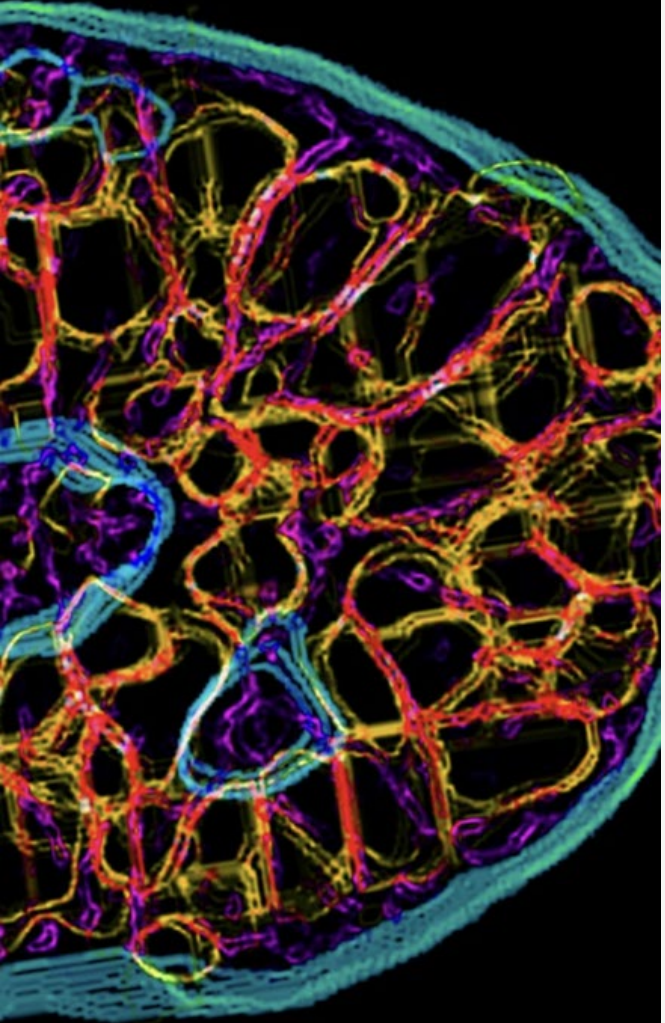


https://www.crownbio.com/model-systems/in-vitro/organoids

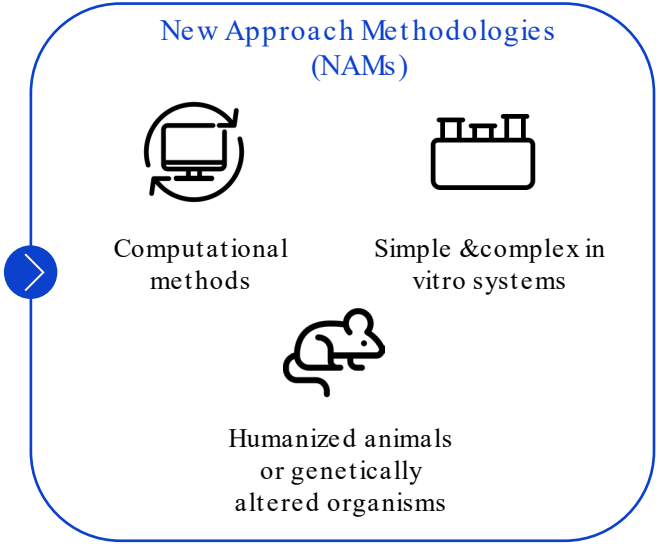
The use of New Approach Methodologies in the pharmaceutical industry: Lessons learned and perspective from the “end user”

Remi Villenave
Roche Innovation Center, Basel

ICCVAM- 2025

Definition of New Approach Methodologies (NAMs)

- NAMsis an FDA-defined term which includes new *in vitro*, *in chemico*, and *in silico* methods used to assess the safety of drugs.
- *In vivo* methods are also considered NAMs when they improve predictivity, shift studies to lower animals, or help replace, reduce, and refine animal use in development programs.



Avila AM et al. An FDA/CDER perspective on non clinical testing strategies: classical toxicology approaches and new approach methodologies (NAMs). Regul Toxicol Pharmacol. 2020;114, 104662.

