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Opportunities and insights from pharmaceutical companies on the current use of new approach methodologies in nonclinical safety assessment

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Sharing New Approach Methodology (NAM)-based regulatory experiences is crucial for improving human risk assessment and reducing animal use in drug safety testing. To foster broader adoption, the Biotechnology Innovation Organization surveyed companies about NAM usage and collected case studies showcasing NAM-based regulatory filings for

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biotherapeutics, where NAMs replaced large animal studies for safety assessment. These scientifically justified approaches were generally accepted by global health authorities, particularly in the context of species relevance limitations, prior target modulation experience, and/or when addressing severe disease. Despite successes with NAM-based global regulatory filings, there are concerns from companies about global regulatory harmonization and clinical translatability. NAMs have the potential for greater uptake with enhanced guidance and industry–regulatory agency collaboration being key to their adoption.

Keywords: nonclinical safety; New Approach Methodologies (NAMs); replacement of animals; 3Rs; nonhuman primate; regulatory submission; case examples; pharmaceuticals; drug development; industry consortium

Introduction

Due to their biological similarity to humans, non-human primates (NHPs) have traditionally been used in preclinical safety evaluation for Investigational New Drug (IND) applications especially for biotherapeutics. NHPs are important for biotherapeutics development because they are often the only pharmacologically relevant species.[p1],[p2] However, ethical concerns, and more recently NHP supply limitations, have highlighted the need to reduce animal usage. Additionally, translating findings from animal models to clinical outcomes remains challenging, especially with emerging novel biologic drug modalities such as chimeric antigen receptor (CAR) T-cell therapies. Consequently, there is growing interest in leveraging New Approach Methodologies (NAMs) to enhance drug development through improved scientific insights.[p3],[p4]

NAMs include a variety of methods such as *in vitro*, *in chemico*, *in silico*, and *in vivo* models[p3] that can reduce reliance on animals and have the potential to improve nonclinical to clinical translation.[p5] Avila *et al.* stated that *in vivo* models can be considered NAMs when they improve predictivity; shift studies to phylogenetically lower animals; or otherwise help replace, reduce, and refine animal use (i.e., the 3Rs) in development programs.[p3] NAMs can provide human-relevant data for risk assessment, especially when there are no pharmacologically relevant nonclinical species.[p6],[p7] Despite their promise, challenges remain in NAMs adoption in nonclinical drug development. This issue has prompted ongoing dialogue between regulators and the pharmaceutical industry and the formation of multiple working groups and task forces.

The global regulatory and legislative landscape surrounding the use of NAMs has evolved in recent years. Significant advancements have been made in the application of NAMs for chemicals and cosmetics testing.[p8],[p9],[p10],[p11] While in the pharmaceutical sector, recent literature reviews have emphasized the importance of defining context of use, setting validation criteria, and early engagement between developers and regulators.[p12],[p13] The European Medicines Agency (EMA) and United States Food and Drug Administration (FDA) have both emphasized the development and use of NAMs in drug development.[p4] The EMA has set recommendations for 3Rs approaches and NAMs, is moving to revise guidelines for regulatory acceptance of these methods, has formed the joint Committee for Medicinal Products for Human Use/Committee for Veterinary Medicinal Products (CHMP/CVMP) 3Rs Working Party,[p14] and is contributing to the development of NAMs as part of the EMA Innovation Task Force.[p15] Similarly, the FDA supports greater use of NAMs through several programs[p16] including the Alternative Methods Working Group, the New Alternative Methods Program, and the Innovative Science and Technology Approaches for New Drugs (ISTAND) program.[p17] The FDA Modernization Act 2.0, enacted in December 2022,[p18] clarifies that the Agency has legal authority to accept data generated via NAMs for preclinical and nonclinical testing. Several guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) already accommodate the use of NAMs, including the main guidance for biotechnology derived pharmaceuticals (ICH S6(R1)[p19] and the guideline on detection of toxicity to reproduction (ICH S5(R3)[p20] as described in a recent review by Brown and Wange of the FDA Center for Drug Evaluation and Research (CDER).[p21] However, in these guidelines, the scenarios where NAM-based approaches are considered scientifically justified are limited and/or leave significant regulatory uncertainty regarding broader acceptance of a NAM-based approach.

The use of NAMs for nonclinical drug development has been a key topic at recent joint annual meetings of BioSafe, a leadership group within the Biotechnology Innovation Organization (BIO), DruSafe, a leadership group within the International Consortium for Innovation and Quality in Pharmaceutical Development, and the FDA (CDER). For example, in 2021, a discussion on reducing reliance on NHPs focused on the need for alternative methods such as rodent surrogates, genetically modified rodents, and *in vitro* methods.[p22] The dialog continued and prompted the topic at the 2022 FDA/BioSafe/DruSafe annual meeting on the use of *in vitro* models for safety assessment to highlight emerging technologies with the potential to replace *in vivo* approaches. Recognizing that the lack of knowledge on real-world NAM-based regulatory filings may have hindered broader implementation, representatives from the FDA encouraged publishing industry examples of successful application and acceptance of NAMs for regulatory filings. Of note, FDA recently (Oct 2024) published a report focused on driving integration of NAMs for regulatory decision-making.[p23] Interestingly, one recommendation in the report is to compile a centralized NAMs database for FDA. This goal is different from publishing case studies where a series of NAMs are applied to build a safety program.

In response to the recommendation by the FDA to publish current use cases for NAM-based approaches, the BIO formed a NAMs Task Force in 2023. The Task Force surveyed members and requested case studies demonstrating the use of NAM-

based approaches for nonclinical safety and pharmacokinetics (PK). To differentiate from other efforts, the focus of the case studies was NAMs used in regulatory filings to replace animal testing, especially large animals, rather than merely refining or reducing animal use. This manuscript summarizes the outcomes, showcasing current NAM applications, limitations, and opportunities to further adopt NAMs.

Approach to gather industry-wide feedback regarding NAMs usage

The BIO NAMs Task Force developed a survey and case study form and collected responses using Alchemer, a feedback and data collection software. Requests were e-mailed using BIO working group distribution lists encompassing 188 unique entities based on company name. The request contained hyperlinks to the survey and case study form (the original survey and case study request forms can be found in the Supplementary Materials). Responses were accepted until early February 2024. One submission was excluded for not matching the required criteria.

To provide context for the request from BIO, background information was included in the e-mail request as summarized below.

Purpose of the request

The BIO is developing a manuscript on NAM-based nonclinical safety and PK programs for regulatory filings. The goal is to gather industry experience to advocate for the use and regulatory acceptance of NAMs where scientifically justified. The focus is on replacing the use of animals, particularly large animals (NHPs, dogs, minipigs), when no relevant animal species exist or when NAMs sufficiently address safety concerns. The emphasis is on replacement rather than reduction or refinement of animal use. The request seeks industry-wide participation in a survey and case studies on NAM-based nonclinical safety and PK programs.

Definition of NAM

According to the FDA CDER, NAMs include *in vitro*, *in chemico*, and *in silico* methods. *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.[p3]

Scope of the request

The case study request focuses on biotherapeutics due to the goals of the BioSafe (BIO preclinical) committee. This class of therapeutics includes antibody-drug conjugates, which apply safety assessment principles relevant to both small and large molecules. The survey covers all therapeutic modalities, including small molecules.

Questions were developed based on the experience of the Task Force members. Most questions were multiple choice, with options to provide written responses. Written responses were categorized *post hoc* by the authors.

The survey consisted of 12 questions aimed at capturing high-level experiences with NAMs across all modalities. Topics included the category of NAMs used, modality, indication, development stage supported by NAMs, regulatory experiences, and opportunities for and limitations of NAM usage. The respondents for the survey were not limited to responses where the NAM was designed to replace the use of a large animal.

The case study request had 68 questions and aimed to capture a single NAM-based case example, with respondents allowed to complete the form multiple times. Respondents could submit up to three case studies, with an option to indicate if they had more. One company submitted a fourth case study, which was accepted. Each case study could describe multiple individual NAMs. Some terms were defined, while others (e.g., fit-for-purpose, qualified, and validated) were left to respondent interpretation. Defined terms included 'pharmacologic target' and '*in silico* methods.' The Task Force used definitions for pharmacologic target consistent with those proposed by the European Federation of Pharmaceutical Industries and Associations (EFPIA) Safety Reflection Initiative (Beilmann *et al.*, submitted for publication). The authors' intent with the term *in silico* was to encompass any data sets that did not require a wet lab or animal-based experiments. To this end, '*in silico* methods' were described to include literature searches, public and proprietary databases (e.g., Genotype-Tissue Expression [GTEx] database[p24]), weight-of-evidence assessments, modeling methods, and other methods as described by the respondents.

