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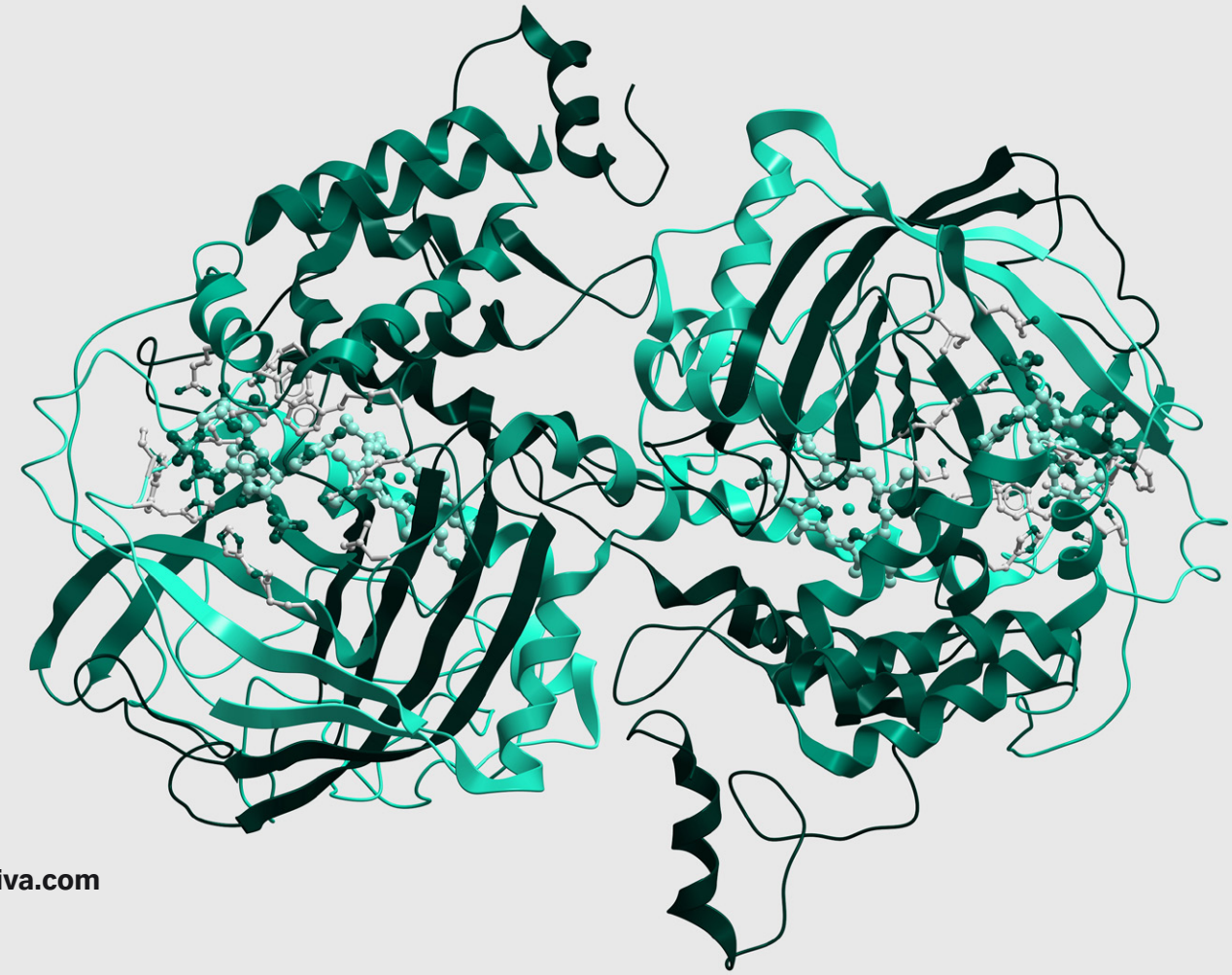
Revolutionizing drug discovery

From “undruggables” to AI



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Drug discovery is the process of identifying novel therapeutic candidates and developing therapeutic strategies. This process comes with many challenges, chances of failure, and costs a substantial amount of time and money. Recent estimates state that bringing a drug to market takes an average of 10 to 15 years, costs $1 to 2.5 billion (USD), and has a failure rate of over 90% (1-4).

