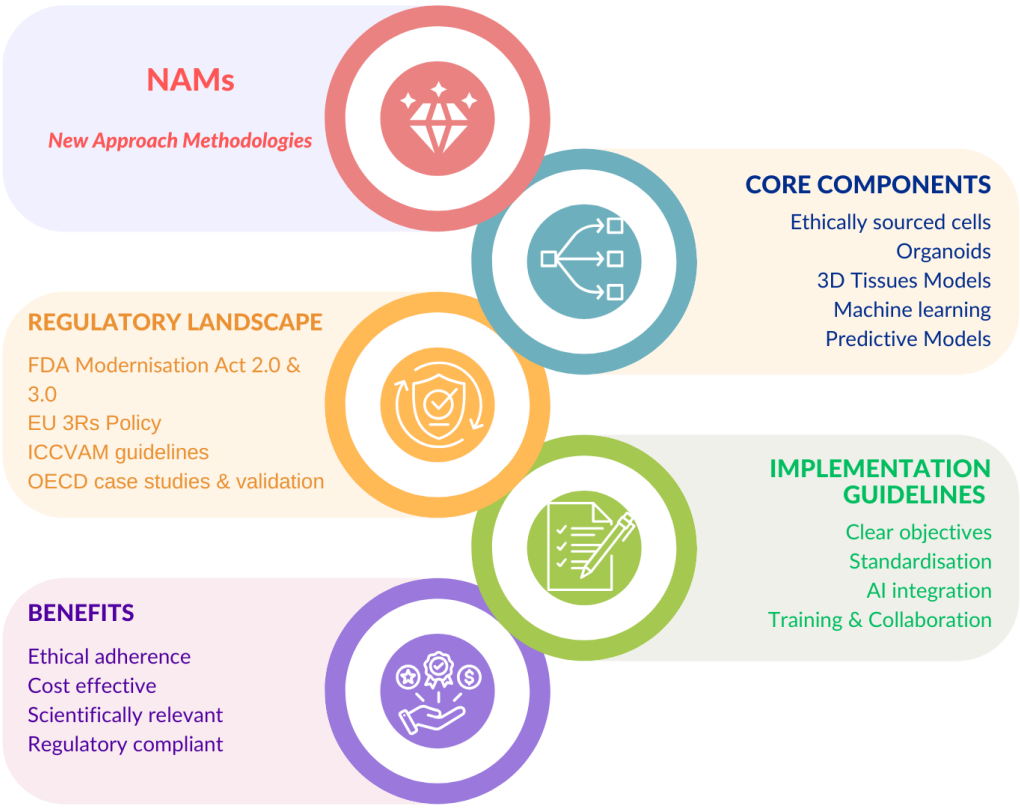
Key Guidance in Adopting and Integrating Non-Animal based New Approach Methodologies

S. Krishnan, PhD, R. Bauri, PhD, P. Parkali, M. Pharm, S. Dravida, PhD

Abstract

Non-animal New Approach Methodologies (NAMs) are transforming pharmaceutical and biotherapeutic research by combining human cell and tissue models with advanced computational tools. These strategies offer ethical, cost-effective, and scientifically relevant alternatives to traditional methods. NAMs are emerging as a revolutionary approach in human drug discovery and assessment, providing improved safety and efficacy evaluations. Non-animal approaches are gaining attention due to concerns with traditional methods, such as ethical issues, low relevance to human physiology, inefficiency, and poor clinical predictability. These innovative approaches enhance physiological relevance and data analysis. As global efforts to promote NAMs grow, clear guidelines and regulatory frameworks are essential for seamless integration. This document provides preliminary guidelines for businesses and authorities in areas lacking established frameworks.



A graphical overview of the all-rounded aspect for implementing non-animal based New Approach Methods (NAMs) in human drug discovery and assessment

Introduction

In the absence of definitive guidelines for non-animal testing methods, it is crucial to establish foundational principles for integrating NAMs into research. Traditionally, animal testing has been the standard for pharmacological assessments, yet its limitations in predicting human responses hinder drug development efficiency. NAMs provide a structured alternative using human-derived biological systems, AI-driven models, and high-throughput *in vitro* screening to enhance accuracy and reproducibility. While global regulatory bodies acknowledge NAMs, challenges remain in achieving uniform implementation.

