J Toxicol Pathol 2024; 37: 45–53

Invited Review

Drug review process advancement and required manufacturer and contract research oraganization responses

Takayuki Anzai[1*], Glenn J. Myatt[2], Frances Hall[2], Brenda Finney[2], Kenshi Nakagawa[1], Hijiri Iwata[3], Reo Anzai[4], Anne Dickinson[5,6], Matthew Freer[6], Dai Nakae[7], Hiroshi Onodera[8], and Takaaki Matsuyama[1]

[1] Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-0064, Japan
[2] Instem, Diamond Way, Stone Business Park, Stone, Staffordshire, ST150SD, United Kingdom
[3] LunaPath Laboratory of Toxicologic Pathology, 3-5-1 Aoihigashi, Naka-ku Hamamatsu-shi, Shizuoka 433-8114, Japan
[4] Tokyo University of the Arts, 5000 Omonma, Toride-shi, Ibaraki 302-0001, Japan
[5] Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom
[6] Alcyomics, The Biosphere, Draymans Way, Newcastle upon Tyne NE4 5BX, United Kingdom
[7] Teikyo Heisei University, 4-1 Uruido-Minami, Ichihara-shi, Chiba 290-0193, Japan
[8] National Institute of Health Sciences, 3-25-26 Tono-machi, Kawasaki-shi, Kanagawa 210-9501, Japan

Abstract: The United States Senate passed the "FDA Modernization Act 2.0." on September 29, 2022. Although the effectiveness of this Bill, which aims to eliminate the mandatory use of laboratory animals in new drug development, is limited, it represents a significant trend that will change the shape of drug applications in the United States and other countries. However, pharmaceutical companies have not taken major steps towards the complete elimination of animal testing from the standpoint of product safety, where they prioritize patient safety. Nonetheless, society is becoming increasingly opposed to animal testing, and efforts will be made to use fewer animals and conduct fewer animal tests as a natural and reasonable response. These changes eventually alter the shape of new drug applications. Based on the assumption that fewer animal tests will be conducted or fewer animals will be used in testing, this study explored bioinformatics and new technologies as alternatives to compensate for reduced information and provide a picture of how future new drug applications may look. The authors also discuss the directions that pharmaceutical companies and nonclinical contract research organizations should adopt to promote the replacement, reduction, and refinement of animals used in research, teaching, testing, and exhibitions. (DOI: 10.1293/tox.2023-0106; J Toxicol Pathol 2024; 37: 45–53)

Key words: target safety assessment, weight of evidence, investigational new drug, global standard exchange of nonclinical data, artificial intelligence program for toxicology

Environment Surrounding Animal Testing

Regulatory measures and international collaboration

The use of laboratory animals in drug development is under increasing pressure from society, as evident in the "FDA Modernization Act 2.0"[1], which passed in the United States Senate on September 29, 2022. While the replacement, reduction, and refinement (3Rs alternatives)[2] of animals used in research, teaching, testing, and exhibition have been promoted for some time, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) has recently taken the initiative and revised its guidelines on long-term carcinogenicity studies (ICH S1B(R1))[3]. In addition, another initiative supporting the 3Rs that is currently underway is a project aimed at promoting virtual control groups to eliminate the use of control groups in nonclinical studies[4].

Deterioration in economic viability

In addition to growing advocacy for animal welfare, there has been a significant deterioration in the economic viability of drug development. In particular, the prices of laboratory primates are expected to increase sharply from 2022. Such soaring development expenses have increased drug prices, ultimately increasing the financial burden on patients. Such price increases have led pharmaceutical companies and regulatory authorities to question the use of primates in pharmaceutical testing[5].

Received: 8 September 2023, Accepted: 9 November 2023
Published online in J-STAGE: 22 November 2023
*Corresponding author: T Anzai (e-mail: takayuki.anzai@me.com)

Economic security

The environment surrounding animal testing is characterized by both regulatory developments and significant challenges such as deteriorating economic viability, particularly in the use of primates. Countries that produce laboratory cynomolgus monkeys include China, Vietnam, Cambodia, and Mauritius, whereas those that import them are developed countries that are leaders in pharmaceutical development such as Japan, the United States, and European countries. This inevitably has a significant effect on how regulatory authorities of different countries perceive primate studies. It is essential for industry, government, and academia to make coordinated efforts to secure the procurement of laboratory monkeys with the aim of expanding the designation of "specified critical products" under the Economic Security Promotion Act[6] to include laboratory primates that are used for the development of new drugs.