The responses from the survey and the case study requests were evaluated using the Alchemer software and imported into Excel for further analysis.

Surveyed experience with application of NAMs to the nonclinical safety evaluation of pharmaceuticals across 27 pharmaceutical companies

BIO member companies were asked to complete a survey on the current use of NAMs, contextualize NAM utility through regulatory interaction experiences, and summarize perspectives on the opportunities or limitations facing adoption of NAMs. The survey was modality agnostic and captured the use of NAMs for nonclinical safety assessment and PK characterization. Importantly, we did not delineate in the survey whether the NAM replaced or reduced the use of large animals or were utilized as a complementary approach. Twenty-seven companies responded to the survey, with 24 providing complete responses and three only partial responses. Responders could select more than one answer for each question.

A wide variety of NAMs have been used to support nonclinical safety assessment or PK, with at least 50% of companies indicating that they have used *in silico*, human *in vitro/ex vivo* models, humanized or genetically modified animals, or surrogate molecules in rodents (Supplementary Figure S1a). *In silico* and human *in vitro/ex vivo* approaches were most often used. Other approaches shared through written comments included artificial intelligence, non-human *in vitro* approaches, and clinical data from a comparable compound (1 response each). A deeper dive into the type of *in silico* approaches used highlighted a diversity of assessment tools (literature search [78% of respondents], public/proprietary databases [83% of respondents], weight-of-evidence approach [78% of respondents], and modeling methods [67% of respondents]) (Supplementary Figure S1b).

NAMs supported all drug modalities surveyed: monoclonal antibodies, multi-specific antibodies, antibody-drug conjugates,

fusion proteins, recombinant proteins, cell therapies, gene therapies, peptides, oligonucleotides, and small molecules (Supplementary Figure S2a). Half of the respondents reported that NAMs supported their work with monoclonal and multi-specific antibodies, and 30–40% of companies reported that NAMs supported work with oligonucleotides, cell and gene therapies, and small molecules. On the other hand, only 10–20% of companies noted using NAMs to support work for fusion proteins, recombinant proteins, and peptides. While these responses likely reflect several factors, including the pipelines of responding organizations, they highlight that NAMs are being considered regardless of modality. More companies had experience supporting monoclonal and multi-specific antibodies with NAMs than small molecules. The lower likelihood of there being a pharmacologically relevant animal model for antibodies may partially explain this discrepancy, but is not the only explanation, as 54% of responding companies indicated that they had used a NAM or a NAM-based approach even when a large animal species was relevant (Figure 1a). Less concern for off-target effects with antibody-based therapies may also provide more confidence in applying a NAM-based approach versus small molecules. Moreover, NAMs supported the development of molecules for serious and life-threatening diseases (SLTDs) aligned with ICH S9 guidance, i.e., advanced cancer (70% of respondents), SLTDs not under ICH S9 (43% of respondents), and non-life-threatening disease indications without (40% of respondents) or with (35% of respondents) alternative therapeutic options (Supplementary Figure S2b).

While companies have used NAMs to support all stages of drug development, the greatest experience was supporting preclinical development (prior to regulatory submission for first-in-human [FIH] trials) (42% of respondents), FIH studies (21% of respondents), or registration (21% of respondents) (Supplementary Figure S2c). Most companies (74%) sought regulatory guidance on the use of a NAM/NAM-based approach prior to submission (Supplementary Figure S3), with many indicating a pre-IND interaction with the FDA. Of those interacting with the FDA, three companies indicated discussions were held through an FDA INitial Targeted Engagement for Regulatory Advice on Center for Biologics Evaluation and Research (CBER)/CDER ProducTs (INTERACT) meeting, while several others indicated a Type B/C meeting with either CDER or CBER. In addition to the FDA, interactions with global regulatory authorities including Pharmaceuticals and Medical Devices Agency (PMDA; Japan), Paul-Ehrlich-Institut (PEI; Germany), and Medicines and Healthcare products Regulatory Agency (MHRA; United Kingdom) were also noted. Ten companies indicated that they had NAMs accepted by regulators as a supplement to animal testing, with 9/10 gaining this acceptance in multiple regions. Nine companies indicated that they had NAMs accepted by regulators to replace animal testing, with six of the nine gaining this acceptance in multiple regions (in response to Survey Questions 8 and 9; see Survey Form in the Supplementary Materials).

Given the current state of NAM usage and acceptance, we also sought to understand if companies would consider using a NAM-based approach in the future to replace traditional large animal testing. Perhaps not surprisingly, the majority (91%) of companies would consider using NAMs to replace traditional large animals (Figure 1b) and most would consider multiple factors in their decision. The most important considerations for NAMs use for the replacement of large animals (Figure 1c) were scientific justification (91% of respondents), reduction of animal usage (3Rs) (87%), lack of pharmacologically relevant species (83% of respondents), improved translatability/prediction of human toxicity (83% of respondents), and additional mechanistic insight/context for findings in animals or in the clinic (70% of respondents). Less emphasis was placed on shortened timelines (44% of respondents). Individual write-in responses included providing justification for higher starting doses and assurance of regulatory acceptance of the NAM-based approach. Despite the overwhelming suggestion that companies would consider using a NAM, most had multiple concerns for usage of NAMs including regulatory acceptance by FDA, lack of harmonized global regulatory acceptance, concerns about translatability and validity, and added time for the qualification of novel NAMs (Figure 1d).

Experience with replacement of large animals with NAMs-based approaches based on 22 case studies

Twenty-two biotherapeutics case studies representing 10 unique companies were received for review. Six companies submitted multiple case studies, with two companies each submitting three or four case studies. Case study data were evaluated on the aggregate and representative case studies are also briefly described. Aligned with the goals of BioSafe, only biotherapeutics were in scope for the case studies. The modalities represented in the case studies (Supplementary Figure S4a) were predominantly antibody-based therapies (17 of 22), with the remaining five examples being cell therapies. Information received on the mode of action is provided in Supplementary Figure S4b.

Case studies did not focus on the technical details of the NAMs but instead looked at high level considerations, e.g., type of NAM applied, and whether the NAM was newly designed, adapted from an existing NAM, or off-the-shelf (i.e., readily usable from a commercial source or a Contract Research Organization, without additional modification/adaptation). NAMs were predominantly *in silico* only or a combination of *in silico* and *in vitro* approaches (Figure 2a). In addition, most NAMs were reportedly adapted from existing or previously used NAMs or designed for a specific use rather than being off-the-shelf (Figure 2b). For *in silico* approaches, literature search, application of public and proprietary databases, and weight-of-evidence approaches were most frequently cited (Figure 2c).

While NAMs were used to support all stages of development, most were used for early development either at the preclinical stage prior to an initial FIH filing (4 of 22) or to support the FIH stage (12 of 22; Supplementary Figure S5). Four cases mentioned that the programs had reached Phase 3 or registrational trials, while only one case study referred to an approved drug. Among these later development cases, three either included or specifically focused on development and reproductive toxicology (DART) studies, which are generally required to support later stage clinical trials or registration. For the remaining two case studies, the DART package could only be inferred.

