility, platformisation, and regulatory alignment as critical determinants of successful translation. Readiness increases as it progresses from blue (early academic research) to orange to green (operational adoption). Created with BioRender.com.

9. Regulatory impact

Given the FDA's Roadmap to Reducing Animal Testing in Preclinical Safety Studies, it is now essential to distinguish hypothesis-driven research from regulatory readiness. Preclinical studies should not be judged by novelty or innovation (as academic projects are); they should be assessed on whether the evidence is fit for purpose, reproducible, and interpretable in the context of an existing regulatory question. This distinction matters in discussions of NAMs because the recent policy shift is not a blanket endorsement of any human-based model. Rather, it moves toward context-specific acceptance of validated technology platforms with clearly defined performance boundaries.^{1,8,10,11,41}

The FDA's March 2026 draft guidance on general NAM use is significant because it translates the agency's 2025 roadmap into a more practical validation framework. For simple engineered 3D cultures, this framework favors assays with defined materials, explicit endpoints, controls, and site-to-site reproducibility. For organoids, it supports applications where the biological question is narrow enough to bound and report variability, such as patient stratification or mechanism-based translational studies. For MPS, it offers a clearer path toward endpoint-specific qualification in toxicology and pharmacology, as long as sponsors can show consistent engineering performance and comparison against human or accepted reference data. For bioprinted systems, the message is equally clear: print geometry, bioink composition, and most importantly post-print maturation, and analytical readouts must all be treated as validated assay components, not as flexible laboratory technique variables.^{8,44-46}

The FDA's draft guidance from December 2025 on streamlined nonclinical safety studies for certain monospecific monoclonal antibodies is significant because it clearly highlights a

specific area where animal testing, especially involving non-human primates, can now be minimized. This development is particularly relevant for technologies like immune-enabled liver chips, blood-based cytokine release assays, advanced 3D immune co-cultures, and PBPK-supported risk assessments. These approaches may substitute for some lengthy and expensive animal studies that offer limited information. Moreover, the FDA's updated pyrogen and endotoxin guidance in March 2026 reinforces the idea that non-animal methods are not just for disease models or discovery stages. Instead, they are increasingly being incorporated into release testing and CMC decision-making, especially when performance standards are well defined.⁵⁹

Outside the U.S., regulatory convergence is taking shape. The MHRA's March 2026 guidance states that it is open to applications without animal studies if alternative methods sufficiently ensure safety, and it introduces an option for early review of Module 4 alone. EMA's March 2026 consultation on virtual control groups is more limited but strategically important, as it represents the first qualification opinion for a NAM used in toxicity assessment and shows European regulators' willingness to endorse incremental, context-specific animal-reduction tools. Alongside the approval of FDA Modernization Act 3.0 by both the Senate and the House of Representatives, these developments indicate a progressive shift away from animal-centric language towards a mechanism-based approach to nonclinical evaluation evidence.¹²

The surrounding ecosystem has also evolved. NIH has introduced Complement-ARIE awards to develop, standardise, and qualify NAMs. Europe has launched dedicated Horizon 2026 topics focused on NAM integration and coordination. Innovate UK and NC3Rs have initiated a competition centered on industrial human-relevant drug models. Additionally, ARDF, IAMPs, NAT-Net, UConn, and the Emirates Drug Establishment demonstrate a broader institutional push towards validation infrastructure, training, and market development.^{13–20} For model systems, the implications vary but are significant: MPS now benefit from greater industrial and regulatory backing; organoids are advancing further into translational and precision-medicine applications; simple engineered 3D cultures continue to be the most scalable for deployment; and 3D bioprinting is becoming more important in contexts where standardised production, architectural precision, and supply-chain reliability are essential for regulatory approval trust.^{17,18,44–46}

10. Conclusion

This article highlights the importance of matching NAMs to specific research or commercial applications, understanding which endpoints are currently feasible, and integrating biofabrication and validation standards from the outset. In this context, bioprinting is particularly important in the NAM field. Its main short-term role may not be to replace all other 3D model platforms, but to improve the standardization, auditability, and transferability of advanced models—especially organoids and MPS—across labs and ultimately in regulatory submissions. Organoid and MPS production need greater standardization, and analytical endpoints should be benchmarked with clinically relevant data. Acceptance criteria across agencies also continue to evolve. However, the overall direction is now clearer than ever.