The drug discovery pipeline begins with target identification, which requires a deep understanding of disease mechanisms (5). Target validation follows and involves thorough characterization and “druggability” testing. AI-based tools can aid in identifying therapeutic candidates via high-throughput screenings. Compounds are then optimized for safety and efficacy by testing on animals or organoids before moving on to human trials (phase I-III).

The entire drug discovery process is complex. Your chances of encountering issues and experiencing failures is immense. This complexity offers opportunities for improvement by constant advancements in technologies and methodologies.

In this whitepaper, we discuss four current trends in drug discovery: Targeting undruggables, fashionable models, AI, and paint-by-cell. We focus on how each is poised to solve some of the most critical challenges facing drug discovery, and the hurdles in their adoption.

1. The “undruggables”

An undruggable target refers to a clinically meaningful therapeutic target that is either yet to be drugged, or have been challenging to drug by traditional approaches (6). These targets often have less understood 3D structures, non-catalytic protein-protein interaction (PPI) functional modes, and lack a defined ligand-binding pocket.

There are currently efforts to develop innovative strategies and technologies to discover these inaccessible molecules. Strategies that show promise in improving protein druggability and promoting more targeted therapeutics include multi-specific drugs, oligonucleotides, PPI modulators, and proteolysis-targeting chimeras (PROTACs).

1.1. Multi-specific drugs

Classical drugs, such as small molecule inhibitors and therapeutic antibodies, work on a one-target-to-one-drug (1T1D) principle where a single drug modulates a single and specific therapeutic target via a clearly defined drug-target binding interface. Classical drugs that work via the 1T1D principle exert their effects across all tissues, which can lead to unwanted pleiotropic effects.

Multi-specific drugs interact with multiple therapeutic targets simultaneously (7). They generally work through several distinct modes or a multistep pathway. For example, they may anchor their target close to an endogenous effector, facilitating effector-driven modulation of the target. Their therapeutic effect is exhibited by simultaneously or concurrently inducing the formation of two or more drug-target binding interfaces. This mechanism of action typically leads to a tissue-specific effect, which increases efficacy, allows for lower doses to be used, and reduces the chance of off-target effects including toxicity. Multi-specific drugs can directly and specifically bind to difficult-to-bind therapeutic targets (7,8).

Recent publications have categorized multi-specific drugs into two groups, tetherbodies and concurrent obligate multi-specific drugs (COMMs) (8). Tetherbodies can be classified into two subcategories: Sequential obligate multi-specific drugs (SOMs) and concurrent obligate multi-specific drugs that mediate localization (COMLs). An SOM first binds to the dock, enhancing the abundance of the drug in a precise location. Next, it utilizes a second drug-target interface to engage the target, allowing the target to be modulated by the drug. COMLs have a similar mechanism except the dock and target exist within the same compartment and are bound concurrently by the drug. Clinically relevant examples of SOMs include antibody-drug conjugates (ADCs) (9). Many SOMs showed great potential in clinical trials and have been brought to market. Gemtuzumab ozogamicin is an SOM approved by both the FDA and the EMA for the treatment of acute myeloid leukemia (10). COMLs include antibody-cytokine fusions, and several have been subjects of recent clinical trials (11).

COMMs pull the effector and target entities together, allowing the effector to act upon the target. Often termed molecular matchmakers because of their unique mode of operation, COMMs utilize an endogenous biological mechanism to facilitate their activity. COMMs are broad in terms of their diversity and target diseases and include emicizumab, a bispecific monoclonal antibody used to treat hemophilia A (12).