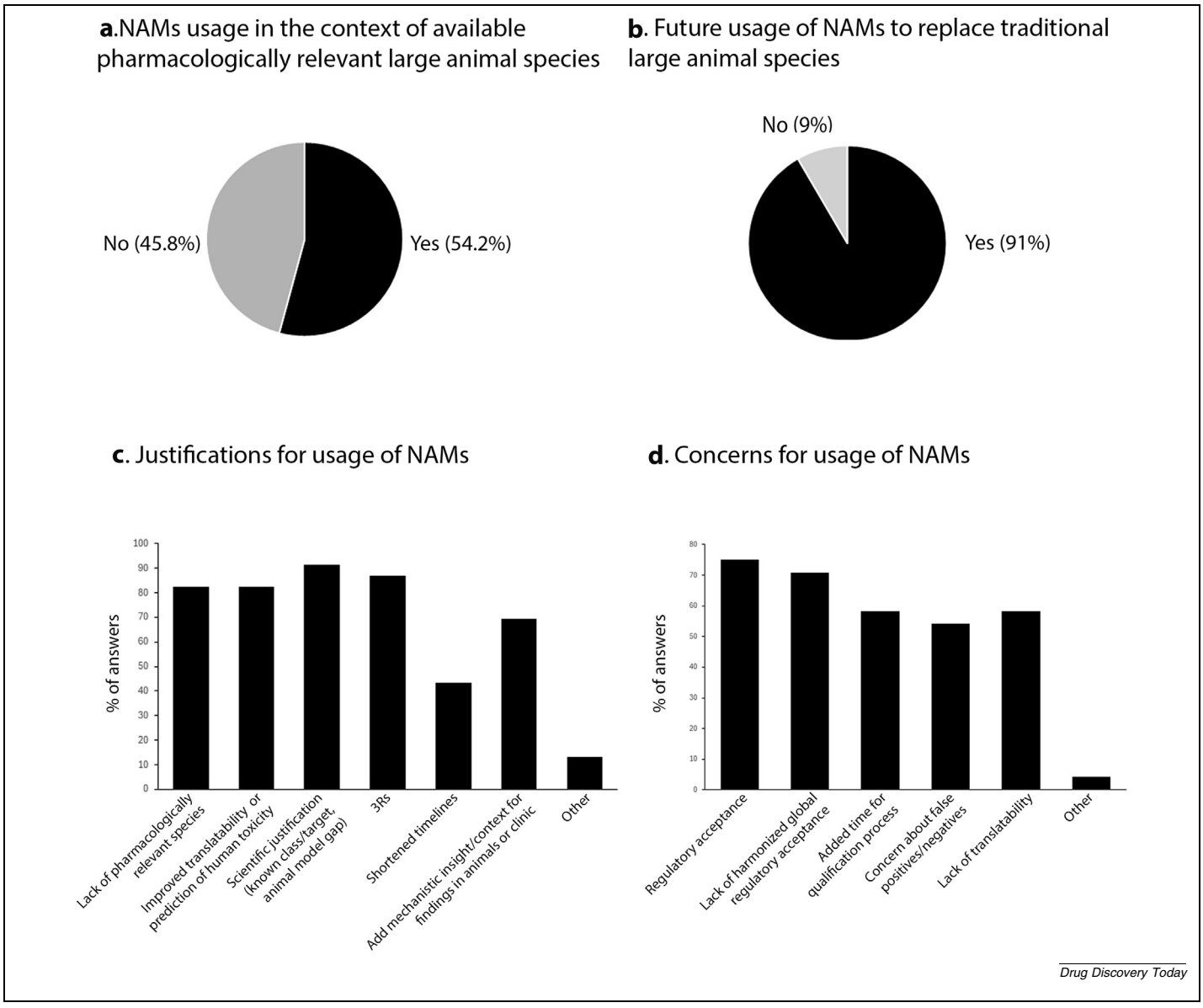


FIGURE 1

Survey results representing (a) current and (b) future usage of NAMs when a pharmacologically relevant large animal species (nonhuman primate, dog, minipig) is available. The justifications or concerns for using NAMs are captured in (c) and (d), respectively. Respondents were asked: (a) 'Has your organization used a NAM approach when there was a pharmacologically relevant large animal (nonhuman primate, dog, minipig) model available' (Question 6)? Response options were limited to 'yes' or 'no'. (b) 'Would your organization consider using a NAM approach in the future to replace traditional large animal (nonhuman primate, dog, minipig) testing' (Question 10)? Response options included 'yes', 'no' or 'other'. (c) 'If yes to the previous question, what would prompt utilization of a NAM approach? Select all that apply' (Question 11). Response options were: (1) Lack of pharmacologically relevant species, (2) Improved translatability/prediction of human toxicity, (3) Scientific justification (well-known class/target, known gap in traditional animal model), (4) 3Rs, (5) Shortened timelines, (6) Provide additional mechanistic insight/context for findings in animals or clinic and (7) Other. For that question, the respondents who selected 'other' wrote the following comments: 'All of the above', 'Regulatory acceptance of NAMs' and '3D complex *in vitro* model may allow/provide justification for higher starting doses'. (d) 'What concerns limit the use of NAMs by your organization (select all that apply or write-in other concerns)' (Question 12)? Response options were: (1) Regulatory acceptance by FDA, (2) Lack of harmonized global regulatory acceptance, (3) Lack of translatability, (4) Concern about false positives/false negatives, (5) Added time for qualification process, and (6) A write in comment box. In all cases, the option 'Other' included an option to write in a response. The Survey Form can be found in the Supplementary Materials.

Overwhelmingly, companies felt the identified risks, based on target safety assessment, modality, and mode of action, could be addressed with non-animal methods (19 of 22 case studies; data not shown). About half of the respondents indicated that the data from the NAM-based approach were used for clinical translation (data not shown), and similarly, about half of the respondents indicated that additional mitigation measures (e.g., starting dose selection using the minimal anticipated biologic effect level [MABEL]) and monitoring were incorporated into human clinical trials (Figure 3).

The perceived risk by Sponsors regarding the submission of NAM-based regulatory packages varied, with respondents evenly

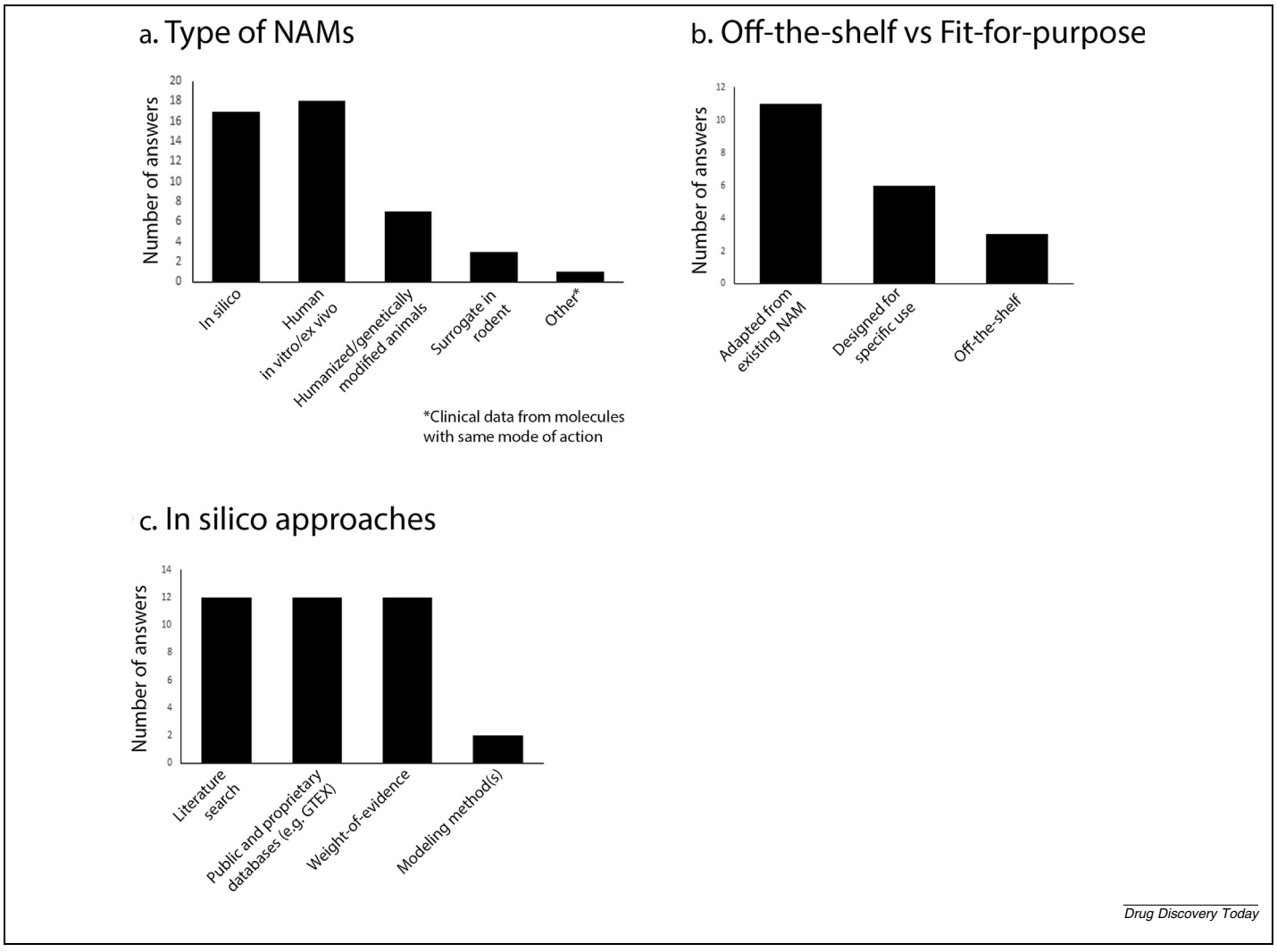


FIGURE 2

General categories of NAMs identified across case studies. Respondents were asked: (a) 'Indicate the categories of the NAM(s) applied to address safety for this case study' (Question 27). Response options included: (1) *In silico*, (2) Human *in vitro/ex vivo*, (3) Humanized animals or genetically modified organisms, (4) Surrogate or tool molecule in rodent, (5) Other. (b) 'Indicate whether the NAM was' (Question 30). Response options included: (1) Designed for this specific use, (2) Adapted from an existing or previously used NAM, (3) Available off-the-shelf, or (4) Other. (c) 'If yes to *in silico*, select all the methods that were applied' (Question 28). Response options included: (1) Literature search, (2) Public and proprietary databases (e.g., GTEX), (3) Weight of evidence assessment (s), (4) Modeling method(s), and (5) Other. In all cases, the option of 'Other' included an option to write in a response. The Case Study Request Form can be found in Supplementary Materials. GTEX, genotype-tissue expression.

