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Review

Modernizing Preclinical Drug Development: The Role of New Approach Methodologies

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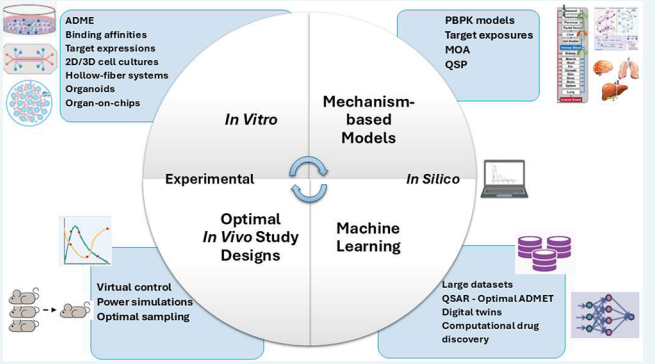
ABSTRACT: Over 90% of investigational drugs fail during clinical development, largely due to poor translation of pharmacokinetic, efficacy, and toxicity data from preclinical to clinical settings. The high costs and ethical concerns associated with translational failures highlight the need for more efficient and reliable preclinical tools. Human-relevant new approach methodologies (NAMs), including advanced in vitro systems, in silico mechanistic models, and computational techniques like artificial intelligence and machine learning, can improve translational success, as evident by several literature examples. Case studies on physiologically based pharmacokinetic modeling and quantitative systems pharmacology applications demonstrate the potential of NAMs in improving translational accuracy, reducing reliance on animal studies. Additionally, mechanistic modeling approaches for drug-induced liver injury and tumor microenvironment models have provided critical insights into drug safety and efficacy. We propose a structured and iterative "a priori in silico" workflow that integrates NAM components to actively guide preclinical study design—a step toward more predictive and resource-efficient drug development. The proposed workflow can enable in vivo predictions to guide the design of reduced and optimal preclinical studies. The findings from these preclinical studies can then be used to refine computational models to enhance the accuracy of human predictions or guide additional preclinical studies, as needed. To conclude, integrating computational and in vitro NAM approaches can optimize preclinical drug development, improving translational accuracy and reducing clinical trial failures. This paradigm shift is further supported by global regulations, such as the FDA Modernization Act 2.0 and EMA directive 2010/63/EU, underscoring the regulatory momentum toward adopting human-relevant NAMs as the new standard in preclinical drug development.



KEYWORDS: *new approach methodologies (NAM), physiologically based pharmacokinetic (PBPK), quantitative systems pharmacology (QSP), organ-on-chip, microphysiological system, preclinical drug development*

Animal experimentation has been used in medical science since ancient history and continues to be a part of the pharmaceutical research and development process.[1] In the 1930s, following the sulfanilamide tragedy, the US Food and Drug Administration (FDA) followed by other regulatory authorities mandated in vivo animal studies, especially for the safety evaluation, of investigational therapeutics.[2] In the last century, in vivo models played a crucial role in advancing drug discovery and development by providing insights into biological and disease mechanisms and for testing drug safety and efficacy.

Key questions to assess during the preclinical drug development stage include identifying the appropriate target receptor, enzyme, or gene, predicting human safety and efficacy, and determining the optimal human dosing regimen, among others. Accurate translation from preclinical studies to the clinical setting is critical for selecting a successful drug candidate for further development. Despite promising preclinical results, over 90% of drugs fail during clinical development. The primary reasons for these failures are lack of efficacy and unacceptable toxicity in humans.[3,4] The key reasons for preclinical in vivo models' inability to accurately

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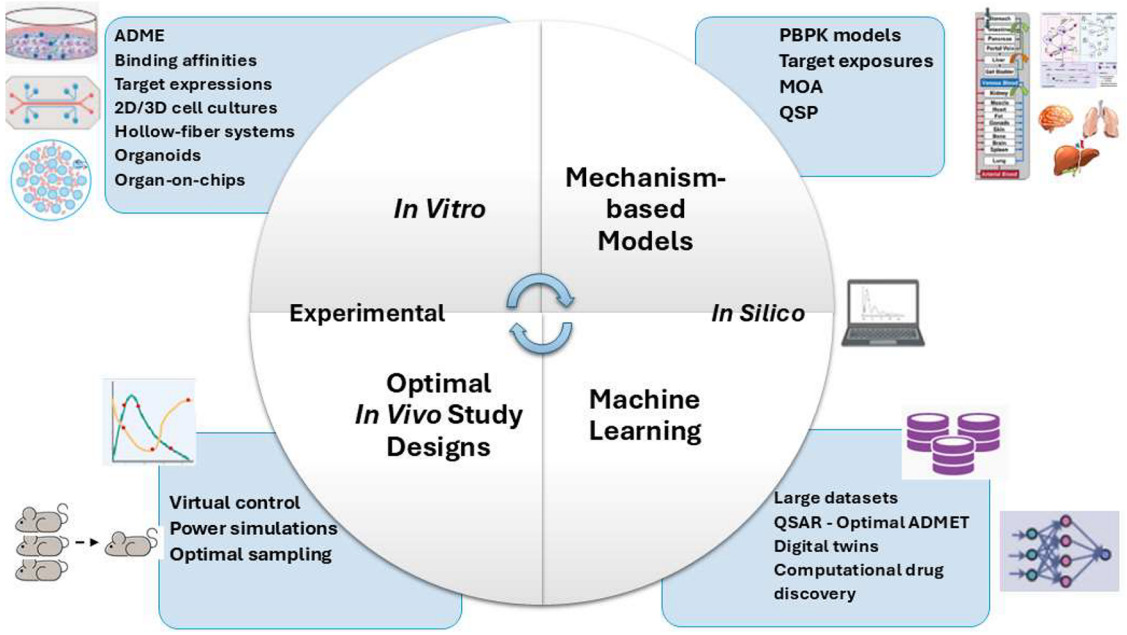