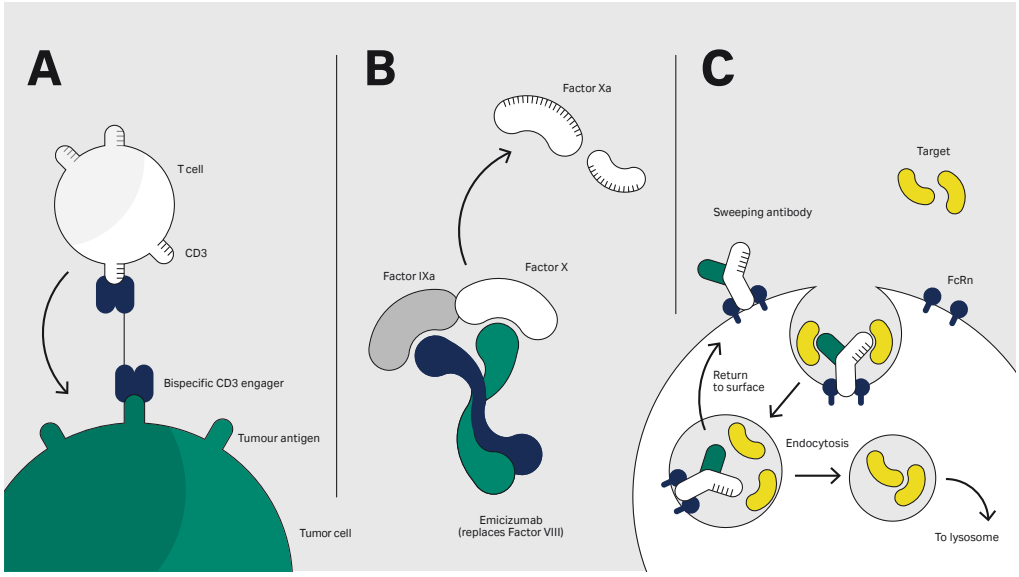

Fig 1. Multi-specific drugs mechanism of action, specific to emicizumab.

1.2. PROTACs

PROTACs are often considered a subcategory of COMMs due to their multi-specific targeting nature, but they work by inducing protein degradation rather than inhibiting activity. They specifically target and induce the degradation of an undruggable target protein via the ubiquitin-proteasome degradation pathway (13). This is facilitated by their bipartite structure: One component is responsible for selectively recognizing and binding to a target, and the other recruits an E3 ubiquitin ligase, subjecting the target to ubiquitination and subsequent degradation (6).

The advantages of PROTACs are similar to multi-specific drugs; they work at far lower dosages compared to classical drugs, and significantly expand the molecules that can be targeted therapeutically since the only requirement is a binder. Almost any target can be degraded (6). They show remarkable selectivity and result in outstanding biological responses (14). Examples include ARV-110 and ARV-471, which target androgen and estrogen receptors to treat prostate and breast cancer, respectively. ARV-110 and ARV-471 both demonstrate excellent clinical outcomes and are undergoing further clinical investigation (15,16).

Research has begun harnessing other endogenous protein degradation pathways therapeutically using the same principles. This includes the endosome-lysosome pathway, targeted with lysosome-targeting chimeras (LYTACs), and autophagy pathways, by employing autophagy-targeting chimeras (AUTACs) (17,18).

1.3. Oligonucleotides

Oligonucleotide therapeutics are designed to interact with specific genetic targets by modulating their expression and targeting the root cause of disease (19). They offer an alternative therapeutic strategy that's beneficial for targeting undruggable molecules at the protein level. Oligonucleotide-based therapeutics were first proposed in the 1970s and have evolved into a well-developed therapeutic strategy. They can be classified into several categories including antisense oligonucleotides (ASOs), interference RNA (RNAi; small interference RNAs [siRNAs] and microRNAs [miRNAs]), CRISPR-based genome editing, and guanine quadruplex (G4) stabilizing. The two most clinically important categories of oligonucleotide therapeutics are ASOs and RNAi and are prominent players in drug discovery, clinical development, and therapeutic applications.