Advancements of Informatics or Target Safety Assessment

Informatics is advancing rapidly across many fields such as material informatics (MI) in the materials sector and bioinformatics (BI) in the pharmaceutical industry. The concept of open science[7] is crucial in informatics. The development of informatics relies heavily on all information available in a standardized data format that is accessible to everyone. One application of informatics concerning pharmaceutical safety is the target safety assessment (TSA)[8]. The concept of TSA, as illustrated in Fig. 1, typically involves the use of informatics methodologies for the comprehensive retrieval, analysis, and evaluation of information to address specific challenges associated with a specific target in a drug development program. With TSA, making predictions based on existing information involves repeated data-gap analysis and read-across. This approach has evolved significantly with the regulations on Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH). The ICH S1B(R1)[3] (Fig. 2) guidelines take the same basic approach as REACH in that they emphasize the importance of the weight of evidence (WoE)[9] as well as fairness and transparency in the evaluation of any information. While REACH adopts the Klimisch score as a measure to assess the value of study data, the concept of WoE has also been explored in the pharmaceutical field. Myatt *et al.*[10] and Johnson *et al.* [11] evaluated the value of *in silico* data and developed an advanced version of data evaluation criteria (Table 1).

Movement towards Open Science and Consortiums

eTRANSAFE

The effective use of informatics relies on abundantly available and user-friendly databases. Pioneering efforts were made to assess the safety of pharmaceuticals and agricultural chemicals in the eTRANSAFE[12] project. eTRANSAFE brought together 29 organizations that included world-class pharmaceutical companies such as Novartis, Bayer, Sanofi, AstraZeneca, Roche, Merck, Janssen, Boehringer Ingelheim, and Eisai, and chemical manufacturers such as

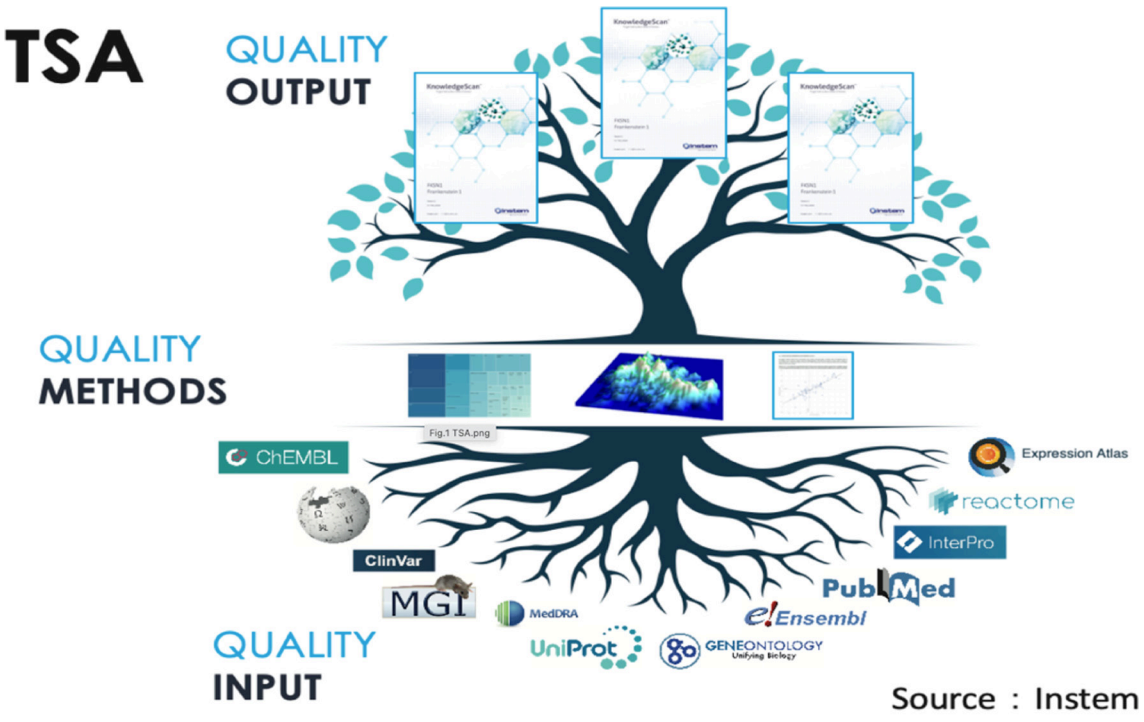

Fig. 1. Graphical image of target safety assessment (TSA).

BASF. To promote the mutual use of study data and access to a common database, they developed the ToxHub platform, which for it to be sustained beyond the consortium and open to world-wide participants, is now being commercialized by Instem. This transition is expected to boost the sharing and utility of data, including legacy data, among companies by enabling subscription to the databases and tools of ToxHub as part of their Centrus® platform.