split on whether they engaged with regulators prior to submission (Figure 4a). The regulatory authorities covered by the case studies included the US (FDA, CDER and CBER), the United Kingdom (MHRA), Europe (EMA and national health authorities), Japan (PMDA), and China (National Medical Products Administration; NMPA) (Figure 4b). Although most case studies were submitted before the issuance of the since withdrawn (with the expiration of the COVID-19 Public Health Emergency) FDA guidance on NHP supply constraints (February 24, 2022 to May 11, 2023) and the FDA Modernization Act 2.0 (September 29, 2022) (Figure 4c), all cases were accepted by health authorities. There were two reports of health authorities requesting animal studies be added to the NAM-based package but subsequently agreeing that animal studies were not required. There was only one case where an animal study was required (a fertility study in NHP).

Of the 22 use cases submitted, 15 indicated some limitations or perceived barriers to application of NAMs. Reported limitations can be grouped into four main topics including (i) the questionable *in vitro* to *in vivo* translatability of NAMs (8 reports), (ii) the limited ability of NAMs to help define optimal dose selection (5 reports), (iii) the limited tissue coverage and availability offered by NAMs (5 reports), and (iv) the technical limitations of NAM-based assays (4 reports).

Based on the case studies received, there are several common situations where NAMs are currently being used in industry. These include advanced cancer (ICH S9) or other serious and

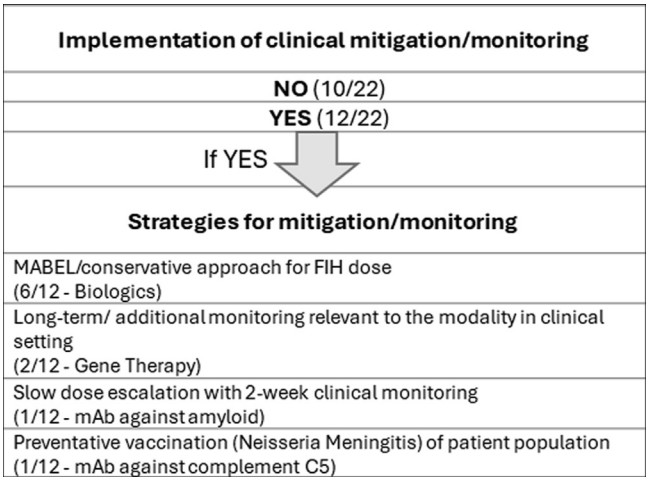


FIGURE 3

Implementation of additional clinical mitigation or monitoring across case studies. Respondents were asked 'Was additional clinical mitigation (this includes starting dose selection and dose escalation strategy) and monitoring required to address risks that could not be addressed with non-animal methods?' (Question 17). Response options were limited to yes or no, but the following question asked for further elaboration if possible using a write-in option. A summary of the write-in information is provided including the incidence of responses and modality. Additional details are provided as shared by the respondent. The Case Study Request Form can be found in Supplementary Materials. FIH, first-in-human; MABEL, minimal anticipated biological effect level.

life-threatening conditions as the intended patient population, lack of a pharmacologically relevant species for nonclinical safety assessment, and prior clinical experience with modulating the target. These common scenarios are described in further detail in the following sections.

Frequency of NAMs-based case studies reflect the seriousness of the disease to be treated

According to the ICH S9 guideline, nonclinical programs for developing pharmaceuticals for advanced cancer can be more flexible than those for other drugs.[p25] Some of the flexibility described in ICH S9 may be applied to other SLTDs on a case-by-case basis. The FDA guidance on Rare Diseases, for example, emphasizes broad flexibility in applying the statutory standards for drugs treating severely debilitating or life-threatening rare diseases, while preserving appropriate standards of safety and effectiveness.[p26] Factors considered for determining nonclinical flexibility include the characteristics of the drug, clinical investigation design, disease severity, available therapies, and potential human risks. Therefore, it is expected that early adoption of NAMs would predominantly involve ICH S9 cases followed by pharmaceuticals in development for other SLTD.

Indeed, most of the submitted case studies focused on biotherapeutics in development for advanced cancer (16 of 22 cases; Figure 5a) with 15 of 16 supporting FIH and/or Phase 1 clinical trials. One of these cases had also progressed through to registration with a NAMs only based nonclinical safety package (a T-cell receptor CD3 bispecific antibody; Case 14). Another advanced cancer case study focused on a NAM-based risk assessment for developmental and reproductive toxicity (Supplementary Figure S6, Case 8).

For advanced cancer (ICH S9) cases supporting FIH and other Phase 1 trials, most NAM-based programs were focused on *in silico* data combined with *in vitro*/*ex vivo* assessments (Table 1 and Supplementary Figure S7, Cases 1 and 7); however, some respondents also leveraged pharmacology or PK studies in rodents (6 cases) and/or large animals (3 cases) to bolster their NAM-based safety package (Supplementary Figure S8).

Six of twenty-two cases were being developed for non-oncology indications. These cases were of particular interest due to the perceived reduced flexibility in nonclinical safety programs for pharmaceuticals being developed for diseases out of scope for ICH S9.

Three of the six non-ICH S9 cases (cases 11, 13, and 19) were reportedly in development for patients with SLTD (not under the scope of ICH S9) with one case reporting that patients had other therapeutic options (case 13). These indications included serum amyloidosis, human immunodeficiency virus, and a series of rare diseases. Unlike in the NAM-based cases for advanced cancer where *in silico* combined with *in vitro* packages were common, for pharmaceuticals in development for these indications, mouse models were used for safety assessment in two of three cases (serum amyloidosis and rare disease). For the serum amyloidosis program (Case 11), safety endpoints (e.g., clinical pathology and macro and microscopic pathology assessments) were included in pharmacology studies of the anti-amyloid component P monoclonal antibody (mAb) in a human serum amyloid A knock-in mouse model to support the FIH clinical trial following consultation with MHRA. Notably, this antibody was not considered first-in-class, i.e., there was prior clinical experience with modulating this target. In the rare disease case, the NAM-based approach leveraged a series of mouse toxicity studies where a mouse cross-reactive surrogate antibody was tested (Case 19). It is inferred by the authors that the mouse cross-reactive surrogate antibody also supported evaluation of toxicity to development and reproduction (Supplementary Figure S6).

The final non-ICH S9 but SLTD case (Case 13) was for a bispecific T-cell engager targeting a peptide-MHC complex (pMHCxCD3) for the treatment of HIV. The approach taken was like that taken for T-cell engagers targeting tumor antigens (including human-specific pMHC antigens). Specifically, the FIH-enabling toxicology package consisted of *in silico* assessments (review of public and proprietary databases [e.g., GTEX]), *in vitro* cell-based functional assays, and secondary pharmacodynamic (PD) studies (where off-target cross-reactivity with healthy tissue was assessed). The NAM-based assessment supported hazard identification, risk assessment, and FIH dose selection based on a MABEL. The FIH nonclinical safety program was submitted to and accepted by MHRA and EMA.