Figure 1. Overview of new approach methodologies to streamline preclinical drug development. The NAM methods include — in vitro and in silico approaches. In silico methods can be further divided into mechanistic modeling and machine learning based approaches. To fully realize the predictive power of NAMs, a combinatorial approach is essential — the one that integrates mechanistic mathematical models, advanced computational methods such as machine learning and strategically designed in vitro and in vivo experiments. Well-designed and advanced in vitro experiments are foundational to inform mechanistic models, such as PBPK and QSP. Such in silico models when combined with ML methods can provide explainable insights from large data sets. This framework can support efficient and optimal in vivo study designs to support successful clinical translation. ADMET = absorption, metabolism, distribution, elimination, and toxicity; ML = machine learning; MOA = mechanism of action; NAM = new approach methodology; PBPK = physiologically based pharmacokinetic modeling; QSAR = quantitative-structure activity relationship; QSP = quantitative systems pharmacology.

predict efficacy and safety in the clinical setting are mechanistic differences between animal models and humans, a lack of disease representation in animal models, and the homogeneous nature of the animal sample population.[5,6] Traditional preclinical to clinical translation methods using in vivo studies are becoming even less suitable for clinical projections with advancements in drug modalities, such as bispecific antibodies, antibody-drug conjugates (ADCs), targeted protein degraders, and cell and gene therapy.[4,7] Moreover, preclinical in vivo studies often involve a high financial burden and raise ethical concerns.[8] The average preclinical development cost per successful drug was estimated to be $237.8 million. After accounting for failed clinical trials, the median total cost of bringing a new drug to market was estimated to be $985.3 million (95% CI, $683.6−$1228.9 million). The immense burden of failed preclinical to clinical translation drives up the cost of the marketed drugs. Moreover, rising awareness of animal sentience and suffering has sparked significant resistance to animal research.[9] Overall, there is a growing need to explore alternative approaches to animal testing to improve translational research accuracy and efficiency.

Due to scientific, financial, and ethical reasons, there has been a growing push to develop and validate human-relevant "new approach methodologies" (NAMs) and to reduce and replace animal use in drug research and development.[9−14] Global regulatory committees have adopted the "3Rs" principles (reduction, refinement, replacement) for guiding decisions on animal experimentation.[10,11] Human-relevant NAM approaches, including advanced in vitro systems, in silico mechanistic models, and computational techniques such as artificial intelligence and machine learning, can improve preclinical study design. The NAM methods include in vitro and in silico approaches; however, to fully realize the predictive power of NAMs, a combinatorial approach is important. Such an approach can integrate mechanistic mathematical models, advanced computational methods such as artificial intelligence (AI) and machine learning (ML), with strategically designed in vitro and in vivo experiments.[12] By combining the strengths of each methodology, we can enhance translational relevance, improve predictive accuracy, and ultimately streamline the preclinical drug development process.[13,14]

Here, we first review examples of NAM-based approaches for pharmacokinetics (PK), pharmacodynamics (PD), efficacy, and safety predictions for preclinical to clinical translation (Figure 1, Table 1). We also review case studies of innovative in vivo study designs and the current regulatory landscape for NAM approaches. Next, we propose an alternative "a priori in silico" preclinical development workflow that integrates standard and advanced in vitro experiments and in silico models to support efficient and effective preclinical study designs and discuss future considerations for the development and validation of such approaches. Lastly, we discuss the challenges and future perspectives for the adoption of NAM-based approaches in drug discovery and development.

PBPK MODELS FOR EFFICIENT TOXICOKINETIC AND PHARMACOKINETIC PRECLINICAL EVALUATIONS

Physiologically based pharmacokinetic (PBPK) models, combined with other quantitative methods, i.e., quantitative structure activity relationships (QSAR) and in vitro to in vivo extrapolation (IVIVE) of drugs' absorption, distribution,

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Table 1. In Silico NAM Approaches: Key Strengths, Limitations, and Use Cases[a]

NAM type	key strengths	limitations	use cases	refs
PBPK	predictions of species-specific drug plasma/serum PK profiles in vivo based on physiological data and drug-specific in vitro data, i.e., physicochemical and binding properties	requires extensive input data	"a priori" PBPK approach showed a reasonable agreement with the observed dose-ranging PK data for eight small molecules in rats and monkeys[23,24] and 22 monoclonal antibodies in humans[25]	23−25,30−32
	first-in-human dose projections	combinatorial organ-on-chip + QST: requires interdisciplinary expertise and data integration		
	predictions of sites of action drug exposures, i.e., CNS and tumor site drug distribution	a PBPK model with CNS subcompartments, developed from in vitro data for a tau-targeting antibody, accurately predicted serum, CSF, and brain interstitial fluid concentrations in rats and monkeys[26]		26−30
		a combined PBPK−Krogh cylinder model, informed by physicochemical data and calibrated with two systemic parameters, predicted heterogeneous and perivascular tumor distribution of T-DM1 consistent with experimental and clinical observations[29]		
	when combined with advanced in vitro models, such as organ-on-chip systems, they can offer improved predictive performance, mechanistic insights, and allow for predictions of interindividual variability in PK		a combined liver-on-chip and PBPK approach successfully translated systemic clearance of propranolol[34] and captured interindividual variability in six small molecules metabolism and effectively predicted human PK[33]	33,34
QSP	in vitro to in vivo translation of dose/exposure-response relationship for target engagement, pharmacodynamic markers, and treatment effects	high model complexity; requires robust knowledge of biology	a QSP platform model for bispecific T-cell engagers was calibrated using extensive in vitro experimental data for numerous cell lines and different experimental conditions successfully predicted clinical data for three bispecific T-cell engagers[38]	4,30−32,37,38
	first-in-human dose projections	needs high-quality dose−response in vitro data	Thiele modulus analysis predicted target-site receptor saturation based on drug transport and target properties, offering mechanistic insights into tolerability of approved ADCs and checkpoint inhibitors[4]	
QST	enables predictions of system-specific drug-induced adverse effects, such as hepatotoxicity, nephrotoxicity, cardiotoxicity, and hematotoxicity	high model complexity; requires robust knowledge of biology	DILIsym model has been used to assess liver toxicity risks and drug interactions—such as ferroquine-acetaminophen—informing clinical trial protocols and supporting safe drug use[43−46]	43−50
	when combined with advanced in vitro models, such as liver-on-chip or kidney-on-chip, provides improved predictive performance by providing mechanistic insights that computational approach alone may not offer	needs high-quality dose−response in vitro data	a mechanistic PK−toxicodynamic model using in vitro cardiac data successfully translated doxorubicin and terfenadine toxicity to preclinical and clinical levels, predicting reduced cardiotoxicity with dose fractionation[48,52]	
		combinatorial organ-on-chip + QST: requires interdisciplinary expertise and data integration	a QSP model integrating kidney-on-chip biomarker data accurately predicted clinical nephrotoxicity for three drugs, supporting its use in dose optimization to reduce kidney injury risk[47]	
machine learning-enhanced mechanistic models	leverages large data sets, including molecular structure, omics, and high throughput data	often lacks mechanistic interpretation generalizability issues	QSAR-based machine-learning in combination with PBPK modeling framework allowed preclinical exposure prediction of novel small molecules[56,57,59]	56,57,59
	development of digital twins for personalized, mechanistic insights into factors impacting drug responses leading to more accurate predictions		generative adversarial network-based digital twins for rats developed based on data from prior preclinical studies predicted hepatotoxicity potential of novel drugs, enabling predictions of toxicity profiles across various chemical structures and drug classes and the findings from simulations correlated closely with human findings[61]	60−62