ASOs are short (12 to 30 nucleotides), synthetic, single-stranded oligonucleotides that utilize Watson-Crick base pairing to bind to complementary DNA or RNA and modulate gene expression through various mechanisms (19-21). Some ASOs bind to mRNA and target it for enzymatic cleavage and degradation — effectively eliminating the disease-causing gene product. They can also influence RNA by modifying splicing to alter RNA structure and the protein it encodes, or block the binding of RNA to ribosomal subunits to inhibit translation and protein production. ASOs are highly versatile and can be designed to target a wide variety of RNA sequences. An example of a clinically promising ASO is AZD4785, a high-affinity constrained ethyl-modified ASO, which showed remarkable outcomes in *in vitro* and *in vivo* studies at downregulating KRAS mRNA, and consequently progressed to clinical trials, though the results remain to be revealed (22).

Similarly, RNAi-based therapeutics aim to reduce the expression of a specific protein by inducing the degradation of a target RNA. It is a commonly used approach for targeting undruggable proteins, particularly using siRNA molecules (19). Teprasiran is an siRNA-based therapeutic, currently in the phase III clinical trial stage that aims to treat acute renal failure by specifically targeting mutant p53 (23).

1.4. PPI modulators

PPI modulators are molecules designed to influence the interactions between proteins within a cell. By targeting PPI interfaces known as hot spots, PPI modulators can affect critical cellular pathways such as cell growth, proliferation, differentiation, signal transduction, and apoptosis (19, 24). This innovative approach offers opportunities for therapeutic intervention in diseases where undruggable proteins are implicated, as many of these molecules act as part of larger, complex PPI networks. PPI modulators are classified

based on their binding mechanism (covalent or non-covalent) and their mechanism of action (orthosteric or allosteric). Orthosteric modulators target PPIs by pinpointing and targeting hotspots, while allosteric modulators work on PPIs without defined hotspots and exert their effects on non-interaction regions which cause conformational changes in the target proteins (25). A broad range of PPI inhibitors have been studied and clinically developed to target several undruggable proteins involved in PPI networks: RAS, Bcl-2, p53, and Myc (19).

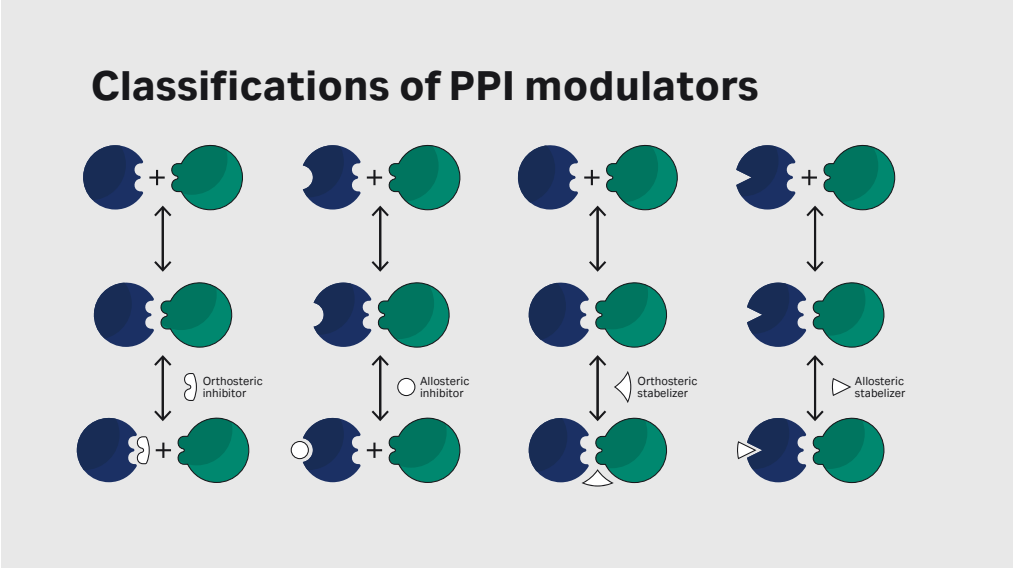

Fig 2. Orthosteric and allosteric mechanisms for PPI inhibition and stabilization.

Although PPI modulators hold promise as a strategy for targeting undruggable molecules across various field, significant challenges remain (26, 27). One major challenge is achieving specificity and selectivity for the target protein interaction. Many proteins engage in multiple interactions, and it's crucial to avoid unintended effects on other cellular processes. PPIs generally involve large, flat interfaces without clearly defined binding pockets which are more challenging to target than the well-defined protein-ligand interactions associated with enzymes, ion channels, or receptors (27).