G-SEND

The effective use of informatics and data standardization is mutually important. This is reflected in the Standard Exchange of Nonclinical Data (SEND)[13] promoted by the U.S. Food and Drug Administration (FDA). The objectives of SEND are outlined in the FDA's 5- and 10-year information technology plans[14], including expedited reviews and rapid responses to adverse events. However, an important objective is the strategic use of artificial intelligence (AI) and informatics, which was predicted in a column titled

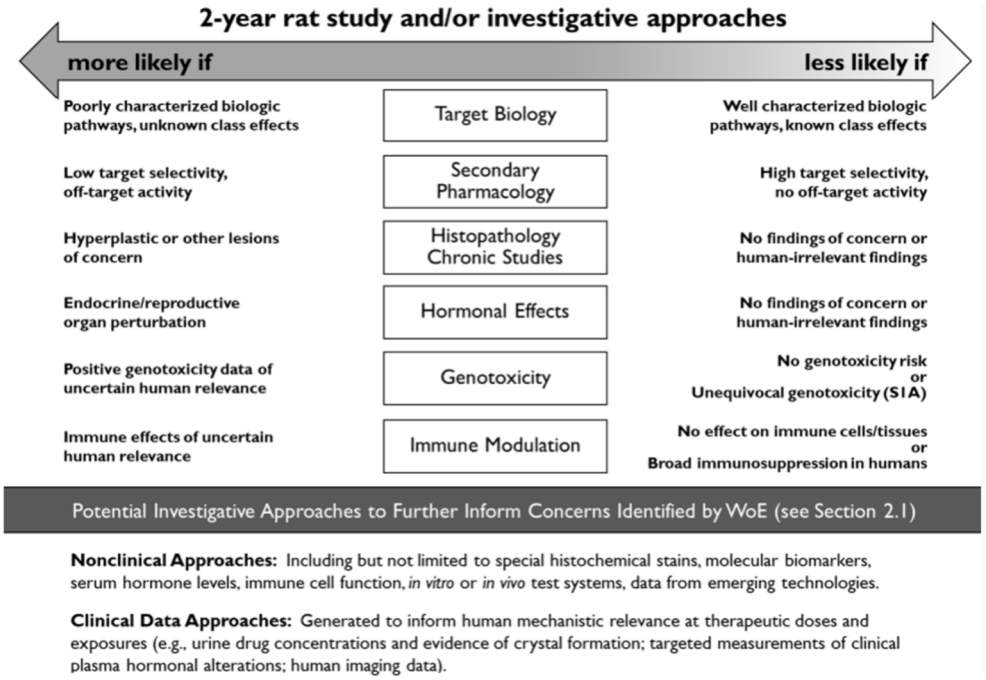

Fig. 2. International Council for Harmonisation (ICH) S1B(R1).

Table 1. Description of Reliability Score

Reliability score	Klimish score	Description	Summary
RS1	1	Data reliable without restriction	Well documented and accepted study or data from the literature Performed according to valid and/or accepted test guidelines (e.g., OECD) Preferably performed according to good laboratory practices (GLP)
RS2	2	Data with restriction	Well documented and sufficient Primarily not performed according to GLP Partially complies with test guideline
RS3	-	Exert review	Read-across Expert review of *in silico* result(s) and/or Klimisch 3 or 4 data
RS4	-	Multiple concurring prediction results	
RS5	-	Single acceptable *in silico* result	
RS5	3	Data not reliable	Inferences between the measuring system and test substance Test system not relevant to exposure Method not acceptable for the endpoint
RS5	4	Data no assignable	Not sufficiently documented for an expert review Lack of experimental details Referenced from short abstract or secondary literature

OECD: The Organization for Economic Cooperation and Development.

"SEND, FDA, and Industry Movement" posted in June 2019 on the website of AMED iD3 Catalyst Unit from the University of Tokyo[15]; the prediction was proven correct 3 years later by the FDA's Artificial Intelligence (AI) Program for Toxicology (AI4TOX), which was announced on July 7, 2022[16]. Several organizations have actively maximized the benefits of SEND[17]. Notably, the Global SEND Alliance (G-SEND) is a non-profit organization in which contract research organizations (CROs) play a vital role[18], and 26 organizations from five countries participate. While its primary focus is to address the common challenges in SEND data generation, G-SEND also explores ways to use SEND data effectively, including AI-based next-generation safety assessment methods that combine animal test data with *in silico* information. CROs are vast and diverse repositories that provide data on various test substances. If they take collective action, they will contribute significantly to advancing next-generation safety assessments. Regardless of the form it may take, open science calls for multiple industry peers to form groups that require high levels of compliance. In this regard, the EU REACH, which has experience in data sharing among industry peers, can serve as a valuable reference for consortium management and other elements[19, 20]. G-SEND adopts consortium management.