Actions taken by the FDA, MHRA, and EMA, along with significant funding and coordination efforts in the U.S., Europe, the UK, Australia, and the UAE, indicate a shift from advocacy to practical implementation. Human 3D cell cultures and tissue models now lead this transition, delivering more predictive data and driving broader adoption of bioprinting for its control and reproducibility as part of a complex biofabrication technology platform.

Acknowledgments

Figures 1–3 were created using BioRender.com.

Funding

The authors acknowledge funding from the Max Planck Queensland Centre (MPQC) and the Australian Research Council (ARC DP230102934 awarded to D.W.H.). R.B. and M.M.S. acknowledge funding from the Engineering and Physical Sciences Research Council (EP/T020792/1). M.M.S. acknowledges funding from the Department of Science, Innovation and Technology (DSIT) and the Royal Academy of Engineering under the Chair in Emerging Technologies programme (CiET2021\94), the University of Oxford Strategic Research Fund, and the Rosetrees Trust.

Conflict of interest

Prof. Dietmar W. Hutmacher is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. C.M. is a shareholder, Director, and CEO of Gelomics. P.L. is a shareholder, Director, and COO of Gelomics. T.T., T.K., M.M.S., G.R., and D.W.H. act as strategic advisors to Gelomics; T.K. and D.W.H. also hold shares in the company, and M.M.S. holds share options. R.B. and M.M.S. are inventors on a patent (2414356.2/GB/PRV) related to a novel bioengineered microphysiological chip.

Author contributions

Conceptualization: Christoph Meinert, Peter Levett, Travis Klein, Molly M. Stevens, Dietmar W. Hutmacher

Funding acquisition: Molly M. Stevens, Dietmar W. Hutmacher

Writing—original draft: All authors

Writing—review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

Further disclosure

The authors acknowledge the use of ChatGPT (GPT-5.5, OpenAI, Grammarly) to assist with manuscript organisation and language refinement. The graphical abstract was generated with the assistance of OpenAI ChatGPT based on instructions provided by the authors and subsequently refined by the authors.

References

1. U.S. Food and Drug Administration. Roadmap to Reducing Animal Testing in Preclinical Safety Studies. Published online 2025. <https://www.fda.gov/media/186092/download>
2. Wong CH, Siah KW, Lo AW. Estimation of clinical trial success rates and related parameters. *Biostatistics*. 2018;20(2):273-286. doi:10.1093/biostatistics/kxx069
3. Filipova D, Boomgaarden W, Fox L, et al. Need for NAMs: A systematic evidence synthesis revealing over half a century of drug development failure. *NAM Journal*. 2026;2:100119. doi:10.1016/j.namjnl.2026.100119
4. Mullane K, Williams M. Alzheimer's disease beyond amyloid: Can the repetitive failures of amyloid-targeted therapeutics inform future approaches to dementia drug discovery? *Biochemical Pharmacology*. 2020;177:113945-113945. doi:10.1016/j.bcp.2020.113945
5. Cummings J, Lee G, Nahed P, et al. Alzheimer's disease drug development pipeline: 2022. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*. 2022;8(1):e12295-e12295. doi:10.1002/trc2.12295
6. National Academies of Sciences, Engineering, and Medicine. Nonhuman Primate Models in Biomedical Research: State of the Science and Future Needs. *The National Academies Press*. Published online June 2023. doi:10.17226/26857