For the remaining three non-ICH S9 case studies, two addressed unmet medical needs without being classified by the respondent as SLTD (Alzheimer's disease [Case 15] and a respiratory disease [Case 10]), and the final case was for a condition that the respondent indicated was not life-threatening and where alternative therapeutic options were available (hemophilia A

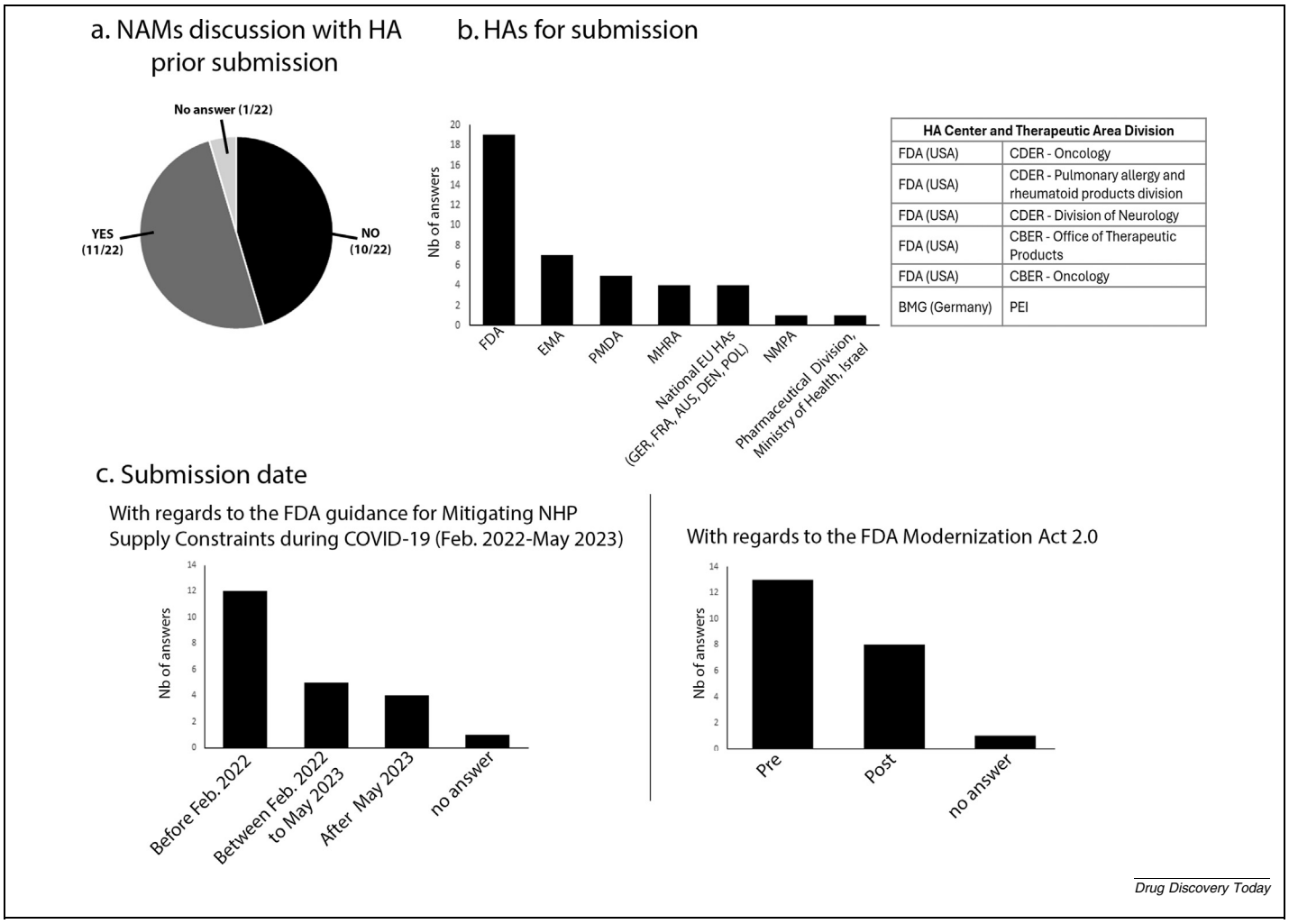


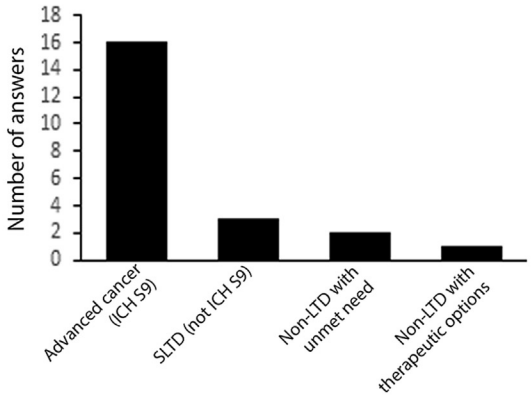
FIGURE 4

Health authorities' interactions across case studies (a, b) and dates of submission with regard to the FDA guidance for mitigating NHP supply constraints and the FDA Modernization Act (c). Respondents were asked: (a) 'Were the NAMs discussed with the health authority prior to the submission? (Question 57). Response options were limited to 'yes' or 'no'. (b) 'Indicate to which health authorities the safety package in this case study was submitted (e.g., FDA, MHRA, PMDA, a specific EMA national competent authority, etc.) (Question 58) and 'Indicate the relevant Center (e.g., CDER or CBER for FDA, BfArM or PEI for Germany) and Therapeutic Area Division of the Health Authority, if appropriate (e.g., Oncology) (Question 59). Responses were provided as write-ins and subsequently collated. (c) 'Indicate the timing of the regulatory interaction in relation to the publication by the FDA of the guidance titled 'Nonclinical Considerations for Mitigating Nonhuman Primate Supply Constraints Arising from the COVID Pandemic' (published Feb 2022 and expired May 2023)' and 'Indicate the timing of the regulatory interaction in relation to the signing into law of the FDA Modernization Act 2.0 (Dec 2022)' (Questions 62 and 63). EMA, European Medicines Agency; FDA, Food and Drug Administration; MHRA, Medicines and Healthcare products Regulatory Agency; PMDA, Pharmaceuticals and Medical Devices Agency. AUS, Austria; DEN, Denmark; FRA, France; GER, Germany; POL, Poland. BMG, Bundesministerium für Gesundheit; CBER, Center for Biologics Evaluation and Research; CDER, Center for Drug Evaluation and Research; NMPA, National Medical Products Administration; PEI, Paul Ehrlich Institut.

[Case 4]). Like the non-ICH S9 case for serum amyloidosis (see above Case 11), this Alzheimer's disease case also leveraged a transgenic mouse model (presumably a human knock-in mouse) for safety assessment (Supplementary Figure S7, Case 15).

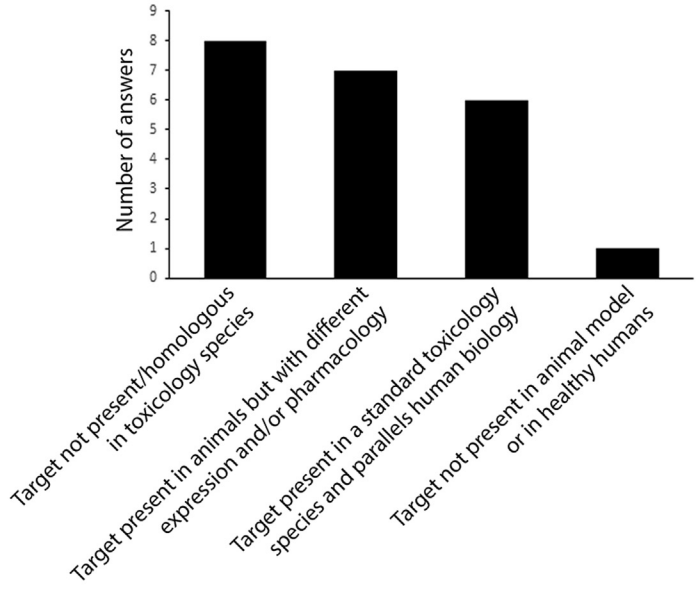
The respiratory case (Case 10) was focused on an enhanced pre- and postnatal development (ePPND; ePPND studies are described in ICH S5) study waiver (i.e., it is agreed between Sponsor and health authority that a study is not required to inform the risk assessment) for a fragment crystallizable (Fc)-modified mAb targeting a cytokine. The target was not novel; there were already approved therapies. The company was successful with obtaining an ePPND study waiver despite NHP being a pharmacologically relevant species (Supplementary Figure S6). The NAM-based approach to the waiver was based on *in silico* data. The respondent reported using a model of IgG placental transfer to predict only a modest increase in placental transfer due to an increased binding of the antibody Fc to neonatal Fc receptor (FcRn) (the significance of FcRn binding in transplacental transfer of maternal IgG to the offspring is explained in (p27)) compared to a predecessor molecule as part of the weight-of-