The push for NAM adoption has evolved over two decades, driven by organizations like the Organization for Economic Co-operation and Development (OECD) and Food and Drug Administration (FDA) advocating for alternatives in cosmetic and chemical testing. NAMs

offer efficiency, cost-effectiveness, and ethical advantages, accelerating their acceptance despite regulatory hurdles. Industry leaders now focus on reducing, refining, and replacing animal testing, with AI and *in vitro* technologies revolutionizing drug discovery.

Regulatory bodies such as the FDA, European Medical Agency (EMA), OECD, and Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) increasingly endorse NAMs, yet inconsistent guidelines hinder widespread adoption. Case study of Dasari et al. highlighted the effectiveness of digital potency measurement as an alternative to animal-based antisera testing[1], while Taylor et al. emphasized alternative toxicology assessment methods[2]. Additionally, Health Canada's Bill C-47 reinforces ethical non-animal testing practices[3] . This article examines NAMs applications, global case studies, and key considerations for their integration into biological research.

Core Components of NAMs

New Approach Methodologies (NAMs), in a broad perspective, comprise of *in vitro*, *in chemico*, and *in silico* approaches with comparable endpoints to the traditional approaches (Fig. 1). These can be implemented independently, synergistically, or additively to achieve more precise outcomes. While biopharmaceutical companies have recently and innovatively focused on *in vitro/ex vivo* systems, with or without artificial intelligence integration, recent breakthroughs in stem cell research and the creation of human microphysiological systems offer promising alternatives. These advanced *in vitro* models closely recreate physiological conditions, enhancing relevance and accuracy. The combination of artificial intelligence and advanced *in vitro* systems generates vast datasets, enabling more precise translational predictions from the laboratory to the human body and ultimately reducing drug failures in clinical trials[4,5].

A brief discussion of the major components of NAMs relevant to the biopharmaceutical industry follows below:

1. **Human Microphysiological Systems**

NAMs employ various human-relevant approaches, with human microphysiological systems (hMPS) being a key method. Using ethically sourced progenitor cells, hMPS replicates organ- and tissue-specific environments, enhancing disease modeling, drug research, and toxicology studies. While hMPS is a widely investigated and targeted model for recreating the

physiological system, it is not the only viable option. Other advanced alternatives, such as computational models, 3D bioprinting, and organoids hold significant potential in specific situations to further improve research accuracy and reliability.

The study by Dasari et al. on 2022 emphasized alternative models, demonstrating the use of human surrogate primary progenitor stromal cells for *in vitro* profiling[6]. Similarly, Smirnova et al. in 2024 reviewed *in vitro* models replicating human physiological processes, highlighting their utility in neurotoxicity and developmental toxicity assessments[7]. Their findings stress the need for refined human-relevant methodologies to enhance predictive accuracy in research. Aligning with this, Dasari et al. in 2024, introduced a novel strategy using human microphysiological systems to assess neurovirulence and neurotoxicity in vaccine development[8]. These findings underscore the need of adopting non-animal approaches for safer, more accurate biomedical outcomes. Considering the potential of microphysiological system in revolutionizing drug development, the WHO Expert Committee also highlighted the importance of 3D tissue models for antiviral drug development[9] in 2024.

2. Artificial Intelligence-Driven Analytical Framework

Artificial intelligence (AI) is driving a global transformation in drug discovery and assessment. AI accelerates target identification, optimization, testing, and trial efficiency[10,4]. Key industry players are increasingly adopting generative and predictive AI in their discovery pipelines. According to Notified-GlobeNewswire, the global AI healthcare market, reflecting its wide-ranging applications across the sectors, was valued at $7.9 billion in 2021 and projected to reach $201.3 billion by 2030[11]. AI applications in NAM-based research include optimizing experimental protocols, trend identification, and processing extensive datasets with real-time and predictive analysis. The integration and high quality of large datasets, rigorous training, and multi-class data significantly enhance model efficacy. AI improves data accuracy, robustness, regulatory compliance, and reduces experimental variability[12,13]. Machine learning aids in novel biomarker identification and accelerates drug discovery, leading to cost-effective preclinical and non-clinical setups[14].

AI enhances precision by integrating genetic, molecular, and pharmacokinetic data in NAM-based research. Standardized AI-driven models improve regulatory compliance and

experimental reliability. Machine learning further optimizes drug discovery, minimizing the need for costly preclinical trials.

The potential of integrated AI and *in vitro* models was demonstrated by Dasari et al. (2022), who examined toxicogenomic patterns in human microphysiological systems[15]. Liu et al. (2023) and others explored AI-driven innovations in NAM research[16], while Liron et al. studied regulatory validation of AI-assisted models[17].