[a]ADME = absorption, distribution, metabolism, and elimination; CNS = central nervous system; PD = pharmacodynamics; PBPK = physiologically based pharmacokinetic; PK = pharmacokinetics; QSAR = quantitative structure activity relationship; QSP = quantitative systems pharmacology; QST = quantitative systems toxicology.

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metabolism, and elimination (ADME) characteristics, have been extensively evaluated for predictions of small molecules and biologics PK characteristics.[15−22] Whole-body and minimal PBPK (mPBPK) models incorporating key mechanisms playing a role in ADME have been thoroughly investigated for preclinical species and humans. These models have established a robust framework for predicting in vivo PK properties using species-specific physiological and drug-specific physicochemical properties. However, preclinical PBPK models are frequently fitted to and qualified using independent in vivo data sets, which typically demand a large amount of data. In the absence of such large data sets, it could be said that the potential of PBPK models for streamlining preclinical development remains under-explored to date. Case studies have demonstrated that the predictions of models based on "a priori in silico" approach using in vitro data and prior knowledge have shown reasonable agreement with in vivo observed data for numerous small molecules and biologics.[23−25] Additionally, such an approach can provide valuable mechanistic insights into drugs' PK characteristics early. For example, the 'a priori in silico' approach, as demonstrated in the case of PBPK modeling of 22 monoclonal antibodies, accurately predicted nonspecific binding, FcRn interactions, pinocytosis, and transcytosis processes, highlighting its potential to reduce preclinical in vivo requirements.[25] Thus, PBPK models offer a promising framework for predicting drug PK properties and reducing the reliance on extensive preclinical in vivo data.

It is not always feasible to conduct preclinical studies to measure drug concentrations at the target site of action. The number of case studies where generic PBPK models were coupled with expanded mechanistic models for organs of interest is described in the literature.[15,21,26,27] For instance, a PBPK model expanded to include CNS subcompartments developed for a therapeutic antibody targeting tau protein using in vitro and physicochemical data demonstrated reasonable agreement with observed serum, CSF, and brain interstitial fluid data from rats and monkeys.[28] In another example, a Krogh cylinder model integrated within PBPK models for predictions of drug penetration into tumor tissues based on drug physicochemical properties and tumor vasculature-related parameters captured the heterogeneous tumor distribution of T-DM1, matching experimental results in tumor-bearing mice and predicted the perivascular distribution observed at the clinical dose in patients.[29] This framework has been extensively used in supporting first-in-human dose predictions for oncology drug development, reducing the need for in vivo studies evaluating tumor drug penetration.[30−32] Overall, the PBPK models allow predictions of drug exposures at target sites of action.

The integration of computational models with advanced human-relevant in vitro technologies, for example, organ-on-chip, 3D cultures, and organoids, can enhance the predictive power of preclinical PK studies.[33] For instance, a mathematical framework for liver-on-chip systems-DigiLoCs (digital liver-on-chip simulator) was first used to simulate drug clearance in liver-on-chip systems.[34] The model consists of three compartments—media, interstitial, and intracellular space—to represent the drug distribution and metabolic processes occurring within the liver-on-chip. By incorporation of drug-specific physicochemical properties, chip-hardware-specific details, and biological parameters, the DigiLoCs distinguished between active biological processes (i.e., metabolism) and passive processes (i.e., permeability and partitioning). A proof-of-concept translation to human PK for propranolol was performed by integrating human clearance values predicted from DigiLoCs into a whole-body PBPK model to simulate human drug kinetics. The simulations from these models closely matched clinical observations. In another example, interindividual variability in hepatic drug metabolism for six small molecules was evaluated using cryopreserved hepatocytes from five donors cultured in a liver-on-chip system.[33] Metabolic depletion profiles, along with gene expression and functional viability markers, were incorporated into PBPK models to translate to human PK. The findings suggested substantial interdonor variability in drug metabolism, gene expression, and liver-specific functions and demonstrated the potential of this approach for predictions of human PK along with interindividual variability. Overall, PBPK models based solely on standard or advanced in vitro experiments and physicochemical data can be used to predict in vivo PK profiles to gain valuable mechanistic insights into a drug's PK properties. Findings from such models can then be used to design smaller and more efficient preclinical toxicokinetic and PK study designs to support further development and qualification of the models.