a. Indication

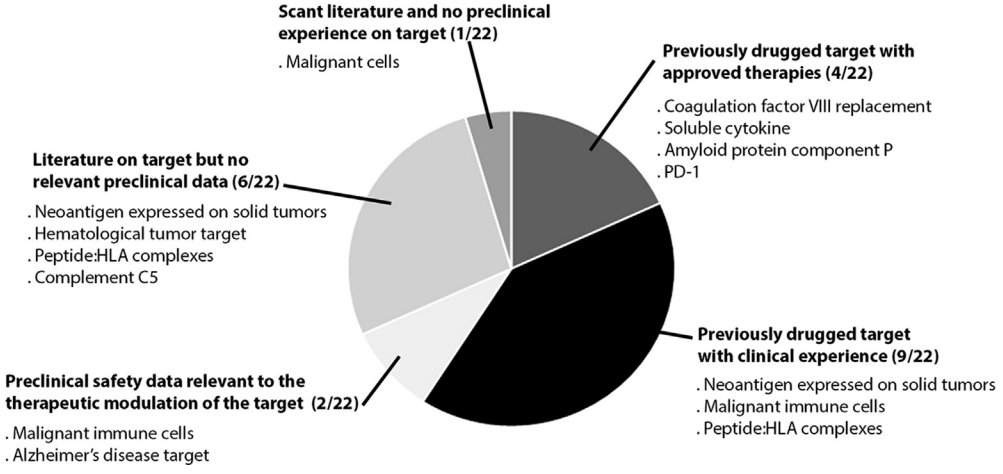


Advanced cancer (ICH S9)	Unspecified malignancies
	Hematological Malignancies
SLTD (not ICH S9)	Serum amyloidosis
	HIV
	Rare diseases (paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, generalized myasthenia gravis and neuromyelitis optica spectrum disorder)
Non-LTD with unmet need	Respiratory disorder
	Alzheimer's disease
Non-LTD with therapeutic options	Hemophilia A

b. Target in toxicology species



c. Previous experience with target



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evidence approach. This NAM-based approach to DART was accepted by multiple health authorities (FDA [CDER], EMA, and PMDA).

Of particular interest was the case for hemophilia A (Case 4) because it was considered to have the lowest benefit to risk ratio based on the respondent's classification and would therefore be assumed to have the least flexibility in approach to the nonclinical safety program. Nevertheless, a NAM-based nonclinical safety program was supported by multiple health authorities (Supplementary Figure S7, Case 4). The NAM-based assessment reportedly informed FIH dose selection and clinical translation (dose selection, clinical monitoring, exclusion criteria) and was accepted by regulatory agencies due to lack of an appropriate nonclinical safety species.

Lack of a pharmacologically relevant species was a common factor for NAMs-based regulatory filings

NHPs are often the only pharmacologically relevant species for nonclinical safety assessment for biopharmaceuticals. It is much less common that rodents, a standard second species for assessment of small molecule therapeutics, are a pharmacologically relevant species for biotherapeutics safety assessment. In addition, it is not common to conduct nonclinical safety studies for biotherapeutics in dogs or minipigs for reasons including lack of species cross-reactivity, concerns about anti-drug antibody formation that can impact study interpretation, differences in target or pathway biology, lack of historical background data, and a comparable (to NHP) lack of suitable assays and reagents for *ex vivo* assessments. It was not our intent to encourage replacement of NHPs with other large animal species (dogs, minipigs) for safety assessment, but rather to seek case studies where it was scientifically appropriate to replace large animals for the safety assessment of the potential medicine.

Because the safety of biotherapeutics is generally related to on-target, exaggerated, or undesired pharmacology, it is critically important to conduct safety studies in pharmacologically relevant species and use of irrelevant species is discouraged (ICH S6 (R1)). In cases where no relevant nonclinical species is available, Sponsors are required to propose alternative methodologies for safety assessment. Therefore, we anticipated that many of the NAMs case studies we received would involve situations where no relevant nonclinical safety species exist, given the limited alternatives besides NAMs in such cases.

As expected, normal animals were deemed irrelevant for safety assessment of the clinical candidate in most cases due to considerations related to the pharmacologic target and/or the human-specific modality (Supplementary Table S1). Reasons considered relevant to the pharmacologic target (Figure 5B) included differences in target expression in nonclinical species compared to human, a lack of expected or observed pharmacology in the nonclinical species, and/or a lack of cross-reactivity of the clinical candidate in nonclinical species generally used for nonclinical safety assessment. In cases where modality was cited, the rationale was based on the modality being a human-specific cell therapy (e.g., Supplementary Figure S7, Case 1), highlighting that traditional animal-based nonclinical safety assessment paradigms are not appropriate for these regenerative medicines.

Prior experience with modulating the target was often cited in the NAMs-based case studies

Prior experience with modulating a target can increase conviction in both the potential safety and efficacy of a new therapy targeting the same or a similar pathway. One can also apply principles described in recent draft FDA guidance documents (e.g., Generally Accepted Scientific Knowledge in Applications for Drug and Biological Products [May 2023][p28] and Platform Technology Designation Program for Drug Development [May 2024] (FDA 2024))[p29] to a NAM-based weight-of-evidence. For example, one may expect that the success of a NAMs-based regulatory filing would increase when Sponsors leverage prior knowledge from their previously submitted applications that may include animal-based safety assessment studies. Therefore, it could be

FIGURE 5

Indication (a) and target-related assessment: presence of the target in toxicology species (b), previous experience with the target (c) across case studies. (a) Respondents were asked about the risk:benefit considerations in the intended patient population (left panel) and indication (right panel). Left panel: 'Indicate the tolerance for risk in the patient population on a scale of 1 to 5 as described below. Choose the best answer' (Question 5). Responses for selection were: (1) The initial development program is performed in patients with serious and life-threatening malignancies (ICH S9), (2) The initial development program is performed in patients with serious and life-threatening diseases (not ICH S9), (3) The initial development program is performed in patients with non-life-threatening diseases but with an unmet medical need (i.e., absence of available therapy or a patient population refractory to available therapy), (4) The initial development program is performed in patients with non-life-threatening diseases and with alternative therapeutic options, (5) Other – write in. Right panel: 'State the intended indication (e.g., overweight or obese children, asthma, advanced cancer)'. Responses were limited to write-in. The write-in responses were paired with the risk:benefit responses. LTD, life-threatening disease; SLTD, serious and life-threatening disease. (b) Respondents were asked to 'Select the most appropriate category regarding the pharmacological target' (Question 9). Six options were included and options 2 to 5 were aligned with the EFPIA Safety Reflection Initiative. Options included: (1) The pharmacologic target is present in a standard toxicology species, and target expression and pharmacology sufficiently parallel human biology, (2) The pharmacological target is not present or has insufficient homology in any standard non-clinical animal species, (3) The pharmacological target is present in animals but has different cross species expression and/or pharmacology leading to questionable translational value of animal models, (4) The pharmacological target is not present in the animal model or in healthy humans, (5) Drug candidates for which adverse events observed in clinical trials were not predicted form animal studies, (6) None of the above. (c) Respondent were asked to 'Indicate the novelty of the target, from well-characterized to completely novel' (Question 7). Responses for selection included: (1) Previously drugged target with approved therapies, (2) Previously drugged target with clinical experience, (3) Preclinical safety data relevant to the intended therapeutic modulation of the target are available, (4) Literature on target but no preclinical data relevant to the safety of modulating the intended target, and (5) Scant literature on target and no preclinical experience with intended therapeutic modulation of target. Clarifying comments could be written in and are reported under each category of responses.

TABLE 1

NAM-based approaches for advanced cancer indications relied largely on NAMs.

Case	Description[a]	Types of NAMs applied in safety assessment		
		In silico	*In vitro/ ex vivo*	*In vivo*[b]
1	T-cell receptor engineered T cells against pMHC	Yes	Yes	No
2	Multi-specific against a solid tumor TAA	Yes	Yes	No
3	*Ex vivo* gene therapy (presumed cell therapy)	Yes	Yes	Yes[c,d]
6	TCE against pMHC	Yes	Yes	No
7	Bispecific antibody targeting T cells and a TAA (presumed TCE)	Yes	Yes	No
9	TCE against a solid tumor TAA	Yes	Yes	Yes[d,e]
12	CAR T-cell therapy against a solid tumor TAA	No	Yes	Yes[c,f]
14	Bispecific protein TCRxCD3 against pMHC	Yes	Yes	No
16	Multi-specific Ab against hematology TAA	No	Yes	Yes[c,d]
17	TCE against a hematology TAA	Yes	Yes	Yes[c,d]
18	Multi-specific Ab: bispecific co-stimulator against a hematologic TAA; non-CD3-based mAb	Yes	Yes	Yes[c,d,e]
20	Engineered T cells against oncogene antigen	Yes	Yes	No
21	Bispecific TCR-based therapeutic that targets and activates T cells based on expression of oncogene peptide	Yes	Yes	No
22	CAR T-cell therapy	Yes	Yes[g]	
23	Multi-specific Ab TCE against malignant immune cells	Yes	Yes	No

CAR, chimeric antigen receptor; mAb, monoclonal antibody; pMHC, peptide-major histocompatibility complex (MHC); TAA, tumor-associated antigen; TCE, T-cell engager; TCR, T-cell receptor.
Note: Case Study 8 was focused on DART and not included in this table. Although we reported on 22 case studies, case study numbers ranged from 1 to 23 because one case study was excluded.