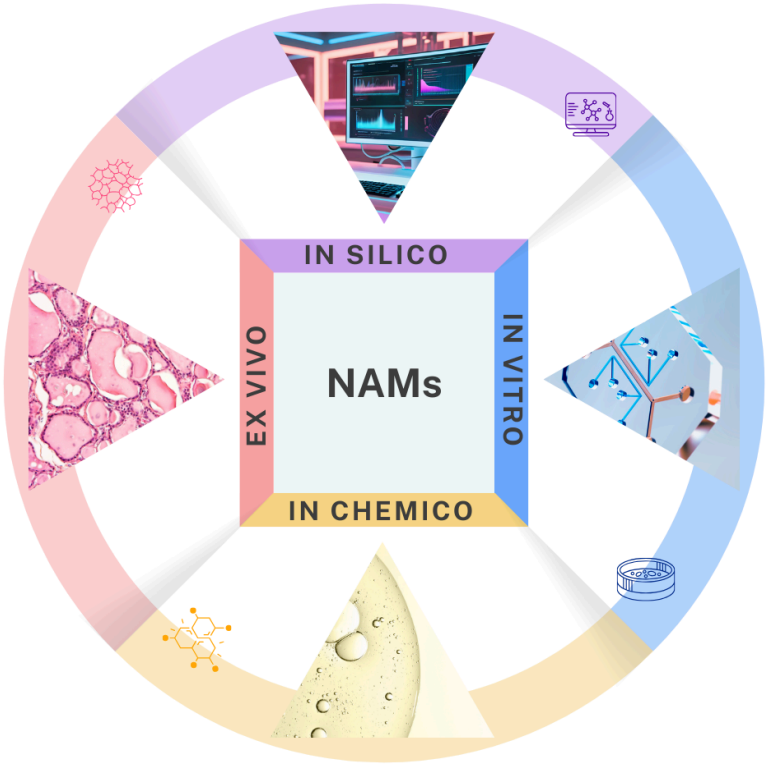


Fig 1: Components of New Approach Methodologies (NAMs)[18]

Preliminary Guidelines for the Implementation of NAMs

A. Establish Explicit Objectives

Implementing organizations should define clear objectives for integrating New Approach Methodologies (NAMs) in drug development, toxicity screening, and disease modeling. Aligning goals with regulatory expectations ensures credibility and acceptance. For instance, reducing animal testing by validating *in vitro* and *in silico* models for early-stage drug screening is a concrete objective. Regulatory bodies like EMA and FDA recognize

computational models such as PBPK modeling for predicting drug absorption, distribution, metabolism, and excretion[19,20].

For toxicity screening, human-derived cell systems in high-throughput tests can assess chemical toxicity efficiently, as demonstrated by the EPA's ToxCast program[21]. In disease modeling, human microphysiological systems enhance translational success and predict drug-induced organ toxicity[22]. Regulatory bodies increasingly recognize these models. Guidelines like the OECD's Good In Vitro Method Practices (GIVIMP) ensure reliability and regulatory acceptance[23].

B. Utilize Ethical Resources

Compliance with legal and regulatory standards is ensured by using ethically sourced human tissues and cells. Transparent procurement documentation and adherence to bioethics guidelines reinforce credibility and facilitate regulatory approvals.

C. Invest in Standardization and Protocol Development

Standardizing NAMs protocols ensures reproducibility, regulatory adherence, and scientific acceptance. Collaboration between regulatory bodies and research institutions can establish benchmarks for NAM-based methodologies. Key aspects include:

a. **Interlaboratory Validation:** Multi-site validation studies assess protocol robustness.

b. **Harmonized Data Reporting:** Aligning with frameworks like OECD Test Guidelines ensures cross-study comparisons.

c. **Quality Control & Benchmarking:** NAMs must undergo proficiency testing and adhere to GLP guidelines[24].

d. **AI & Computational Model Integration:** Standardized validation frameworks ensure AI-driven toxicology predictions meet regulatory standards.

e. **Regulatory-Scientific Cooperation:** Continuous collaboration aligns regulatory approvals with scientific advancements.

D. Integrate AI Technologies

AI-driven platforms efficiently synthesize biological data. AI models trained on validated datasets can predict drug toxicity and efficacy, enhancing preclinical research. Additional AI validation may be required to ensure accuracy and consistency.