New Alternative Methodologies (NAMs)

The global regulatory and legislative landscape surrounding the use of NAMs has evolved

FDA

Potential Approaches to Drive Future Integration of New Alternative Methods for Regulatory Decision-Making

A Report to the Science Board to the Food and Drug Administration from the New Alternative Methods Subcommittee

OCTOBER 2024

By Chad P. Nelson[1], Paul Brown[2], Suzanne Fitzpatrick[3], Kevin A. Ford[4], Paul C. Howard[5], Tracy MacGiff, Edward E. C. Morgenroth[1], Jacqueline O'Shaughnessy[6], Tucker A. Patterson[6], Rakesh Raghuwanshi[6], Rodney Rouse[7], Selen Stromgren[1], Kyung E. Sung[8], Luis G. Valerio Jr.[9], Jeffrey L. Ward[10], Namandjé N. Bumpus[11]

January 3, 2023 02:49 PM EST | R&D, Pharma

Passage of FDA Modernization Act 2.0 clears the way to reduce animal testing

December 12, 2024

United States Senate passes the FDA Modernization Act 3.0

The bill compells the U.S. FDA to wind down the practice of animal testing in drug development.

Advancing Alternative Methods at FDA / Implementing Alternative Methods

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Implementing Alternative Methods

Agency-wide efforts for advancing development, qualification, and implementation of new alternative methods for product testing

4.2.2. Alternative approaches for addressing EFD Risk

4.2.2.1. Use of alternative assays

A number of alternative *in vitro*, *ex vivo*, and non-mammalian *in vivo* assays (alternative assays) have been developed to detect potential hazards to embryo-fetal development. They have been used as drug discovery screens for adverse effects on EFD and have assisted in the understanding of the mechanism of toxicity, which can be useful for translating nonclinical data to human risk (especially for human-specific targets).

The continued use of alternative assays for these purposes is encouraged.

ICH S5 (R3) Guideline on detection of reproductive and developmental toxicity for human pharmaceuticals - Scientific guideline

FDA

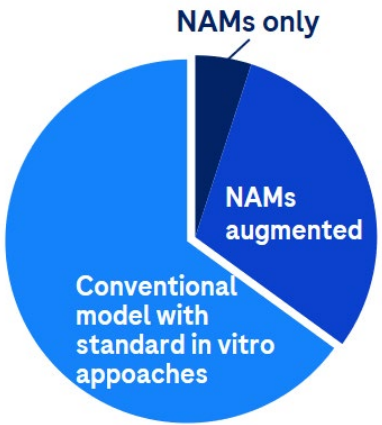
Roadmap to Reducing Animal Testing in Preclinical Safety Studies

FDA NEWS RELEASE

FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

For Immediate Release: April 10, 2025

Leveraging NAMs for internal safety assessment



NAMs and advanced in vitro models have been used for ~a decade with dozens of platforms tested overtime (very simple-> very complex), looking for compelling use cases

Of the currently supported programs, ~65% use conventional animal models, **~30%** use a **NAMs augmented** approach, ~**5%** use a **NAMs only** approach (in the absence of relevant species)

We foresee NAMs to be **increasingly used for portfolio support** due to e.g. new complex modalities, human specific MoA, limited or lack of cross reactivity (target expression/functionality/tissue distribution) with preclinical species and incentives from the regulators (e.g FDA NAMs roadmap)

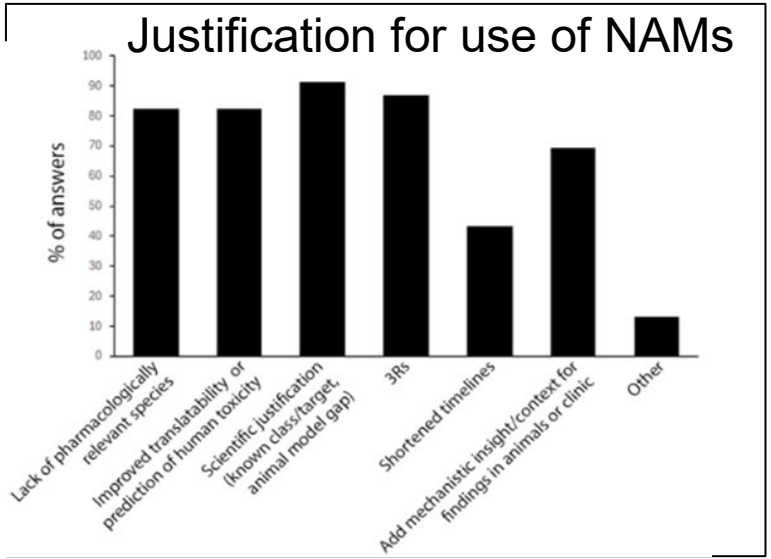
NAMs for safety testing: EIH in the absence of relevant species