[a] Case description is based on write-in text by the respondent. Assumptions listed are those of the authors.

[b] None of these *in vivo* studies were dedicated nonclinical safety studies.

[c] Pharmacology studies in mice (e.g., immunocompromised models) contributed to the weight-of-evidence for safety assessment.

[d] PK studies in mice (e.g., immunocompromised, humanized, or FcRn transgenic mice) contributed to the weight-of-evidence for safety assessment and/or the justification of the clinical dose selection.

[e] PK studies in large animals (e.g., NHPs or minipigs) supported nonclinical safety.

[f] SCID mice injected with patient derived tumor were administered CAR T-cell therapy and at the end of the study all tissues were macroscopically and microscopically examined. These data were utilized to support clinical safety (cross-reactivity with murine target was established prior to study conduct).

[g] Respondent reported that no safety endpoints were added to the pharmacology studies but adverse findings in the pharmacology studies would have been investigated although mice were not a relevant species.

expected that many of the NAMs case studies would include reference to prior Sponsor-submitted packages and/or publicly available data sets.

In line with this expectation, 13 of 22 NAM-based approaches were applied to previously drugged targets (Figure 5c). Of these, 10 were in development for advanced cancer (e.g., Supplementary Figure S7, Cases 1 and 7); one was in development for other SLTDs (non-ICH S9), i.e., serum amyloidosis (Case 11); and two were in development for indications considered not to be life-threatening (hemophilia A, Supplementary Figure S7, Case 4 or respiratory disease Case 10) by the respondent. In several cases, 'previously drugged' included leveraging an internal predecessor molecule against the same target as part of the NAM-based weight of evidence (e.g., Supplementary Figure S7, Case 9). In this latter case, for a T-cell engaging bispecific antibody in development for advanced cancer, the nonclinical safety assessment of a new molecule, with expected pharmacology in a large animal species, was based on prior data (including NHP data) with a predecessor molecule, in addition to a molecule-specific *in silico* and *in vitro* data package (Supplementary Figure S7, Case 9). As a reminder, the authors consider leveraging data from a predecessor molecule to support a novel therapeutic as part of a weight-of-evidence approach and therefore an *in silico* NAM.

For later development case studies focused on DART, there were two cases with different experiences. Unexpectedly, an NHP fertility study was requested for an anti-programmed cell death protein 1 (PD-1) mAb in development for advanced cancer. Although multiple health authorities (FDA [CDER], MHRA, PMDA, EMA) granted a waiver of an NHP (species inferred by the authors) ePPND study based on a weight-of-evidence assessment, the respondent reported that there was a request from one health authority (unnamed) to complete a stand-alone fertility study in sexually mature NHPs. By contrast, for an anti-cytokine mAb developed for a respiratory condition (Case 10; see Supplementary Figure S6), instead of conducting an ePPND study, researchers used an *in silico* weight-of-evidence approach. This approach included ePPND data from a predecessor molecule with weaker FcRn binding affinity and successfully supported a study waiver.

For the remaining 8 of 22 cases, where NAM-based approaches were used for targets without prior clinical experience, 5 of the 8 cases were for therapeutics in development for advanced cancer, 2 were in development for another SLTD (non-ICH S9), i.e., human immunodeficiency virus (Case 13) and rare disease (Case 19), and 1 was in development for a disease not considered life-threatening by the respondent (Alzheimer's disease; Supplementary Figure S7, Cases 15).

Discussion

The goal of this Task Force was to publish current use cases for NAM-based approaches, in response to a recommendation by the FDA to share this knowledge broadly. To gather use cases, we surveyed BIO members and requested relevant case studies

for nonclinical safety and PK, with a focus on NAM-based regulatory filings that replaced animal testing, large animals in particular. The more general survey portion highlighted the diversity of NAMs that have been used to date, with over half of respondents indicating that they have used *in silico*, human *in vitro*/*ex vivo* models, or other alternative methods. Most case studies received were for: (1) therapeutics indicated for advanced cancer or other SLTDs; (2) programs that lacked a pharmacologically relevant nonclinical safety species; or (3) molecules targeting previously drugged pathways with existing safety data. Use of NAM-based approaches to support risk assessment for therapeutics in development for SLTDs is consistent with current regulatory guidance that allows increased flexibility as the severity of disease increases. In keeping with recommendations in ICH S9, NAM-only approaches continue to be successful when supporting clinical development beyond FIH-enabling studies, partly because less emphasis is placed on developmental and reproductive toxicity. Although NAM-based regulatory submissions were less common for non-SLTDs, several case studies supporting indications of lesser severity were submitted. Among NAM-based regulatory submissions supporting FIH trials, a majority also lacked a pharmacologically relevant nonclinical safety species, making a NAM-based approach the only option. In these cases, the use of surrogate antibodies or transgenic animals for safety assessments was more common for therapeutics in development for non-advanced cancer indications, likely due in part to the anticipated future need to evaluate DART and the anticipated reduced flexibility in approach outside of advanced cancer. Finally, the NAM-based regulatory submissions for molecules targeting previously drugged pathways demonstrates that prior examination of safety concerns strengthens the NAM-based approach.

Although companies are applying NAM-based approaches, the survey results and nature of the case studies submitted pointed to concerns about the translatability of NAMs for informing human risk assessment and the regulatory acceptance of a NAM-based preclinical approach. These concerns limit the uptake of NAMs beyond the categories observed in the submitted case studies. Pursuing a NAM-based strategy for safety assessment requires significant tolerance for regulatory risk given the lack of globally aligned and accepted guidelines, as well as resource diversion from innovation to scenario planning to avoid significant delays should additional studies be requested by health authorities. Many companies (81% of survey respondents and 50% of case study examples) seek pre-filing advice from one or more health authorities to reduce regulatory uncertainty; however, based on survey responses, a potential lack of global regulatory acceptance plays a large role in whether companies are willing to use NAMs to replace animal studies when scientifically justified. However, submitted case studies clearly indicate that NAM-based approaches are being accepted by global health authorities with no obvious differences in acceptance reported among them (except for the NHP fertility study requested for a PD-1 mAb).

In recent years, European and U.S. government agencies have shown a growing interest in advancing the use of NAMs in drug development. The EMA has established a 3Rs Working Party whose goal is to monitor and supervise the different 3Rs activities required to achieve the strategic goals,[(p14)] in line with the EMA Regulatory Science Strategy 2025[(p30)] and the 3-year workplan of the nonclinical domain, currently under revision. FDA and the National Toxicology Program (NTP) have published roadmaps that demonstrate their recognition of the value NAMs can bring to predictive toxicology and safety assessment.[(p31)],[(p32)] While both publications are helpful to understand what aspects of the models the FDA/NTP consider important to evaluate, neither provide specific guidance on the contents of a submission package for NAMs. Additionally, although the FDA Modernization Act 2.0[(p18)] authorizes the FDA to accept NAMs, clear guidance on the information needs, i.e., what the FDA expects out of a product application that leverages a NAM-based strategy, is still lacking. FDA also recently published a draft guidance on Platform Technology designations[(p29)] that would allow Sponsors to leverage certain nonclinical safety data from prior products that used the designated platform technology such that a new product-specific assessment might not be warranted. The practice of leveraging prior information has always been available to Sponsors via right-of-reference, but this new designation could help educate and accelerate uptake of NAMs in safety assessment.

While we are encouraged that the EMA and FDA are moving ahead with developing their roadmaps for policy development on NAMs, global harmonization such as regulatory standards via the ICH will be critical for increased NAM adoption. Moreover, while this publication has focused on feedback received and emerging precedent observed by Industry from applying NAM-based strategies to safety assessment, it is equally important that health authorities provide their perspective from reviewing NAMs-based applications. These collective experiences could inform: (1) guidance outlining the drug development scenarios under which NAMs are being accepted to replace animal safety studies, including examples of NAMs-based strategies that have been deemed acceptable by reviewers to date (see Figure 6, current state for ICH S9); and (2) guidance clarifying the extent to which NAMs should be qualified or validated to be included in a drug application in the form of clear evidentiary requirements. An ICH guideline (for example an update to ICH S9 [Figure 6, future state] and/or ICH S6(R1)) based on the emerging precedent would help the field move towards greater adoption of NAMs when scientifically justified.