MECHANISTIC DOSE−RESPONSE EVALUATIONS FOR PHARMACODYNAMICS AND EFFICACY

Mechanistic models, informed by rigorous in vitro experimentation, can establish robust dose−response predictions to support PD and efficacy preclinical study designs. The Thiele Modulus calculation can be used to readily predict antigen receptor saturation at a target site given antigen density, drug permeability, and drug-antigen binding.[4] Such a calculation can be readily used to understand the extent of target engagement and therapeutic activity relative to other drugs of similar classes or mechanisms of action. For example, an analysis of approved ADCs and checkpoint inhibitors for solid tumors provided mechanistic insights into tolerability and receptor saturation potential at clinically approved dosing.[4] In another example, a mechanistic model of growth and kill dynamics for rapidly growing and persistent bacterial subpopulations, informed by hollow-fiber infection systems, accurately predicted intralesional drug exposures and treatment outcomes for various combination antimicrobial regimens in tuberculosis patients.[35,36] Disease- and modality-specific mechanistic models are effective tools for rigorous evaluations of dose-PD-effect relationships.[37−39] For example, the efficacy and toxicity of bispecific T-cell engagers are dependent on various factors, such as target affinity, avidity, tumor characteristics, and immunological conditions. A mechanistic platform model for bispecific T-cell engagers, calibrated using extensive in vitro experimental data for numerous cell lines and different experimental conditions, and validated against clinical data for three bispecific T-cell engagers, can be useful to perform in silico evaluations to support the development of follow-up bispecific T-cell engagers.[38] Along these lines, mechanistic models, informed by in vitro data, provide valuable insights into dose−response relationships, target engagement, and therapeutic efficacy, supporting preclinical study design and drug development.

Digital twins (DTs) provide computational replicas of biological systems, enabling virtual simulations of drug effects. For example, a mechanistic tumor microenvironment DT successfully predicted the response to antiangiogenic therapies,

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optimizing dose selection in preclinical studies.[40] A mechanistic tumor microenvironment model incorporating angiogenesis and fluid dynamics along with mouse xenograft DTs was demonstrated to be an efficient predictive tool for studying the effects of antiangiogenic cancer treatments, including successful combination treatment strategies.[41] The DTs provided mechanistic insights into interplays between different mechanisms of action of antiangiogenic cancer treatments, which usually cannot be evaluated in vivo. Overall, these approaches demonstrate how mechanistic modeling coupled with well-designed and efficient in vitro experiments can predict the clinical success of investigational drugs more accurately.

MECHANISTIC DOSE−RESPONSE EVALUATION FOR SAFETY

Evaluation of the potential adverse impact of investigational agents on various organ systems prior to first-in-human-trial initiation is essential and is required by regulatory agencies. Numerous mechanistic models enabling predictions of system-specific drug-induced adverse effects, such as hepatotoxicity, nephrotoxicity, cardiotoxicity, and hematotoxicity, have been developed, and such models for other systems are of interest.[42] Combinatorial NAMs, for example, by integrating in vitro experimental data with mechanistic models, can improve translational safety predictions.

Hepatotoxicity. A quantitative systems toxicology (QST) model of drug-induced liver injury (DILI) for assessing hepatotoxicity risk of investigational agents developed over a period of a decade has now become an important decision-making platform supporting regulatory submissions.[43,44] The DILIsym platform, widely used in regulatory submissions, integrates liver cell populations, biochemical systems, and metabolism processes to assess hepatotoxicity risk. Recent applications in regulatory decision-making have demonstrated its ability to refine clinical dose selection. This model, combined with in vitro dose−response experimental data, has been used to evaluate drug-induced liver injury potential and adjust clinical trial protocols for multiple small-molecule new drugs, including combination treatments, i.e., ferroquine-acetaminophen, solithromycin-metformin. The model has also been useful to understand the mechanistic details of the liver toxicity pathway and early discontinuation of an investigational small molecule, ORM-48824.[45] BIOLOGXsym combined liver toxicity data from the liver microphysiological system with PBPK and DILIsym QST models to simulate the liver toxicity potential of two biologics, tocilizumab and a discontinued investigational agent, cimaglermin alfa. The model was able to recapitulate clinically observed liver toxicity following treatment with tocilizumab and cimaglermin alfa.[46]

Nephrotoxicity. A QSP model informed by kidney-on-chip experimental data was able to successfully predict the impact of three different drugs on kidney injury biomarkers.[47] The model predictions aligned well with observed clinical data for three drugs, cisplatin, rifampin, and gentamicin, demonstrating their potential of NAMs for effective in vitro to in vivo translation.

Cardiotoxicity. In silico cardiac electrophysiology models have been extensively evaluated, encompassing a range of models including translational multiscale QST models, QSAR-based approaches, physics-based models, and atom-level interaction models.[48−51] A mechanistic PK−toxicodynamic cardiac tissue was used in conjunction with data from in vitro 2D and 3D cell culture experiments, including cardiomyocytes and AC16 cells exposed to doxorubicin over time.[48] The model successfully translated in vitro experimental findings into preclinical- and clinical-level data from the literature and was used to suggest doxorubicin dose fractionation to reduce cardiotoxicity.[52] A similar example includes PK/PD modeling of liver-cardiac organ-on-chip data for terfenadine to assess CYP3A4-mediated metabolism and downstream cardiac toxicity (QT prolongation) effects driven by its metabolite. The model predictions reasonably agreed with the observed changes in field potential duration in both humans and in vivo models.[33]

Hematotoxicity. Myelosuppression is one of the most common adverse effects associated with oncology therapeutics, and QSP models of hematopoiesis and granulopoiesis have demonstrated successful in vitro to human translation of drug-induced myelosuppression effects.[53−55]

Overall, these examples demonstrate the crucial role of combinatorial NAM approaches, by integrating in vitro experiments and mechanistic models, to minimize the risk of drug-induced toxicity.