Advancement in Science and Technology

Use of human tissues

Although informatics plays a critical role in addressing the anticipated reduction in animal testing, there is a need for safety data derived from new technologies that do not involve the use of laboratory animals. As global research and development efforts are ongoing, pharmaceutical safety studies using human tissues and cells are promising. One such effort that has garnered significant attention is an *in vitro* assay developed by Alcyomics, a company spun out by Newcastle University in the United Kingdom. Alcyomics utilizes 4 mm (round) skin biopsies and a blood sample from healthy volunteers to develop their Skimune® assay for predicting adverse immune reactions including Type IV hypersensitivity reactions to compounds (chemicals, cosmetics, and pharmaceuticals), as well as cellular therapies. Skimune® has a unique and patented readout that demonstrates histopathological damage in increasing severity from Grade I (normal skin pathology) to Grade IV. Damage includes vacuolization of the epidermis and dyskeratosis (Grade II), subepidermal cleft formation (Grade III), and complete separation of the dermis and epidermis (Grade IV). This manifests as skin rash, blebbing of the skin, and acantholysis. Grades II and higher are considered a positive response. The assay incorporates T cell proliferation and cytokine and/or chemokine analyses, and has been shown to be highly predictive of clinical outcome[21] and response to sensitisers or non-sensitisers[22]. This assay has also been used to assess the safety of cellular therapies[23], oligonucleotides, nanomedicines, and viral vectors.

Use of non-mammalian organisms

Zebrafish are commonly used for environmental toxicity studies. Although these have been used in pharmaceutical safety and efficacy studies, their use is limited to screening assays. Although the effectiveness of zebrafish as a laboratory fish species is widely known, and they have been used in carcinogenicity studies[24, 25], their use has thus far been limited in the field of pharmaceutical development because of the challenges associated with their exposure to test substances. Typically, zebrafish are exposed to test drugs in fish tanks. However, this mode of exposure poses issues regarding the precision of drug exposure and is unsuitable for lipophilic compounds. Therefore, zebrafish have been mostly avoided in applications beyond screening assays. Recently, however, research has produced a groundbreaking technique that potentially solves this issue through non-anesthetized oral administration[26], which has garnered significant attention. Improvements in testing methods, such as this, could contribute to increasing the reliability of non-mammalian testing and represent a promising means to compensate for the decrease in mammalian safety studies. Zebrafish are not intended to replace mammalian testing. It is merely one of the several alternative models with its own translational challenges to overcome.

More Sophisticated Animal Testing

Omics

With the decrease in the number of safety studies conducted or the number of animals used, the quality of information obtained from animal studies needs to be higher. Although toxicogenomics and other omics have already been used for safety assessments, they are currently not common techniques. To allow fewer studies to be conducted using fewer animals, more advanced forms of testing are required to compensate for this reduction by increasing the information produced by each study. Testing the genetic changes and activities that occur within cells after exposure to test substances as part of safety studies may assist in more specifically identifying the underlying causes of the changes observed in animals after drug administration. Understanding toxicity at a fundamental level may allow researchers to obtain more precise safety data, even with fewer tests and fewer animals.

Imaging science

Animal testing has already benefited from the use of medical imaging-related technologies such as image science, image information theory, and image formation theory, as well as the theory and technology behind generating images of the human body and other living organisms. Imaging modalities for animals, such as computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, radioisotopes (RI), functional imaging, and compositional imaging, are well known, and advancements in 3D imaging and AI-supported rapid diagnosis are remarkable. These imaging technologies, along with omics, will provide effec-

tive means to address the anticipated reduction in animal testing. For example, imaging technologies have been developed to enable the real-time observation of brain activity in zebrafish larvae[27].

Other means to achieve more sophisticated animal testing

In addition to genetic observations and visualization of movements within living organisms discussed above, it is increasingly important to incorporate assessments of hormonal effects and immune regulatory functions into safety studies. Furthermore, using TSAs to ensure that all necessary test items are included in safety studies may help eliminate the need for additional tests to be conducted when they can be avoided.