Internal experience so far

	#1	#2	#3	#4	#5	#6	#7	#8	#9/10
LM EIH tox program	in vitro only	in vitro only	in vitro + tg mouse	in vitro only	in vitro only	in vitro + tg mouse	in vitro only	in vitro only + hNSG mice	in vitro only + hNSG mice*
Justification	epitope not express in NHP	epitope not expressed in NHP	No NHP orthologue	antibody not binding to orthologue in NHP	target not expressed in NHP	no NHP orthologue	binding epitope not present in NHP	similar binding but no NHP in vitro functionality	no in vitro functionality in normal cells
FDA/EU HA	accepted	accepted	accepted	accepted	accepted	accepted	accepted	accepted	accepted

- NAMs only packages accepted before FDA modernization act 2.0, when scientifically justified
- NAM-focussed approaches for EIH so far are only applicable to biologics (high specificity/low off-target risk)
  - NAM approaches are not "animal-free": animals are still used in e.g. pharmacology studies
  - No requests received to use cross-reactive homologous ("surrogate") mAbs
- To our knowledge, so far only accepted in oncology - in the absence of relevant species
- To mitigate for a higher risk, the starting dose is often low(er)
- Translatability of NAMs for clinical outcomes needs to be established (insufficient clinical data so far)

A survey of Industry -wide use of NAMs in regulatory filings



- **BioSafe NAMs TF** survey
- 27 companies responded
- 10 had NAMs accepted by regulators as **a supplement to animal testing** , 9/10 in multiple regions
- 9 had NAMs accepted by regulators to **replace animal testing** , 6/9 in multiple regions
- Majority would consider using a NAM based approach in the future to replace traditional large animal testing

EFPIA Safety Reflection Group

Sharing NAMs use cases

nature reviews drug discovery

https://doi.org/10.1038/s41573-025-01182-9

Perspective

Check for updates

Application of new approach methodologies for nonclinical safety assessment of drug candidates

Mario Beilmann[1], Karissa Adkins[2], Harrie C. M. Boonen[3], Philip Hewitt[4], Wenyue Hu[5], Robert Mader[6], Susanne Moore[7], Payal Rana[8], Thomas Steger-Hartmann[9], Remi Villenave[6] & Terry van Vleet[10]

Categories:

1. Animal species lack the target
2. Cross-species target issues
3. Non-mammalian targets
4. Unpredicted clinical events

Category	Example	Drug candidate	Species used	Type of NAM applied	Key results	Outcome
1. Animal species lack the target	1	NBE: IMCgp100 (tebentafusp) BiTE	Human (in vitro)	2D human cancer cell lines and primary cells in co-cultures, complemented by in silico approach	Calculated MABEL and identified safe starting dose for FiH studies	Advanced into clinical development
	2	NBE: MEDI-565 BiTE	Human (in vitro)	Co-cultures of human peripheral blood mononuclear cells and CEA-positive human tumour target cells	Calculated MABEL and identified safe starting dose for phase I clinical studies	Advanced into clinical development
	3	NBE: EpCAM-targeted TCB and CEA-targeted TCBs	Human (in vitro)	Patient-derived intestinal organoids co-encapsulated with immune cells in hydrogel	Identified potential immune-related intestinal toxicities of drug candidates	Advanced into clinical development
2. Cross-species target issues	1	NCE: PDE3A–SLFN12 complex inducer BAY 2666605	Rats and NHPs (in vivo), human and NHPs (in vitro)	Human and NHP primary cells (aortic smooth muscle and endothelial cells)	Identified potential risks and safety margin	Advanced into clinical development
	2	NBE: anti-CD38 antibody SAR444559	NHPs (in vivo), human and NHP (in vitro)	NHP and human blood samples	Identified NHP-specific on-target finding	Advanced into clinical development
	3	NBE: trastuzumab and trastuzumab emtansine	Mice and rats (in vivo), human (in vitro)	Rodent in vivo studies and panel of HER2-positive and negative human tumour cell lines	Identified lack of cross-reactivity in rodent species and target-specific cytotoxicity	Advanced into clinical development
3. Non-mammalian targets	1	NCE: PeEF2 inhibitor M5717	Not applicable	In silico approaches including BLAST analyses	Identified selectivity for parasitic target	Advanced into clinical development
	2	Nucleoside analogues: AZT, fialuridine; nucleotide analogue: sofosbuvir	Various (in vivo), human (in vitro)	Comparison of in vivo studies and in vitro assays with relevant human cell lines	Identified off-target effects such as mitochondrial toxicity	Advanced into clinical development
4. Unpredicted clinical events	1	NCE: AKR1C3 inhibitor BAY 1128688	Rats and NHPs (in vivo), human (in vitro)	Repeat-dose toxicity studies, in vitro assays and DILIsym in silico modelling	Identified human-specific toxicity, most likely BSEP inhibition	Stopped owing to safety concerns
	2	NBE: humanized CD154-specific monoclonal antibody Hu5c8	Human (in vitro)	Microengineered model 'Vessel-Chip' composed of human endothelium and blood components	Revealed prothrombotic effect of Hu5c8	Identified potential risk for thrombosis
	3	Oligonucleotide: antisense oligonucleotide SPC5001	Human (in vitro)	Kidney proximal tubule-on-a-chip model	Induced cytotoxicity and increased levels of kidney injury biomarkers as indication of acute kidney injury observed in clinical but not in preclinical studies	Identified nephrotoxicity