Given the current state of how NAMs are being applied in industry, we would advocate that the use of NAMs could be extended to additional situations where animal studies would not add to human risk assessment beyond the data set developed with a NAM-based approach. These situations include NAM-based regulatory submissions when there is a pharmacologically relevant species for safety assessment for molecules in development for advanced cancer indications (Figure 6, future state). For example, a NAM-based safety program for a new mAb could provide *in silico* and *in vitro* data confirming the expression profile of the target, *in vitro* data confirming the specificity of the molecule to the intended target, prior *in vivo* data from nonclinical studies targeting the intended pathway (e.g., with a prior molecule), and evidence for clinical monitorability and manageability of expected safety findings based on experience in patients where the target biology has previously been manipulated. First-in-human starting dose could be based on *in vitro*

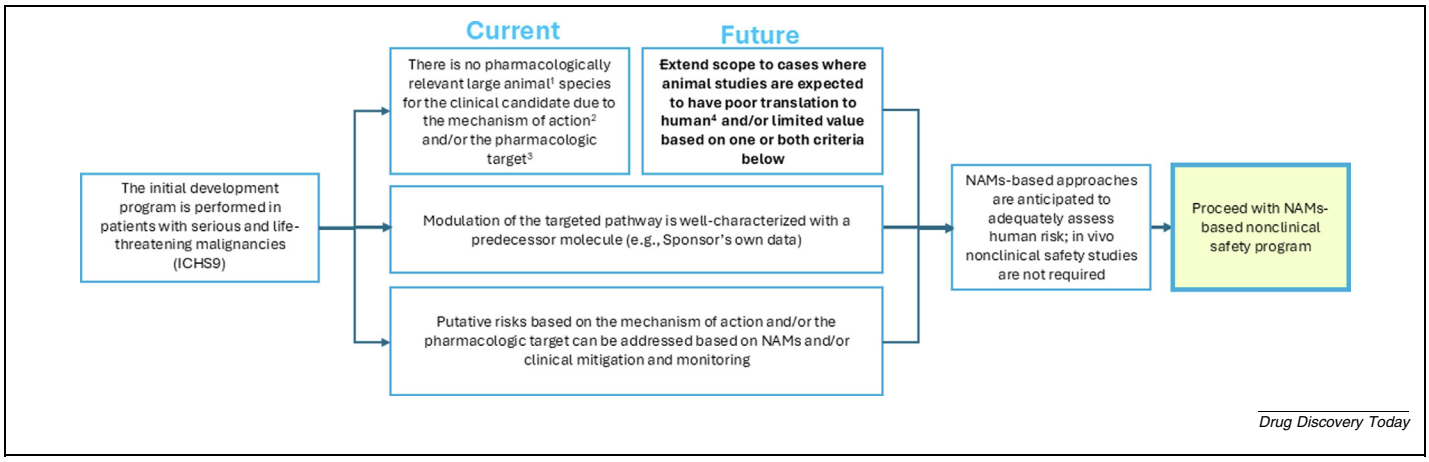

FIGURE 6

Application of NAM-based nonclinical strategies for large molecules for ICH S9. For biotherapeutics in development for advanced cancer, NAM-based regulatory filings have been accepted by global health authorities. NAM-based approaches were generally triggered by cases where there was a lack of a pharmacologically relevant species. In the absence of a pharmacologically relevant species, prior experience with modulating the target, and/or the ability to address putative risks using NAMs and/or clinical mitigation and monitoring, were foundational for the NAM-based submission. We propose a future state where the presence or absence of a pharmacologically relevant species is no longer a trigger to adopting a NAM-based approach. Instead, prior experience with modulating the target, and/or the ability to address putative risks using NAMs and/or clinical mitigation and monitoring, will be the triggers for a NAM-based filing irrespective of the availability of a pharmacologically relevant species. Capturing this in an ICH guideline such as ICH S9 would reduce uncertainty in approach and result in greater adoption of NAM-based regulatory filings where scientifically justified. [1]Dog, minipig, nonhuman primate (NHP). NHP is the most common pharmacologically relevant species for large molecule therapeutics. [2]Example, a human cell-based modality such as allogeneic CAR T cells. [3]The pharmacologic target is not present or has insufficient homology in any standard non-clinical animal species (see definitions as provided by EFPIA in Beilmann *et al.*, submitted for publication). [4]Example, the pharmacologic target is present in animals but has different cross-species expression and/or pharmacology leading to questionable translation value of animal models (see definitions as provided by EFPIA in Beilmann *et al.*, submitted for publication). *The FDA Center for Drug Evaluation (CDER) 'considers NAMs to include a broad range of methods such as *in vitro*, *in chemico*, and *in silico* methods. *In vivo* methods can also be considered NAMs when they improve predictivity, shift studies to phylogenetically lower animals, or otherwise help replace, reduce, and refine animal use (i.e., the 3Rs) in development programs'.[(p3)]

cell-based assays and potentially mouse efficacy models rather than a no-observed-adverse-effect-level (NOAEL) or highest non-severely toxic dose (HNSTD) in an animal toxicity study. A theoretical example is the development of an antibody-like molecule against a novel B-cell target where the intended mechanism of action is to deplete malignant B-cells for the treatment of hematologic cancers. While NHPs are the only pharmacologically relevant species, dedicated safety studies in NHPs are unnecessary. Extensive data on B-cell depletion in NHPs from other similar drugs, combined with substantial clinical experience and established monitoring guidelines, already sufficiently address human risk assessment. Similarly, extending the use of a B-cell-targeted cytolytic antibody developed to treat hematologic malignancies into non-advanced cancer patient populations with other B-cell-mediated diseases could leverage the prior nonclinical and clinical experience for the oncology indication with limited to no nonclinical safety studies required in NHPs to support the less serious indication (from the authors experience these strategies are already being applied but are not broadly visible beyond individual Sponsor and health authority). An update to ICH S6(R1) outlining scenarios where NAM-based approaches are acceptable would address this lack of visibility. The use of NAM-based approaches when expanding into patients with less severe and life-threatening conditions is also expected to increase trust in their scientific translatability and pave the way for their application, when scientifically justified, in the initial development of therapeutics for less serious conditions.

Although our focus was on NAM-based nonclinical safety programs, the responses we received indicated that large animals are often utilized for PK studies (Supplementary Figure S8). The incorporation of safety endpoints into PKPD studies or the use of *in silico* methods for PKPD could bolster the justification that dedicated large animal safety studies are not necessary on a case-by-case basis. Therefore, continued innovation in NAM-based approaches for PKPD prediction could potentially lead to a further reduction in large animal usage.

Concluding remarks

Our intent is to showcase examples of NAM-based regulatory filings when scientifically justified. We hope the case studies presented educate and inspire greater confidence within the industry and with other stakeholders to apply similar approaches in comparable situations and to publish or otherwise share experiences. We were encouraged to see the recent proposal for FDA to develop a NAMs database.[(p23)] We agree that cataloging individual NAMs is an important step forward but also recommend cataloging how NAMs were used together to address multiple aspects of the safety program. We also recognize that even with the progress demonstrated herein in replacing animals with NAMs, there are still limitations and obstacles to be addressed, and, in most cases, there remains a need for animals for safety assessment at this time (reviewed by Stresser *et al.*[13]). This idea is also implicit based on receipt of just 22 case studies from a request that was distributed to 188 companies. Nevertheless,

the success of using scientifically justified NAM-based regulatory submissions noted in the survey and amongst submitted case studies is encouraging and highlights that with continued dialogue between regulators and industry and scientific innovation in the field, the use of NAM-based approaches to replace animal studies when scientifically justified can continue to expand.

Authors' contributions

Conceptualization: **JS, IB**
Methodology: **JS, MO**
Investigation: **JS, IB, MO, EH**
Validation: **LW, H-MD, RV, MMcE, BP, TK, CLF, EH, CW**
Visualization: **JS, EH**
Data curation: **SG**
Writing – original draft: **JS, IB, MO, RD, LW, H-MD, RV, BP, TK, CLF, KAH, EH, SG**
Writing – review & editing: **JS, IB, MO, LW, H-MD, MMcE, BP, TK, CLF, KAH, EH, CW**
Project administration: **SG**
Supervision: **JS**

Data availability

The authors do not have permission to share data.

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Where animal studies were conducted, they were conducted in accordance with each company's policy on the care, welfare and treatment of laboratory animals and were reviewed by the Institutional Animal Care and Use Committee (IACUC) and/or by an appropriate ethical review process within each company and/or at the institution where the work was performed, if outsourced.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors are employed by pharmaceutical companies or contract research organizations, as per their affiliations. Sam Gunter was an employee of BIO until the original submission of this manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT to abbreviate and/or enhance the language used in some sections. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.drudis.2025.104328.

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