Future Forms of New Drug Application

Although it is difficult to predict the future with certainty, reasonable predictions can be made using the current knowledge base. The Investigational New Drug (IND) Application model presented in Fig. 3 is not intended to propose an ideal future; rather, it provides a simplified representation of one possible application model. It should also be noted that different non-clinical studies will have been conducted depending on the type of pharmaceutical product. Because it is unlikely that animal testing will be completely banned in the near future, it is conceivable that INDs will be based on WoE, with some animal tests omitted. In such cases, it is necessary to explain the validity of complementary data such as *in silico* data and data from alternative tests that serve as substitutes for omitted tests.

In addition, TSAs are essential if certain tests are omitted because they highlight that risks have not been identified in particular areas; therefore, the testing requirements are not clear. However, if toxicity-related changes are predicted in a TSA, the necessary tests should be conducted and TSA can be used as evidence to support this testing. However, it should be noted that discrepancies in guidelines for testing drugs that may affect the central nervous system could contribute to varying drug abuse among countries. Therefore, in some countries, it is difficult to omit tests based solely on TSAs.

Challenges

Although the WoE concept has long been used in REACH, it is relatively new in the pharmaceutical industry. Before it could be used in REACH, however, there was extensive discussion regarding the strength of evidence (SoE)[28], which involves weighting evidence as a means to solve the challenges associated with WoE. As it is further implemented in the pharmaceutical industry, WoE requires fairness based on clear evaluation criteria, which in turn requires the SoE to measure the reliability of the data. For evaluation items without an established set criteria, it is necessary to offer sufficient explanation in an executive summary as part of the description of the data package; such an executive summary must be highly reliable. To ensure this, it must be reviewed by toxicology authorities, among other requirements. For new technologies to be used as evidence of alternative data, reviews by experts in their respective fields are required to accompany the article. In such "customized" new drug applications, the pre-IND meeting with regulatory authorities will be crucial to establish prior agreement.

Suggested Direction for Non-clinical CROs

Paradigm change in CRO management

Recent social and economic pressures surrounding animal testing, along with advancements in alternative methods and bioinformatics, strongly suggest that a decrease in animal testing is inevitable. While this might initially appear to pose a financial crisis for non-clinical CROs, it is not necessarily the case. As Table 2 shows, CROs may not survive by solely adopting traditional and simple business strategies to increase revenue through facility expansion. The model in Fig. 3 represents future business portfolios that CROs may need to adopt. In the past, the products offered were the results of outsourced studies, namely animal testing data. In future, these offerings will become composite products that

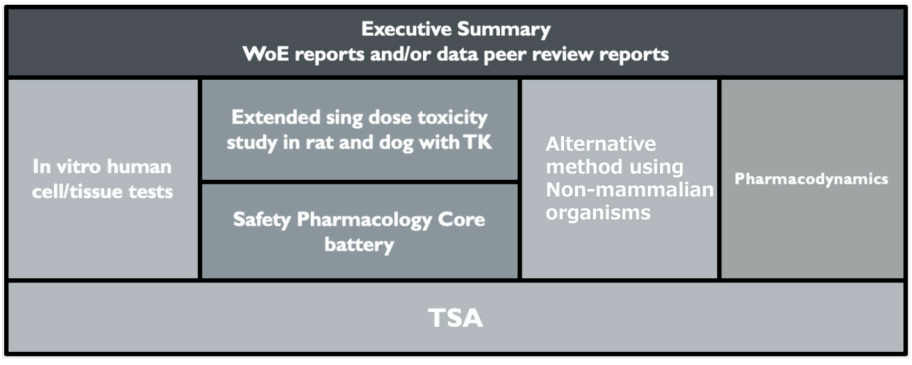

Fig. 3. Example of next generation investigational new drug (IND). TK: toxicokinetics; TSA: target safety assessment.

Table 2. Future Contract Research Organization (CRO)

New requirement	Examples	Future of CROs
Improved quality of information obtained from a single study	Omics / advanced image analysis + TSAs	Higher testing fees, resulting in increased and steady revenue stream
Introduction of informatics	Services only a CRO and/or their informatics partner can provide, e.g., gathering alternative information and providing it in a form of application document or combining it with animal testing; provision of information that compensates for omitted animal testing	CROs are in best placed to offer a lineup of bioinformatics-based services, and bioinformatics makes a new profit center for them
Development of new alternative tests	- Tests using human cells/tissues - Use of non-mammal organisms such as fishes	Tests using non-mammal organisms or cells will become diversified as such organisms or cell are selected for each drug candidate; competition may be avoided, and specialization may also be possible
Advancements of SEND	- Offers higher-quality, more extensive information of higher quality from a single study (Increasing/expanding domains) - Generates SEND data based on in vitro studies that supplement animal testing, alternative methods, and tests using laboratory fish, etc.	- More advanced SEND services/specialists - More extensive SEND services

TSA: target safety assessment; SEND: standard exchange of nonclinical data.

combine the results of animal testing with information obtained through open science or informatics, thereby complementing a smaller body of animal testing data. This trend is evident in the FDA AI4TOX (Fig. 4) activities. In any case, successful non-clinical CROs in the next decade or two are likely to be recognized as "IT-centric CROs", positioned more or less as an information industry player.