AZT, azidothymidine; BiTE, bispecific T cell engager; BLAST, basic local alignment search tool; BSEP, bile salt export pump; CEA, carcinoembryonic antigen; DILI, drug-induced liver injury; EpCAM, epithelial cell adhesion molecule; FiH, first-in-human; HER2, human epidermal growth factor receptor 2; MABEL, minimum anticipated biological effect level; NAM, new approach methodology; NBE, new biologic entity; NCE, new chemical entity; NHP, non-human primate; TCB, T cell-engaging bispecific antibody.

https://www.nature.com/articles/s41573-025-01182-9

NAMs safety assessment framework

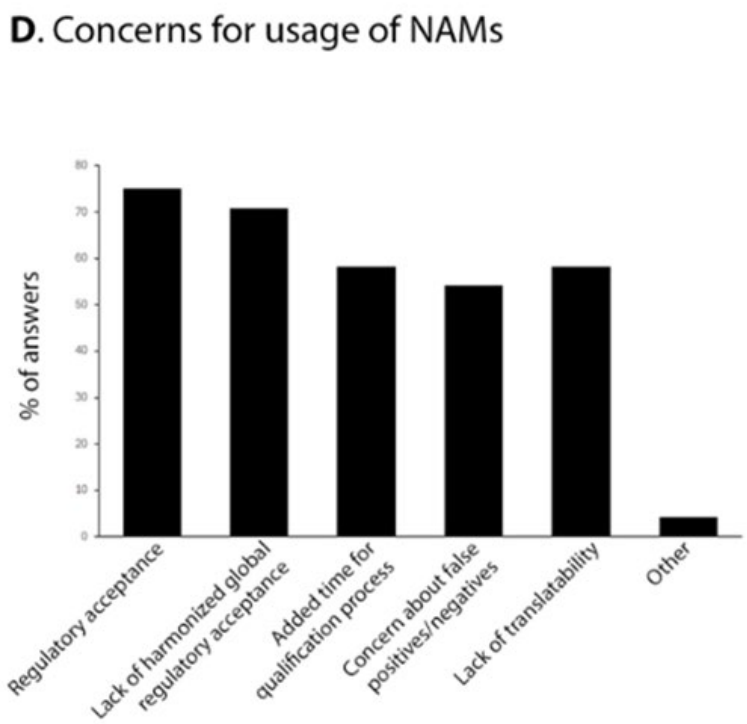
1 - Biologics	2 - Biologics / CT	3 - Biologics	4 - Small molecules
High target specificity*, no cross-reactivity, no pharmacological relevant animal species -Oncology	**High target specificity**, cross-reactive and +/- pharmacological relevant animal species, clinically validated target & MoA	**High target specificity**, cross-reactive and pharmacological relevant animal species, new target/MoA	Often **lower target specificity than biologics**, pharmacology relevant animal species

Acceptance by health authorities

Challenge to replace animal models

Modalities	**Antibodies** (Multi-specific, T-cell engagers, co-stimulators)	**Cell therapy** (Engineered T cells)	**Antibodies** (Anti-amyloid)	**Antibodies** (Bispecific T-cell engager)	**Antibodies** (Anti-cytokine mAb (Fc-modified)	Hemophilia A therapy (?)	Rare diseases (?)
Indications	**Advanced cancer** (pMHC targets, solid tumors, hematology, ICH S9, DART)	**Advanced cancer**	**Serum amyloidosis** (SLTD, non-ICH S9)	**Virology** (SLTD, non-ICH S9)	**Respiratory disease** (Non-LTD, unmet need)	**Hemophilia A** (Non-LTD)	**Rare diseases** (SLTD, non-ICH S9)
Case Numbers	2, 14, 16, 18, 23, 6, 7, 9, 12, 17, 8	1, 3, 12, 20, 22,	11	13	10	4	19