E. Standardize Assay Protocols

Structured training programs for research teams are essential for utilizing NAMs platforms and interpreting AI-generated data. Cross-disciplinary collaborations and continuous education enhance proficiency in non-animal methodologies.

F. Invest in Training and Infrastructure

Providing training on NAMs platforms minimizes errors and biases. Continuous education on NAMs advancements helps teams stay informed and boosts productivity.

G. Collaborate

Regulatory agencies, academic institutions, and industry leaders must collaborate to refine and validate NAMs. Cross-validation studies support broader adoption into regulatory frameworks. Transcell Biologics' DART platform exemplifies AI-driven and human-relevant testing models facilitating this transition.

H. Remain Contemporary

Organizations must stay updated on evolving regulations such as the FDA Modernization Act, the EU's 3Rs Strategy, and policies in Canada and Japan. Aligning NAMs strategies with regulatory developments ensures a smooth transition from animal-based to human-relevant testing approaches.

Perspectives on Regulation: Handling the Changing Environment

Regulatory frameworks for NAMs remain inconsistent across regions. While organizations like the FDA in the U.S. have begun integrating NAMs into guidelines, others require more validation before widespread acceptance. The European Medicines Agency (EMA) supports the 3Rs principle (Replacement, Reduction, Refinement) and endorses alternative methods when scientifically viable[25].

The FDA Modernization Act 3.0 (2023) "removed the mandatory requirement for animal testing in drug development, enabling non-animal methodologies to be considered in regulatory submissions"[26]. Similarly, Canada's Bill C-47 "encourages the implementation of non-animal testing methodologies to promote ethical compliance in drug safety assessments"[3].

Despite progress, global standardization challenges persist. Regulatory agencies demand consistent, reproducible data, necessitating internationally recognized guidelines through harmonized validation studies and cross-regional collaborations. To establish smooth regulatory guidance and harmonized validation, regulatory bodies encourage the development and implementation of alternative methodologies to supplement, diminish, and optimize animal use in testing human and veterinary medicinal products[2]. Regulations for NAMs in veterinary pharmaceuticals are also outlined in the EMA/CHMP/CVMP/3Rs (2016) reflection paper[27].

These updates highlight evolving regulatory perspectives on NAMs and ongoing efforts to standardize alternative testing across regions.

Conclusion

These General guidance establish a preliminary framework for the integration of NAMs in the absence of established policies. Utilizing human microphysiological systems, AI-driven analytics, and standardized testing methods enables enterprises to achieve enhanced precision, cost efficiency, and ethical compliance in biomedical research. Continuous collaboration among academic institutions, regulatory agencies, and industry participants will be crucial in enhancing and broadening the global applicability of NAMs.

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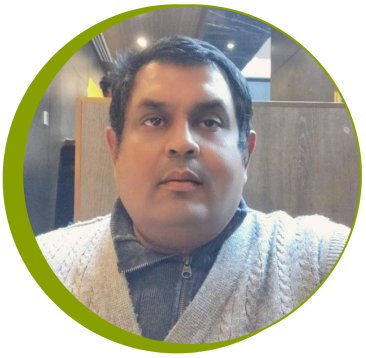
About the authors



Krishnan Sivaraman, PhD, Science Consultant

Krishnan Sivaraman is a PhD in the field of chromatin biology with vast experience in genetic engineering, epigenetics, and mammalian cell culture. His current position in Transcell Biologics focuses on integrating New Approach Methodologies (NAMs) for biopharmaceutical testing and advancing non-animal testing models.

Rathin Bauri, PhD, Application Specialist

Rathin Bauri is a dedicated life sciences researcher with expertise in cytobiology and molecular biology. He is currently associated with Transcell Biologics Pvt. Ltd. He is responsible for establishing a bridge between science and commercial application of Non animal based methods.

Praveen Parkali, M. Pharm, Sales & Application Specialist

Praveen has a diverse background and holds a master's degree in Pharmaceutical Sciences. Driven by a passion for science and the commercialization of technology. He is currently associated with Transcell Biologics Pvt. Ltd. where he bridges the gap between scientific innovation and commercial applications of DART as a part of Business Development.

Subhadra Dravida, PhD, Founder & CEO

Subhadra Dravida is an experimentalist turned bioentrepreneur, the Founder of Transcell Group companies. She is an innovator and manages the business of Transcell Biologics that offers a breakthrough animal-free testing strategy as a next gen enterprise solution to the global pharma and biopharma markets.