Key investment items

Given the predictions discussed earlier, the investment portfolio for CROs, namely, the investment items and their allocations, will experience a shift in focus from investments in facility construction to investments in the information technology field, as follows:

i) Investment in data standardization (SEND)

ii) Investment in the automation of seamless data flows and analysis (e.g., laboratory information management system (LIMS) directly connected to SENDs and bioinformatics)

iii) Investment in securing a bioinformatics organization/human resources

iv) Investment in specialized analytical equipment (e.g., genetic analysis, hormone analysis, and image analysis devices)

Important work performed by a bioinformatics organization at a CRO includes interpreting in-house animal testing data using bioinformatics, providing supplementary data, and proposing supplementary non-animal tests. These services can create a new profit center for a CRO, whereas SEND forms the backbone. Many leading CROs will prioritize SEND in the future. It is expected that SEND or electronic standardization of application data will be required for all regulatory submissions. Meanwhile, a LIMS, which was previously seen as a fixed cost similar to facility-related expenses, will be recognized as a strategic investment for the automatic generation of SEND data, or as a vital piece of infrastructure for bioinformatics.

Fig. 4. FDA Artificial Intelligence (AI) Program for Toxicology (AI4TOX).

Mergers and acquisitions

One possible way for a CRO to keep up with this paradigm shift, by reducing the time required to secure and develop human resources, is to acquire an IT vendor experienced in bioinformatics. However, such a drastic approach may only be feasible for larger CROs, and smaller CROs with less robust financial capabilities may find large-scale investments challenging. For such small-sized CROs, potential options include a merger of equal or one by absorption between industry peers, or the acquisition of a CRO by an IT vendor.

Fig. 5. Schematic view of the next generation investigational new drug (IND). TSA: target safety assessment.

Future Directions for Pharmaceutical Companies

Most pharmaceutical companies outsource a significant proportion of their safety studies to CROs. Consequently, they often suffer from a shortage of in-house personnel capable of evaluating study data. As shown in Fig. 3, the future of new drug applications will not offer an "off-the-shelf" menu but will require customized filing tailored to individual drug candidates. In the future, human resources capable of creating made-to-order designs as well as those capable of writing or reviewing executive summaries will play a vital role in pharmaceutical companies, and developing such human resources will be critical. For instance, to explain the data package shown in Fig. 3, one needs to be experienced in animal testing, including toxicology and pathology, proficient in using bioinformatics, and familiar with new emerging fields. Building a team, forging partnerships to build capabilities, or having an individual on staff with expertise and capabilities is crucial for pharmaceutical companies. Although many tasks can be delegated to CROs, pharmaceutical companies must have people, either internally or with partners, who can write or review executive summaries accurately.

Information Regulatory Authorities Need and Their Responsibilities

The FDA is promoting the use of AI4TOX in IND and New Drug Application (NDA) processes (Fig. 4). The program was developed in four areas, and its specific applications as an AI tool are considered to support IND and NDA submissions. For example, SafetAI is designed as a tool to assist the IND review process, where it determines an IND application data as either "positive" or "negative"; if the response is "positive", the system will alert the reviewer on missing data. Contrarily, it proceeds to the regular review procedure if the response is "negative". Although challenges such as precision, scope, and reliability of prediction still need to be overcome, AI technology is poised to play a critical role in the process of new drug reviews (Fig. 5).

Although the FDA is already in the process of introducing AI into new drug reviews, regulatory authorities outside the United States, including Japan, have not introduced a system for SEND-based data reviews. The EU has started moving towards the introduction of SEND (the CDISC standard) but has not yet turned to the full-scale use of SEND data. Pharmaceutical companies rarely file new drug applications exclusively in regions outside of the United States. Instead, many companies focus on the FDA's new drug review process for drug development. Countries other than the United States are likely to require support for made-to-order customized application data packages. The following four areas should be considered when using bioinformatics to supplement animal testing:

Validation of the presence of counter-evidence: No reports in the open science field show data that are unfavorable to the applicant or contradict the results presented by the applicant.

Storyteller reliability: The storyteller in the executive summary is reliable.

Expert reliability: The executive summary was peer-reviewed by experts in relevant fields.

Validation of WoE: Establishing a review method.