7. Stewart A, Denoyer D, Gao X, Toh YC. The FDA modernisation act 2.0: Bringing non-animal technologies to the regulatory table. *Drug Discov Today*. 2023;28(4):103496. doi:10.1016/j.drudis.2023.103496
8. U.S. Food and Drug Administration. General Considerations for the Use of New Approach Methodologies in Drug Development. April 29, 2026. Accessed May 15, 2026. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-considerations-use-new-approach-methodologies-drug-development>
9. U.S. Food and Drug Administration. Monoclonal Antibodies: Streamlined Nonclinical Safety Studies. December 3, 2025. Accessed May 15, 2026. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/monoclonal-antibodies-streamlined-nonclinical-safety-studies>
10. Medicines and Healthcare products Regulatory Agency. MHRA approach to medicines using non-animal methods. GOV.UK. Accessed May 15, 2026. <https://www.gov.uk/guidance/mhra-approach-to-medicines-using-non-animal-methods>
11. European Medicines Agency. EMA consults on virtual control groups to help reduce animal use in medicines development. March 31, 2026. Accessed May 15, 2026. <https://www.ema.europa.eu/en/news/ema-consults-virtual-control-groups-help-reduce-animal-use-medicines-development>
12. U.S. Congress. S.355 - 119th Congress (2025-2026): FDA Modernization Act 3.0. December 17, 2025. Accessed May 15, 2026. <https://www.congress.gov/bill/119th-congress/senate-bill/355>
13. National Institutes of Health. NIH invests \$150 million in human-based research to reduce use of animal models. Accessed May 15, 2026. <https://www.nih.gov/news-events/news-releases/nih-invests-150-million-human-based-research-reduce-use-animal-models>
14. European Health and Digital Executive Agency (HaDEA). 2026 Horizon Europe “Health” calls for proposals - “Developing and using new tools, technologies and digital solutions for a healthy society.” Accessed May 15, 2026. https://hadea.ec.europa.eu/news/2026-horizon-europe-health-calls-proposals-2026-02-12_en
15. Innovate UK. Contracts for Innovation: industrial human relevant drug models. March 2, 2026. Accessed May 15, 2026. <https://www.ukri.org/opportunity/contracts-for-innovation-industrial-human-relevant-drug-models/>
16. Alternatives Research and Development Foundation. Annual Open Grants - Alternatives Research and Development Foundation. Accessed May 15, 2026. <https://www.ardf-online.org/ardf-grants.html>
17. Industry Alliance for Microphysiological Systems. Industry Leaders Announce World’s First Microphysiological Systems Industry Association (IAMPS). Accessed May 15, 2026. <https://iamps.eu/industry-leaders-announce-worlds-first-microphysiological-systems-industry-association-iamps/>
18. NSW Health Office for Health and Medical Research. The Non-Animal Technologies Network welcomes first independent executive chair to guide future growth. Office for Health and Medical Research. March 10, 2026. Accessed May 15, 2026. <https://medicalresearch.nsw.gov.au/about/news/2026/non-animal-technologies-network-welcomes-chair>

19. Murray S. Gift Seeds Non-Animal Testing Organ-on-a-Chip Initiative at UConn School of Pharmacy. UConn Today. February 10, 2026. Accessed May 15, 2026. <https://today.uconn.edu/2026/02/gift-seeds-non-animal-testing-organ-on-a-chip-initiative-at-uconn-school-of-pharmacy/>
20. Emirates News Agency. Emirates Drug Establishment showcases 'Organ on Chip' project at World Health Expo 2026. Accessed May 15, 2026. <https://www.wam.ae/en/article/byoqwwn-emirates-drug-establishment-showcases-'organ-chip'>
21. Xie R, Pal V, Yu Y, et al. A comprehensive review on 3D tissue models: Biofabrication technologies and preclinical applications. *Biomaterials*. 2024;304:122408. doi:10.1016/j.biomaterials.2023.122408
22. Ingber DE. Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nature Reviews Genetics*. 2022;23(8):467-491. doi:10.1038/s41576-022-00466-9
23. Zhao Y, Landau S, Okhovatian S, et al. Integrating organoids and organ-on-a-chip devices. *Nat Rev Bioeng*. 2024;2(7):588-608. doi:10.1038/s44222-024-00207-z
24. Lawlor KT, Vanslambrouck JM, Higgins JW, et al. Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation. *Nat Mater*. 2021;20(2):260-271. doi:10.1038/s41563-020-00853-9
25. Wang H, Brown PC, Chow ECY, et al. 3D cell culture models: Drug pharmacokinetics, safety assessment, and regulatory consideration. *Clin Transl Sci*. 2021;14(5):1659-1680. doi:10.1111/cts.13066
26. Abuwatfa WH, Pitt WG, Hussein GA. Scaffold-based 3D cell culture models in cancer research. *J Biomed Sci*. 2024;31(1):7. doi:10.1186/s12929-024-00994-y
27. MacArron R, Banks MN, Bojanic D, et al. Impact of high-throughput screening in biomedical research. *Nature Reviews Drug Discovery*. 2011;10(3):188-195. doi:10.1038/nrd3368
28. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459(7244):262-265. doi:10.1038/nature07935
29. Thorel L, Perréard M, Florent R, et al. Patient-derived tumor organoids: a new avenue for preclinical research and precision medicine in oncology. *Experimental & Molecular Medicine*. 2024;56(7):1531-1551. doi:10.1038/s12276-024-01272-5
30. Mukhopadhyay C, Paul MK. Organoid-based 3D in vitro microphysiological systems as alternatives to animal experimentation for preclinical and clinical research. *Arch Toxicol*. 2023;97(5):1429-1431. doi:10.1007/s00204-023-03466-8
31. Benam KH, Novak R, Nawroth J, et al. Matched-Comparative Modeling of Normal and Diseased Human Airway Responses Using a Microengineered Breathing Lung Chip. *Cell Syst*. 2016;3(5):456-466.e4. doi:10.1016/j.cels.2016.10.003