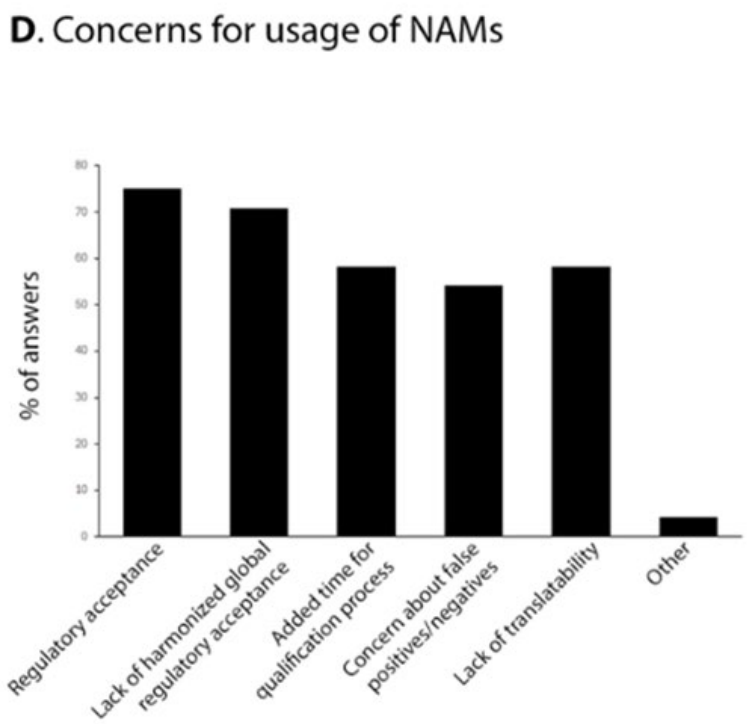
Perceived barriers to NAMs usage



Despite the data suggesting that NAMs-based programs were being accepted, most respondents had multiple concerns for usage of NAMs including:

- Regulatory acceptance by FDA
- Lack of harmonized global regulatory acceptance
- Concerns about translatability
- Added time for the qualification/validation of novel NAMs
- Concern about false positive/negative

Perceived barriers to NAMs usage



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Validation

Conclusions

- Scientifically justified NAM-based regulatory submissions are accepted globally.
- Key justifications are no relevant species, prior experience with the target, and disease severity.
- Opportunity exists to scientifically justify NAM-based approaches in broader settings.
- Remaining concerns about global regulatory harmonization and clinical translatability -> need for validation when cross-reactive animal species exist
- Global effort: leverage cross-industry consortia and join forces to validate assays with common list of reference compounds for large amounts of contexts of use

Doing now what patients need next

Challenges and opportunities for NAMs adoption

- Limited internal confidence in the translatability of in vitro models compared to conventional approach
- Dose selection restricted to conservative estimates (e.g. MABEL)
- Technical limitations of NAMs-based assays
- NAMs used as complement to conventional approach

- Expand NAMs use cases to improve confidence in their predictive value and increase their regulatory acceptance
- Create parallel approaches and push for systematic back translation
- Establish KPIs to measure short (*projects supported, submissions*) and long term impact (*improved risk/benefit assessment, reduce attrition, increase productivity*)
- Increase dialogue with health authorities (indirect &direct)
- Publish use cases and reviews

Conclusions

Improve confidence, increase regulatory acceptance, enhance risk/benefit assessment

- Economical (study cost, limited animal supply), Scientific (limited animal translatability) and Ethical (3Rs, public) reasons are pushing the pharma industry to explore NAMs for safety assessment
- Efforts are ongoing across the pharma industry to assess the use of NAMs for safety assessment (e.g. push across the pipeline and creation of IHB)

  -> Push whenever it makes sense
- Regulatory acceptance of NAMs is a direct consequence of the confidence in their predictive value

  -> Generate and share use cases to increase confidence
- FDA position on animal toxicity studies vs NAMs: “There are no alternative methods that can replace repeat dose toxicity studies in animals” but “when the state of science establishes that an alternative approach is equally capable of assessing risks, the FDA accepts the alternative approach”
  - -> Help HA to improve regulatory framework and show impact (Modernization act 3.0)
- Strike the right balance between enthusiasm and realism, manage expectations and be honest with cost/benefit of NAMs

No relevant animal species: simple internal qualification

Replacement of in vivo study: need for extensive validation (for specific context of use)

Beyond lack of cross reactive species, when animal species are available : need for extensive validation for specific context of use.