Conclusion

Advancements in AI technology are expected to significantly impact drug development. Interestingly, this progress means that reliable human experts with real-life experience will become even more important. These people will be those who can write good executive summaries to explain and peer-review AI-aided assessments. AI cannot file new drug applications; therefore, it is important to know who tells the story and provides the information required for these AI-related applications.

Disclosure of Potential Conflict of Interest: The authors declare no conflicts of interest.

Acknowledgment: We wish to thank the US FDA, CDISC, PhUSE, and member companies of G-SEND that cooperated in preparing this report.

References

1. U.S. Food and Drug Administration. FDA Modernization Act 2.0, S5002–117th Congress (2021–2022), from Library of Congress, from Congress.gov website: https://www.con-

gress.gov/bill/117th-congress/senate-bill/5002/text.
2. USDA National Agricultural Library. Animal Use Alternatives (3Rs), from USDA website: https://www.nal.usda.gov/animal-health-and-welfare/animal-use-alternatives.
3. European Medicines Agency. Committee for Medicinal Products for Human Use, ICH guideline S1B(R1) on testing for carcinogenicity of pharmaceuticals Step 5, from EMA website: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s1br1-guideline-testing-carcinogenicity-pharmaceuticals-step-5_en.pdf.
4. Steger-Hartmann T, Kreuchwig A, Vaas L, Wichard J, Bringezu F, Amberg A, Muster W, Pognan F, and Barber C. Introducing the concept of virtual control groups into preclinical toxicology testing. Altern Anim Exp. **37**: 343–349. 2020. [Medline]
5. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER), Oncology Center for Excellence (OCE), Pharmacology/Toxicology. Nonclinical Considerations for Mitigating Nonhuman Primate Supply Constraints Arising from the COVID-19 Pandemic Guidance for Industry. February 2022.
6. Cabinet Office. Act on the Promotion of Ensuring National Security through Integrated Implementation of Economic Measures (Act No. 43 of 2022). Promulgated on May 18, 2022.
7. Shaw DL. Is open science the future of drug development? Yale J Biol Med. **90**: 147–151. 2017. [Medline]
8. Brennan RJ. Target safety assessment: strategies and resources. Methods Mol Biol. **1641**: 213–228. 2017. [Medline] [CrossRef]
9. European Chemicals Agency. Weight of evidence, from ECA website: https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/weight-of-evidence.
10. Myatt GJ, Ahlberg E, Akahori Y, Allen D, Amberg A, Anger LT, Aptula A, Auerbach S, Beilke L, Bellion P, Benigni R, Bercu J, Booth ED, Bower D, Brigo A, Burden N, Cammerer Z, Cronin MTD, Cross KP, Custer L, Dettwiler M, Dobo K, Ford KA, Fortin MC, Gad-McDonald SE, Gellatly N, Gervais V, Glover KP, Glowienke S, Van Gompel J, Gutsell S, Hardy B, Harvey JS, Hillegass J, Honma M, Hsieh JH, Hsu CW, Hughes K, Johnson C, Jolly R, Jones D, Kemper R, Kenyon MO, Kim MT, Kruhlak NL, Kulkarni SA, Kümmerer K, Leavitt P, Majer B, Masten S, Miller S, Moser J, Mumtaz M, Muster W, Neilson L, Oprea TI, Patlewicz G, Paulino A, Lo Piparo E, Powley M, Quigley DP, Reddy MV, Richarz AN, Ruiz P, Schilter B, Serafimova R, Simpson W, Stavitskaya L, Stidl R, Suarez-Rodriguez D, Szabo DT, Teasdale A, Trejo-Martin A, Valentin JP, Vuorinen A, Wall BA, Watts P, White AT, Wichard J, Witt KL, Woolley A, Woolley D, Zwickl C, and Hasselgren C. *In silico* toxicology protocols. Regul Toxicol Pharmacol. **96**: 1–17. 2018. [Medline] [CrossRef]
11. Johnson C, Anger LT, Benigni R, Bower D, Bringezu F, Crofton KM, Cronin MTD, Cross KP, Dettwiler M, Fredricks M, Melnikov F, Miller S, Roberts DW, Suarez-Rodriguez D, Roncaglioni A, Lo Piparo E, Tice RR, Zwickl C, and Myatt GJ. Evaluating confidence in toxicity assessments based on experimental data and *in silico* predictions. Comput Toxicol. **21**: 100204. 2022. [Medline] [CrossRef]