32. Ikeo S, Tani Y, Sawayama J, et al. Dynamic in vitro platform for mechanical profiling of human pulmonary aciniform organoids via intraluminal access. *Biomaterials*. 2026;331:124094. doi:10.1016/j.biomaterials.2026.124094
33. Wensink GE, Elias SG, Mullenders J, et al. Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. *NPJ Precis Oncol*. 2021;5(1):30. doi:10.1038/s41698-021-00168-1
34. Zhou Z, Cong L, Cong X. Patient-Derived Organoids in Precision Medicine: Drug Screening, Organoid-on-a-Chip and Living Organoid Biobank. *Frontiers in Oncology*. 2021;11:762184-762184. doi:10.3389/fonc.2021.762184
35. Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science*. 2018;359(6378):920-926. doi:10.1126/science.aao2774
36. Sachs N, de Ligt J, Kopper O, et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell*. 2018;172(1-2):373-386.e10. doi:10.1016/j.cell.2017.11.010
37. Tong L, Cui W, Zhang B, et al. Patient-derived organoids in precision cancer medicine. *Med*. 2024;5(11):1351-1377. doi:10.1016/j.medj.2024.08.010
38. Picollet-D'hahan N, Zuchowska A, Lemeunier I, Gac SL. Multiorgan-on-a-Chip: A Systemic Approach To Model and Decipher Inter-Organ Communication. *Trends in Biotechnology*. 2021;39(8):788-810. doi:10.1016/j.tibtech.2020.11.014
39. Ewart L, Apostolou A, Briggs SA, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. *Communications Medicine*. 2022;2(1):1-16. doi:10.1038/s43856-022-00209-1
40. Portela JMD, Paul P, Moriarty O, et al. Review on organs-on-chips for medicines safety assessment: A European regulatory perspective. *ALTEX*. 2026;43(1):98-112. doi:10.14573/altex.2502061
41. Edwards M, Blanquie O, Moriarty O, Beken S, Ehmann F. New approach methodologies: EU regulatory horizons. *Nat Rev Drug Discov*. 2025;24(8):571-572. doi:10.1038/d41573-025-00053-7
42. Rothbauer M, Eilenberger C, Spitz S, et al. Recent Advances in Additive Manufacturing and 3D Bioprinting for Organs-On-A-Chip and Microphysiological Systems. *Front Bioeng Biotechnol*. 2022;10. doi:10.3389/fbioe.2022.837087
43. Tebon PJ, Wang B, Markowitz AL, et al. Drug screening at single-organoid resolution via bioprinting and interferometry. *Nature Communications*. 2023;14(1):1-16. doi:10.1038/s41467-023-38832-8
44. Huang MS, Christakopoulos F, Roth JG, Heilshorn SC. Organoid bioprinting: from cells to functional tissues. *Nature Reviews Bioengineering*. 2024;3(2):126-142. doi:10.1038/s44222-024-00268-0
45. Hua W, Gaharwar AK. 3D biofabricated in vitro models as new approach methodologies for animal alternatives. *npj Biomed Innov*. 2026;3(1):20. doi:10.1038/s44385-026-00073-x