MACHINE LEARNING FOR TRANSLATING LARGE DATA INTO INFORMATION

With advancements in computing power, algorithms, and data accessibility, the rise of AI/ML applications has accelerated significantly. AI/ML algorithms have enhanced the precision and efficiency of predicting relationships between molecular structures and ADME and toxicology (ADMET) properties.[56−58] By leveraging large data sets, these methods enable modeling of complex structure−activity relationships based on molecular, chemical, and biological interactions. These advancements accelerate the identification and optimization of novel small-molecule and biologic therapeutics with favorable ADME and safety profiles, thereby streamlining the drug discovery and reducing reliance on animal testing during the discovery stage. For example, a QSAR graph neural network-based platform with high predictive performance was developed and is available open-source on a server to predict a wide range of ADMET properties based on the molecular structure of small molecules.[57,58] In another example, ML-based QSAR in combination with a PBPK modeling framework allowed full PK time course predictions of novel small molecules.[59] Such models allow for predictions of ADMET, potentially reducing the number of required animal PK studies. AI/ML-based approaches, increasingly recognized by regulatory agencies such as the FDA, have demonstrated potential for predicting drug toxicity. For example, a GAN-based DT accurately predicted the hepatotoxicity potential for a blinded set of novel compounds, aligning with clinical findings. For example, generative adversarial network (GAN)-based DTs for rats were used to simulate the hepatotoxicity potential of novel drugs with high accuracy. The models predicted the hepatotoxicity potential of novel drugs, enabling predictions of toxicity profiles across various chemical structures and drug classes, and the findings from simulations correlated closely with clinical findings.[60−62] Overall, ML-augmented translated approaches to preclinical drug development can enhance drug discovery and research by refining and reducing the use of animals.

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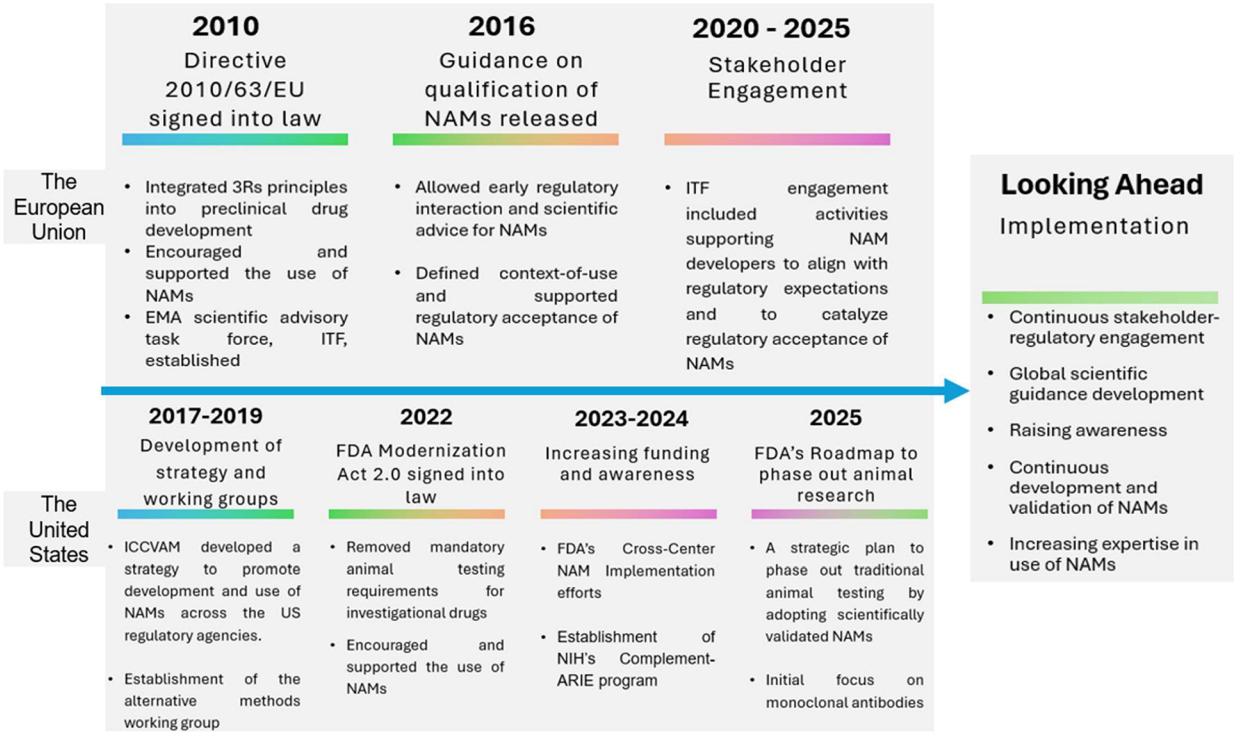

Figure 2. Evolution of the regulatory framework for new approach methodologies use in drug development. The global regulatory authorities are increasingly embracing NAMs through legislative reforms, strategic roadmaps, and collaborative initiatives aiming to modernize drug development by replacing traditional animal testing with more predictive, human-relevant approaches. 3Rs principles = replacement, reduction, and refinement of animal use in research; Complement-ARIE = complement animal research in experimentation; EMA = the European medicines agency; FDA = the food and drug administration; ICCVAM = interagency coordinating committee on the validation of alternative methods; ITF = innovation task force; NAM = new approach methodologies; NIH = the national institute of health.

