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Research highlight

The final stretch of the animal-free approach in new drug research

The U.S. Food and Drug Administration (FDA)'s program documents regarding the animal-free approach for the efficacy, pharmacokinetics, and safety evaluation of new drug preclinical studies have been rolled out gradually. This process has taken the FDA seven years. Starting from the reduction of funding for animal of data from organ-on-a-chip solely for pharmacodynamic studies [1–3], to the explicit proposal of the animal-free approach for pharmacodynamic studies in the FDA Modernization Act in Reference [4], and to the indication in 2025 that safety studies should also prioritize safety research data from *in vitro* microphysiological systems [5], this demonstrates the FDAs determination to gradually achieve the animal-free approach and also signals a profound paradigm shift in the field of drug research and development. This policy direction not only addresses the requirements of ethics and technological advancement but may also reshape the entire new drug development industrial chain.

1. The feasibility of the animal-free approach and the time needed for completely animal-free research

The FDA has suggested using new drug research on research subject for the gradual implementation of the animal-free approach. In the future, the researchon bispecific antibodies and antibody-drug conjugate (ADC) drugs related to monoclonal antibodies can refer to this document.

The initial step in monoclonal antibody drug research is to identify the target. Then, design the antibody structure based on the target and conduct research on the druggability of antibody drugs. Currently, most of the target studies for antibodies are relatively mature, with a large quantity of basic data obtained. The antibodies designed according to the antibody structure of the target are similar. Subsequently, the research on bispecific antibodies and ADCs can also be evaluated based on the basic data of monoclonal antibodies.

Even for antibody research based on new targets, *in vitro* models can address most issues, and the transgenic animals previously utilized can now be replaced by transgenic organoids. The pharmacokinetic study of antibodies mainly focuses on system stability, tissue stability, and cell stability. The safety evaluation mainly examines immunotoxicity and organ toxicity. Both of these can be analyzed using data models and then integrated with wet experimental data from *in vitro* cell lines, organoids, explants, and microfluidic chips. Therefore, the progress of the animal-free research of monoclonal antibodies will rely on microfluidic and the modeling ability based onbig data. That is to say, it has shifted from a competition in life sciences to a competition in physics and mathematics.Whether the drug target has been well-researched or is new, the key is to get as close as possible to the real physiological and pathological states of the human body. The existing animal-based models are increasingly failing to meet this requirement, while various *in vitro* culture models of human tissues and organs have developed to accurately assess drugs. In fact, traditional animal models can be gradually phased out.

New drug research has always been a logically closed-loop study. As the underlying data becomes richer and the accuracy of artificial intelligence (AI) algorithms improves, the pharmaceutical design of new drugs is more logical, which also meets patients' needs for precision drug design and precision clinical treatment. Analyzing biological activity needs to be based on the responses of corresponding organisms. Thus, *in vitro* models derived from human tissues can more realistically represent the responses of antibody drugs. This is indeed a major revolution in the history of pharmacy, but this kind of change didn't happen suddenly. The market has been gearing up for it for over 10 years.

2. Key research technologies needed after animal-free research

In September 2022, an FDA document introduced two concepts: microphysiological systems and *in vitro* complex models. Building these two models involves the use of organoids, explants, organ chips, three-dimensional (3D) printing, microfluidics, data collection, and data detection. The resulting large amount of data has to be analyzed by big data models to avoid bias. Thus, contract research organizations (CROs) should transition from building monkey factories in the past to establishing organ and tissue banks. Meanwhile, these tissue banks should be transformed into digital libraries, which include multi-omics data such as pathology, proteomics, genomics, epigenomics, and drug sensitivity groups.

However, the physiological relevance of multi-organ interaction models remains inadequate. For example, the existing liver-kidney co-culture chips can only simulate 60% of the metabolic clearance pathways, and the modeling error of the communication between the nervous and immune systems is still quite high. The FDA stipulates that new models must be verified by at least three independent laboratories, but currently, very few laboratories can meet this requirement.

3. Opportunities and challenges

Currently, there are already various preliminary prototypes of microphysiological systems. However, more data is required to enhance them. Thus, any new *in vitro* model system should involve the preparation of human tissue samples, the establishment of co-

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culture systems, the design of microfluidic chips, and the accumulation of material systems and fluid parameters. Meanwhile, it is also essential to make comparisons with previous animal models and clinical human data. This creates more opportunities for numerous small data companies.

DeepMind's AlphaFold3 has achieved a prediction error of protein-ligand binding energy <1.5 kcal/mol, which is three times more accurate than traditional molecular docking. In silico medicine used generative AI to design a candidate drug for idiopathic pulmonary fibrosis (IPF). It only took 18 months from target discovery to preclinical trials, and the research and development (R&D) cost was reduced to 1/10 of the traditional model. As researchers in the field of life sciences, even those doing bioinformatics analysis, have the ability to analyze data using raw data. However, it is still quite difficult to build data models through underlying code. In particular, constructing a virtual physiological human (VPH) requires integrating more than 2,000 mathematical models of biological processes. Currently, the HUMAN Project has only completed 48% of the modular modeling of the cardiovascular system. The EU EUToxRisk consortium found that when integrating QSAR predictions and microfluidic liver chip data, the model confidence requires at least 1,000 cross-validation cases. Therefore, in the future, scientific and technological personnel in the life field need to communicate more with those majoring in mathematics and physics, so that they can understand the demands of life science researchers and thus build mathematical models with less deviation based on massive data. This requires the establishment of a technology verification alliance. We can refer to the model of the Innovation and Quality Consortium (IQ Consortium) to build a cross-enterprise organ chip verification database and share cross-comparison data of thousands of compounds. At present, the calculation of clinical dosage requires pharmacokinetic/pharmacodynamic (PK/PD) mathematical models. Therefore, it is extremely urgent to develop a virtual patient system that integrates PK/PD models, organ chip data, and AI predictions.

Right now, not many Chinese companies are qualified to supply clinical samples. There are a few in Europe and the USA. Therefore, in the future, building clinical sample databases is also an area that many pharmaceutical companies should keep an eye on. There are only a handful of companies working on organ chips. However, the current data is pretty scattered and lacks standardization. As a result, the physiological relevance of multi-organ interaction models is still not enough to offer satisfactory CRO services to pharmaceutical companies. But all these situations present opportunities for us, and there's plenty of room for improvement.

The essence of this transformation is the shift of drug research and development from "animal surrogates" to "human subjects" research. Some short-term pain is inevitable. However, institutions that possess core capabilities like human chip engineering, computational model validation, and regulatory strategy design will take the lead in the new safety assessment market, which is worth hundreds of billions of dollars in the next decade. The key is to establish an integrated innovation ecosystem covering bioengineering, data science, and regulatory policies. It can be said that after over 10 years of building microphysiological systems from human tissue sources, the FDA documents have paved the way for the final stage of animal-free new drug research.

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