46. Wu Z, Liu R, Shao N, Zhao Y. Developing 3D bioprinting for organs-on-chips. *Lab Chip*. 2025;25(5):1081-1096. doi:10.1039/d4lc00769g
47. Laubenbacher R, Mehrad B, Shmulevich I, Trayanova N. Digital twins in medicine. *Nature Computational Science*. 2024;4(3):184-191. doi:10.1038/s43588-024-00607-6
48. Raghavan S, Winter PS, Navia AW, et al. Microenvironment drives cell state, plasticity, and drug response in pancreatic cancer. *Cell*. 2021;184(25):6119-6137.e26. doi:10.1016/j.cell.2021.11.017
49. Viceconti M, Pappalardo F, Rodriguez B, Horner M, Bischoff J, Musuamba Tshinanu F. In silico trials: Verification, validation and uncertainty quantification of predictive models used in the regulatory evaluation of biomedical products. *Methods*. 2021;185:120-127. doi:10.1016/j.jymeth.2020.01.011
50. Xu Z, Ren F, Wang P, et al. A generative AI-discovered TNIK inhibitor for idiopathic pulmonary fibrosis: a randomized phase 2a trial. *Nat Med*. 2025;31(8):2602-2610. doi:10.1038/s41591-025-03743-2
51. Haja A, Horcas-Nieto JM, Bakker BM, Schomaker L. Towards automatization of organoid analysis: A deep learning approach to localize and quantify organoid images. *Computer Methods and Programs in Biomedicine Update*. 2023;3:100101-100101. doi:10.1016/j.cmpbup.2023.100101
52. European Commission. Summary Report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2022. Published online 2024. Accessed May 15, 2026. <https://circabc.europa.eu/ui/group/8ee3c69a-bccb-4f22-89ca-277e35de7c63/library/051e5787-7746-46cf-8a0d-310f84fd1900/details>
53. Wang J, Tao X, Zhu J, et al. Tumor organoid-immune co-culture models: exploring a new perspective of tumor immunity. *Cell Death Discovery* 2025 11:1. 2025;11(1):1-17. doi:10.1038/s41420-025-02407-x
54. Dijkstra KK, Cattaneo CM, Weeber F, et al. Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell*. 2018;174(6):1586-1598.e12. doi:10.1016/j.cell.2018.07.009
55. Wagar LE, Salahudeen A, Constantz CM, et al. Modeling human adaptive immune responses with tonsil organoids. *Nat Med*. 2021;27(1):125-135. doi:10.1038/s41591-020-01145-0
56. Maulana TI, Teufel C, Cipriano M, et al. Breast cancer-on-chip for patient-specific efficacy and safety testing of CAR-T cells. *Cell Stem Cell*. 2024;31(7):989-1002.e9. doi:10.1016/j.stem.2024.04.018
57. Hünig T. The storm has cleared: lessons from the CD28 superagonist TGN1412 trial. *Nature Reviews Immunology*. 2012;12(5):317-318. doi:10.1038/nri3192
58. Yao J, Peretz J, Bebenek I, et al. FDA/CDER/OND Experience With New Approach Methodologies (NAMs). *Int J Toxicol*. 2026;45(2):136-156. doi:10.1177/10915818251384270

59. U.S. Food and Drug Administration. Pyrogen and Endotoxins Testing: Questions and Answers. March 18, 2026. Accessed May 15, 2026. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pyrogen-and-endotoxins-testing-questions-and-answers>
60. Blundell C, Yi YS, Ma L, et al. Placental Drug Transport-on-a-Chip: A Microengineered In Vitro Model of Transporter-Mediated Drug Efflux in the Human Placental Barrier. *Advanced Healthcare Materials*. 2018;7(2):1700786. doi:10.1002/adhm.201700786
61. Hughes CS, Postovit LM, Lajoie GA. Matrigel: A complex protein mixture required for optimal growth of cell culture. *PROTEOMICS*. 2010;10(9):1886-1890. doi:10.1002/pmic.200900758
62. Klein L, Hutmacher DW. Straddling the Line Between In Vitro and Ex Vivo Investigations. *Tissue Eng Part C Methods*. 2024;30(10):443-451. doi:10.1089/ten.tec.2024.0246