INNOVATIVE AND EFFICIENT PRECLINICAL STUDY DESIGNS

Model-based approaches are ideal for enabling innovative and efficient preclinical study designs, optimizing the number of animals and samples, and selecting appropriate end points, doses, and dosing regimens. Martin et al. (2016) proposed a compact preclinical chemotherapy safety study design, i.e., collecting PK and PD samples from the same animals, across the full PK/PD profile.[54] Through simulations, it was concluded that the compact design preserved parameter accuracy along with variance estimates compared to those of the standard design. Moreover, the compact design allowed estimation of the impact of interoccasion variability on parameters. Ciecior et al. (2021) proposed the use of xenograft models with heterogeneous tumors to better replicate the tumor heterogeneity observed in human cancer.[63] Their logistic regression-based sample size estimation indicated that utilizing a heterogeneous tumor model design could reduce the required number of animals by 61−78% compared to traditional xenograft study designs, to maintain the minimum power of 80%. Selimkhanov et al. 2017 leveraged a statistical and mechanistic model to quantify the variability in metabolic end points for an antiobesity drug trial, accounting for both inter- and intra-animal variability.[64] Model-based evaluations of virtual cohorts were performed, allowing for estimation of effect sizes and variances to ensure adequate statistical power. By properly powering for primary end points, the authors reduced the number of animals required while minimizing the risk of false conclusions. Virtual control groups represent historical control data collected from prior standardized in vivo studies. The use of virtual control groups allows standardization of control data across studies, allows potential inclusion of potentially larger and high-quality control data, reduces the number of animals required, and may accelerate preclinical development timelines.[65] For example, Gurjanov et al. (2024) evaluated the potential of virtual control data from historical 4-week oral toxicity studies in rats for reproducibility and validity.[66] The study suggested that carefully chosen historical control data can effectively replace concurrent controls without compromising the overall study conclusions. This study established a foundation for future validation of virtual controls in regulatory toxicology. Overall, these case studies provide examples of more accurate, efficient, and ethically responsible preclinical studies.

REGULATORY FRAMEWORK FOR NAM APPROACHES

The limitations of traditional preclinical approaches and the importance of more predictive, human-relevant models have been increasingly acknowledged by global regulatory authorities. This is evidenced by major legislative steps such as the European Medicines Agency's Directive 2010/63/EU and the FDA Modernization Act 2.0 (Figure 2).[9,10] These frameworks have helped accelerate the shift away from mandatory animal testing, allowing for regulatory acceptance of in vitro, in silico, and integrated approaches to evaluate drug safety and efficacy. Global initiatives, such as the EU's Innovative Task Force and the US NIH's Complement-ARIE program, underscore a shared commitment to advancing, validating, and implementing NAMs into drug development.[13,14] The FDA recently

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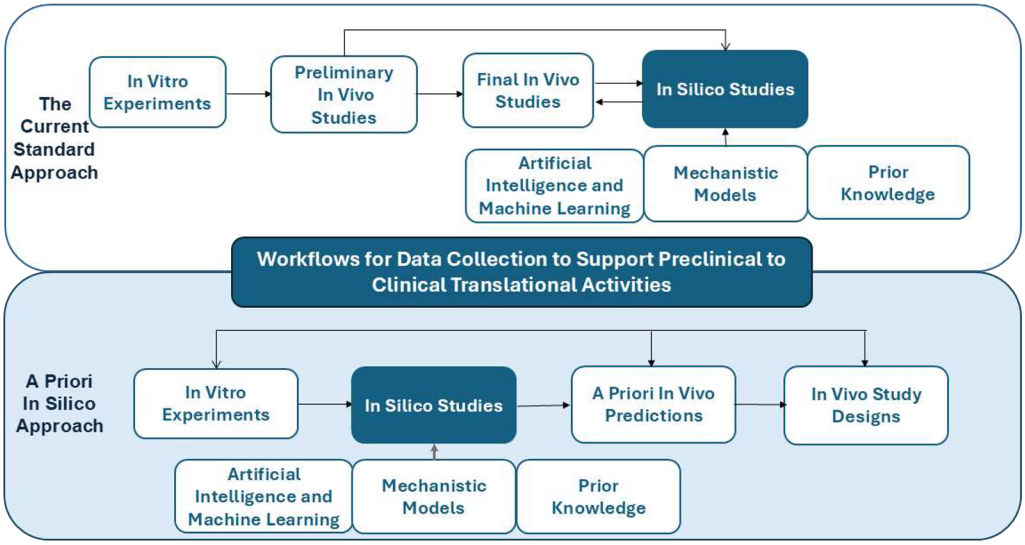

Figure 3. Current standard and proposed preclinical development workflows to support translational predictions for first-in-human study designs. The current standard preclinical development workflow begins with in vitro experiments, followed by preliminary and final in vivo studies. Here, we propose an "a priori in silico" approach that integrates advanced in vitro data with physiological and disease-specific in silico models. The proposed "a priori in silico" approach includes the following steps[1]: conduct initial in vitro experiments to generate data pertaining to biochemical properties, molecular interactions, cellular responses, etc.,[2] integrate in vitro data into computational models, use computational models to analyze and predict species-, drug- and dose-specific response,[3] utilize in silico models to predict preclinical pharmacokinetics, toxicity, and biological efficacy before actual animal testing,[4] optimize experimental conditions, dose selection, and study parameters, and design smaller preclinical studies based on a priori predictions,[5] iterative feedback as needed, i.e., use results from preclinical in vivo studies to refine in vitro, in silico, and in vivo experiments, as needed to improve predictive accuracy.

released a strategic roadmap to phase out animal testing in drug development, with an initial focus on monoclonal antibodies due to their well-characterized mechanisms and predictable pharmacology.[11] This marks a targeted effort to integrate NAMs in areas where scientific readiness supports the immediate regulatory application. Looking ahead, the global roadmaps focus on sustained stakeholder-regulatory engagement, harmonized guidance development, and continued investment to enable the successful transition to scientifically robust, nonanimal methods for streamlined preclinical drug development. While regulatory advances in the U.S. and EU are promising, challenges remain in achieving global harmonization, particularly across emerging markets. Sponsors must, therefore, navigate jurisdiction-specific requirements while contributing to ongoing international efforts to standardize NAM acceptance.

FUTURE PERSPECTIVES AND CONCLUDING REMARKS

We discussed in vitro and in silico approaches, along with case studies, that can be useful for efficient and more accurate preclinical to clinical translation of investigational drug PK, efficacy, and safety characteristics. We also discussed the evolution of the regulatory framework relevant to NAM approaches.