12. Pognan F, Steger-Hartmann T, Díaz C, Blomberg N, Bringezu F, Briggs K, Callegaro G, Capella-Gutierrez S, Centeno E, Corvi J, Drew P, Drewe WC, Fernández JM, Furlong LI, Guney E, Kors JA, Mayer MA, Pastor M, Piñero J, Ramírez-Anguita JM, Ronzano F, Rowell P, Saüch-Pitarch J, Valencia A, van de Water B, van der Lei J, van Mulligen E, and Sanz F. The eTRANSAFE project on translational safety assessment through integrative knowledge management: achievements and perspectives. Pharmaceuticals (Basel). **14**: 237. 2021. [Medline] [CrossRef]
13. CDISC SEND TEAM. Standard for exchange of nonclinical data implementation guide (IG) version 3.1 for public review as of Dec. 4, 2014 after publication on May 19, 2011, from CDISC website: http://www.cdisc.org/send.
14. U.S. Food and Drug Administration. Center for Drug Evaluation and Research. Office of Regulatory Affairs. GDUFA Information Technology/Informatics Plan (Draft) FY 2013–FY 2017. September. 2014, from FDA website: http://www.fda.gov/downloads/ForIndustry/UserFees/GenericDrugUserFees/UCM416856.pdf.
15. Anzai T. SEND, FDA, and industry movement. The University of Tokyo. from AMED iD3 Catalyst Unit website: https://www.id3catalyst.jp/e/column03.html.
16. Tong W. An FDA artificial intelligence (AI) program for toxicology, from FDA website: https://www.fda.gov/about-fda/nctr-research-focus-areas/artificial-intelligence.
17. Anzai T, Kaminishi M, Sato K, Kaufman L, Iwata H, and Nakae D. Responses to the standard for exchange of nonclinical data (SEND) in non-US countries. J Toxicol Pathol. **28**: 57–64. 2015. [Medline] [CrossRef]
18. Anzai T, Matsuyama T, Wasko M, Hatakeyama H, Horikawa SI, Anzai R, Iwata H, Imai N, Mizuhashi F, Tsuboi M, Okuzono T, Takagi H, Cho H, Rong BTS, Masaki F, Nakae D, and Nakae D. Establishment of the Global SEND Alliance (G-SEND) in Japan and efficient creation of electronic SEND datasets between CROs. J Toxicol Pathol. **32**: 119–126. 2019. [Medline] [CrossRef]
19. Anzai T. Survey method for supply chains under REACH. Specialty Chemical Magazine. **29**: 43–45. 2009.
20. Anzai T. REACH consortia management and effective measures. Journal of Economic Maintenance Tribology. **532**: 33–38. 2009.
21. Ahmed SS, Whritenour J, Ahmed MM, Bibby L, Darby L, Wang XN, Watson J, and Dickinson AM. Evaluation of a human in vitro skin test for predicting drug hypersensitivity reactions. Toxicol Appl Pharmacol. **369**: 39–48. 2019. [Medline] [CrossRef]
22. Ahmed SS, Wang XN, Fielding M, Kerry A, Dickinson I, Munuswamy R, Kimber I, and Dickinson AM. An in vitro human skin test for assessing sensitization potential. J Appl Toxicol. **36**: 669–684. 2016. [Medline] [CrossRef]
23. Dickinson AM, Wang XN, Sviland L, Vyth-Dreese FA, Jackson GH, Schumacher TN, Haanen JBAG, Mutis T, and Goulmy E. In situ dissection of the graft-versus-host activities of cytotoxic T cells specific for minor histocompatibility antigens. Nat Med. **8**: 410–414. 2002. [Medline] [CrossRef]
24. Feitsma H, and Cuppen E. Zebrafish as a cancer model. Mol Cancer Res. **6**: 685–694. 2008. [Medline] [CrossRef]
25. Zhao S, Huang J, and Ye J. A fresh look at zebrafish from the perspective of cancer research. J Exp Clin Cancer Res. **34**: 80. 2015. [Medline] [CrossRef]

26. Fujiwara N, Nonaka S, Yanoue Y, Tomiki M, Nishi N, Toyohisa S, and Saika O. Improvement of a fish holding device for oral administration to zebrafish. Fundam Toxicol Sci. **8**: 183–187. 2021. [CrossRef]
27. Muto A, Ohkura M, Abe G, Nakai J, and Kawakami K. Real-time visualization of neuronal activity during perception. Curr Biol. **23**: 307–311. 2013. [Medline] [CrossRef]
28. European Chemicals Agency. Template for weight of evidence/uncertainty in hazard assessment, from ECHA website: https://echa.europa.eu/documents/10162/17169198/template_for_weight_of_evidence_en.docx/eb183c2e-c360-cbce-.