Alternative Preclinical Development Workflow. The integration of NAMs in preclinical development workflows requires not only the development and validation of these methods but also the continuous application of the latest advancements within iterative "learn and confirm" workflows. The current standard preclinical development workflow begins with in vitro experiments, followed by preliminary in vivo studies. Data from these studies inform final in vivo experiments, either directly or through modeling and simulation. Results from the final in vivo studies are used in modeling and simulation to determine first-in-human study doses and dose regimens. We propose an "a priori in silico" approach (Figure 3) that integrates standard and advanced in vitro experiments to inform computational models with drug-specific parameters. Simultaneously, these silico models should incorporate physiological- and disease-specific parameters derived from prior data, models, and knowledge. This framework can enable in vivo predictions to guide the design of optimal and reduced preclinical studies, including determining dose, dosing regimens, biomarkers, end points, and sampling time points. The results from in vivo studies can be used to refine the models to enhance accuracy for human predictions or guide additional preclinical studies, if needed. The proposed workflow would enable conducting well-designed and efficient preclinical studies, providing data necessary for more accurate predictions for human translation. To our knowledge, this is the first review to propose a systematic, iterative "a priori in silico" preclinical workflow that integrates human-relevant in vitro systems with predictive modeling to inform and reduce in vivo experimentation. This approach not only streamlines development but also aligns with emerging regulatory directions, offering a pragmatic path forward for more efficient, ethical, and human-relevant drug development.

Combinatorial NAMs. Integrating the power of mechanistic models, cutting-edge computational techniques like AI/ML, advanced in vitro systems, and well-designed in vivo experiments allows maximizing the predictive potential of these methods, as demonstrated by the mature examples such as the DILIsym platform, the hollow-fiber infection model, the DigiLoCs MPS Digital Twin platform, and the PBPK-ML-based small molecule PK predictor that we discussed.[12] Further work should focus on advancing combinatorial NAM

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Table 2. Current Challenges and Potential Solutions to the Adoption of NAM Methods[a]

challenges	potential solutions	refs
development of regulatory standards and guidelines	academia, industry, and regulatory collaborations	9−11
	cross-disciplinary initiatives	
in vitro, in silico, and combinatorial model development, evaluation, and standardization	comparisons with traditional methods	13,14
	context of use	
interindividual, interlab, and interchip, interexperiment variability	quantification and integration of variability	75
reproducible in vitro and in silico models	data and model standards and guidelines	76,77
	comprehensive documentation	
easy access to validated in silico tools, models, and data	data and model repositories	77−85
	integrate llms for effective reuse of information	
availability of skilled professionals in NAM methods	development and dissemination of training programs for NAM methods by the academia, regulators, and professional communities	14

[a]AI = artificial intelligence; LLM = large-language model; ML = machine learning; NAM = new approach methodologies; PBPK = physiologically based pharmacokinetic.

methods.[13] Like DigiLoCs, computational modeling of in vitro systems can distinguish drug-specific parameters from system-specific ones. These drug-specific parameters can then be integrated into mechanistic models, such as PBPK and QSP models, to increase the robustness of their in vivo predictivity. For example, while in vitro tumor growth inhibition studies using individual patient-derived (PDx) tumor organoids predicted individual chemotherapy response in metastatic colorectal cancer and GI cancer patients, a prospective Phase 3 study that treated patients based on PDx recommendations failed to elicit an objective response.[67−69] Incorporating PDx-based drug effect parameters along with patient-specific immune and mutation data within mechanistic in silico models may improve predictions of individual patient-level responses and investigate the potential gap leading to a lack of observed objective response in the clinical study. Furthermore, integrating different in silico methods, such as hybrid mechanistic-ML models, can provide maximal information from multidimensional and complex data. ML approaches alone can be valuable for pattern recognition and hypothesis generation using large data, such as multiomics and high-throughput; however, the inherently sparse and complex nature of biological systems suggests that combining mechanistic modeling with ML methods, such as using physics-informed neural networks and scientific ML approaches, would allow deeper understanding of factors affecting heterogeneity in disease and population.[70] Moreover, integrating hybrid mechanistic-ML models to generate DTs of patients and animals can facilitate iterative learning and enable the continuous optimization of predictions. Overall, the combination of multiple NAMs is essential to fully unlock their potential.

NAMs for Newer Modalities. One area where NAM methods hold immense potential is the translation from preclinical to clinical settings for cutting-edge therapeutic modalities, such as ADCs, bi- or multispecific antibodies, and cell and gene therapies. Platform QSP models for preclinical to clinical translation for ADCs and bispecific antibodies that integrate mechanistic details of the modality, target, and disease have been developed.[27,32,38] These models incorporate a wide range of in vitro and in vivo data, with in vivo data primarily pertaining to PK from mice and nonhuman primates, as well as tumor growth inhibition data from mouse xenografts. Future efforts can evaluate integrating PBPK models to inform PK parameters and organoids or organ-on-chip experimental data to inform PD, i.e., tumor growth inhibition parameters. Similarly, QSP models can be developed for cell and gene therapies incorporating relevant biology and mechanistic details, including cell or gene therapy vector biodistribution, target and off-target cellular disposition, as well as transgene production, distribution, and elimination and organ-on-chip systems can be used to inform PK and PD parameters of the platform in silico gene therapy models. Moreover, organ-on-chip models or in vivo advanced imaging techniques can be evaluated to collect data from the same animals over time, reducing the number of required animals for in vivo studies. Thus, NAM methods offer significant potential for improving preclinical-to-clinical translation of novel modality therapeutics by integrating mechanistic modeling and organ-on-a-chip systems to refine PK and PD predictions while reducing reliance on animal studies.

Quantification of Predictive Accuracy and Resource Savings. Incorporating NAM-based approaches into preclinical development can significantly reduce the number of required animal tests and thus costs.[71] For example, an evaluation of 870 liver-on-chips and a blinded set of 27 drugs demonstrated high predictive accuracy for DILI, with a sensitivity of 87% and specificity of 100%.[72] This performance surpasses that of traditional preclinical models, which often fail to predict human hepatotoxicity. Switching to liver-on-chip for DILI evaluations could save approximately $3 billion annually by enhancing productivity through earlier and more reliable predictions. Incorporating an in silico approach along with liver-on-chip experiments could be even more beneficial. Evaluating 100 compounds for DILI during the early preclinical phase using traditional in vivo methods only, with a sensitivity of around 50%, requires approximately 5000 animal tests.[73] On the other hand, combining DTs of in vitro microphysiological systems with computational human physiological DILI models can improve sensitivity to over 95%, reducing the need for animal testing to just 50 tests in general.[72] Thus, this transition can reduce the reliance on animals, enabling ethical and cost-effective preclinical testing. Future work should further evaluate the proposed combinatorial NAM-based approaches along with quantitative measurements of their actual impact on costs, prediction accuracy, and success in clinical development.

Current Challenges of NAM Approaches and Potential Solutions. There are some limitations of NAM approaches. Table 1 summarizes the limitations of key NAM

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ACS Pharmacol. Transl. Sci. XXXX, XXX, XXX−XXX

types along with their strengths and use cases. Traditional in vivo studies offer insights at the whole-animal level that in vitro and in silico methods alone cannot achieve. However, when validated in vitro and in silico approaches are integrated, they can together yield human-relevant predictions at the whole-system level. Another key limitation of NAM approaches is the need for rigorous, context-specific validation to ensure reliability and regulatory acceptance (87). However, traditional animal models often fall short in predicting human responses, with high failure rates observed in clinical translation.[4,7,11] Moreover, in silico models rely heavily on a range of physiological and drug-specific parameters that can be derived from either previously generated data or fit-for-purpose in vitro experiments. Thus, if input data or parameters are inaccurate or incomplete, they can introduce errors or uncertainty in the predictions. Therefore, it is important to use high-quality and well-characterized input data to conduct sensitivity analyses and iteratively refine and validate in silico models with experimental or clinical data. Overall, while NAM approaches have limitations, when properly integrated and validated, they can potentially provide more human-relevant mechanistic insights than traditional animal models.[11] We propose a structured and iterative "a priori in silico" framework that integrates NAM components to actively guide preclinical study design—a step toward more predictive and resource-efficient drug development.

The successful development, validation, and implementation of NAM methods will depend on robust international collaborations among academia, industry, and regulators.[12] These collaborations can focus on potential solutions to current challenges to the adoption of NAMs, such as variability, standardization, applicability, reproducibility, and accessibility (Table 2). Moreover, to successfully implement optimal preclinical development workflows, it is essential to share data, models, and tools in open-source, user-friendly, and reproducible formats.[74] Numerous existing repositories and platforms support this need. Open-source model codes can be easily accessed through platforms like BioModels, the Open Systems Pharmacology Community, etc [https://github.com/, https://www.ebi.ac.uk/biomodels/, https://github.com/Open-Systems-Pharmacology/OSP-based-publications-and-content/]. Additionally, data-sharing platforms such as the Critical Path Institute Data Collaboration Center, ImmPort, Project Data Sphere, Gene Expression Omnibus, The Cancer Genome Atlas, and many others offer valuable resources [https://c-path.org/program/data-collaboration-center/, https://www.immport.org/, https://data.projectdatasphere.org/, https://www.cancer.gov/ccg/research/genome-sequencing/tcga, https://www.ncbi.nlm.nih.gov/geo/]. Leveraging these tools and platforms more effectively can significantly enhance preclinical research and development efforts and ultimately replace the need for animal testing.

One major concern for the adoption of NAMs is the above-mentioned applicability in a safety context and their capability to be protective of human health. While NAMs are designed to be of higher human relevance than the classical in vivo animal models, they lack the whole-organism context and, thus, retain uncertainty in their applicability for systemic toxicology. Further collaborative efforts are needed to generate data and expand the complementary in silico pathway and model repositories to further increase the in vivo relevance of the integrated NAM-based approaches for applicability on a holistic translational systemic level. As a framework for pathway expansion, so-called Adverse Outcome Pathways (AOPs), and the underlying causal effect relationships as annotated in established public resources such as the AOP wiki [https://aopwiki.org/] developed for human safety assessment, based on existing biological and disease map- and knowledge graph construction and reasoning, can be utilized. The expansion of these qualitative pathway models and their quantification can serve as a basis for the establishment of systemic QST models and will require annotation of biomarkers specifically relevant for adversity to quantify the qualitative relational, i.e., biological or disease, knowledge maps.[80,83] Such expansion should be conducted in alignment with curated ontologies and ongoing data harmonization and curation efforts, and in collaboration with ongoing initiatives, such as OpenRiskNet, the Comparative Toxicogenomics Database [https://ctdbase.org/], the Biological Expression Language, and the Integrated Network and Dynamical Reasoning Assembler [https://bel.bio/].[81,82] As enabling technologies, large-language models are increasingly explored and hold the potential for efficient data- and information-mining and effective reuse of information from these resources.[83]

The successful adoption of NAM-based methods will depend on having skilled professionals implement them effectively. Therefore, future efforts should prioritize the development and dissemination of training programs for these methods.[13] Lastly, NAMs also need to overcome social barriers and legal barriers that may stifle rather than accommodate or facilitate beneficial and ethical technological development.[84] Overall, in addition to the development and validation of combinatorial NAMs, the effective implementation of approaches to enhance preclinical development predictions and reduce costs will require robust cross-disciplinary collaborations.

CONCLUSIONS

To conclude, despite promising preclinical results, early clinical development of new therapeutics faces high failure rates due to poor translation of PK, efficacy, and toxicity data from preclinical to clinical settings, largely driven by the limitations of animal models. The substantial costs of these translational failures, coupled with ethical concerns, underscore the need for more efficient and reliable preclinical research and development methods. Combining NAMs, including human-relevant in vitro systems, in silico mechanistic models, and advanced computational methods-AI/ML, can enable simulations to support preclinical study designs. This approach provides efficient and more accurate translational predictions for clinical study designs. Future efforts should focus on validating combinatorial NAM approaches for regulatory acceptance, emphasizing international collaborations to establish standardized frameworks. By integrating mechanistic modeling, AI/ML, and advanced in vitro systems, we see that the path toward replacing traditional animal models becomes increasingly viable.

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