In response to the challenge of undruggable molecules in drug discovery, innovative strategies and technologies have emerged to revolutionize therapeutic interventions. Multi-specific drugs offer the benefits of tissue-specific effects, reduced off-target effects, and lower doses, expanding the range of druggable targets. PROTACs provide a distinct mechanism by inducing protein degradation, significantly broadening the scope of targetable molecules. Oligonucleotide therapeutics, such as ASOs and RNAi, precisely modulate gene expression at the RNA level. PPI modulators act to disrupt or enhance cellular pathways by influencing protein interactions, showing promise in complex networks like cancer. While challenges in target specificity and structural characteristics remain, these innovative approaches hold potential to unlock an array of previously inaccessible therapeutic targets, offering hope for more effective and precise treatments.

Discuss your undruggable challenges with a translational medicine advisor today

2. Fashionable models

The Federal Food, Drug, and Cosmetics Act of 1938 mandated animal testing for every new drug development protocol. This approach was developed with the intention that all new therapeutic drugs and devices meet standards of safety and efficacy before being tested in human subjects. The mandate also created many concerns and challenges. First, there are significant ethical and animal welfare issues associated with subjecting animals to experiments. Second, the effectiveness of this method in predicting the safety of drugs for human use has come into question due to instances of experimental failure.

Recent technological and methodological advancements in research processes are beginning to offer viable alternatives to animal testing, coined "fashionable models", which include organoids and organ-on-chip (OoC) systems. These strategies offer the same level of quality assurance on safety and efficacy of therapeutics prior to entering clinical trials and eliminate the need for animal testing. Fashionable models have the added benefit of providing data directly from human cells and offer advantages in terms of predicting outcomes in human subjects.

As a result of the development of viable alternatives to animal testing and moving towards the reduction of animal testing, we have seen a significant shift in legislation surrounding the necessity of animal testing for therapeutic purposes. In December 2022, the FDA Modernization Act 2.0 was signed, which allows for alternatives to animal testing for the purpose of drug and biological product applications (28).

2.1. Organoids

An organoid is an artificially grown mass of cells or tissue derived from stem cells, that resemble an organ. They are a miniaturized, simplified, *in vitro* model of an organ that recapitulates the key structural, functional, and biological characteristics of the corresponding *in vivo* tissue (29). Organoids are established from several major components. First cells are taken from the source, either via a biopsy from human or animal tissues or from *in vitro* cultured cell lines. Following isolation and purification protocols, cells are seeded and expanded on a matrix containing defined extracellular matrix (ECM) or synthetic substrates in the presence of specific growth factors, small molecules, and physical cues that function to drive the differentiation of cells and development into a defined structure resembling the desired organ.

Several organs have been recapitulated as organoids by using defined physicochemical conditions, including the small intestine, colon, stomach, esophagus, tongue, liver, lung, pancreas, heart, ear, and brain (29,30). These tools facilitate significant advancements in basic biology, development and disease modeling, and drug discovery and development. Cerebral organoids provide significant contributions to our understanding of the brain in health and development, and how it responds to stressors like hypoxia or onset of diseases such as Alzheimer's disease (31,32). Cerebral organoids have been developed and employed by biopharma for target and drug discovery purposes (30). An interesting example is the use of cerebral organoids to identify viral entry receptors involved in the Zika viral infection, which laid the groundwork for the use of cerebral organoids for neuroscience drug discovery (33,34).

Despite success stories associated with the use of organoids as a tool for both basic research and target identification, some considerable challenges limit their adoption at a larger scale. There is a high degree of sample heterogeneity both between and within organoid batches, stemming from the inherent variability of patient-derived samples (35). This can be partially overcome by using automated liquid handling and cell culture technologies to reduce systemic errors associated with culturing organoids. A major hurdle to organoid adoption by big pharma is scale. Culture protocols usually involve 96-well plates, because organoids are generally too large for 384- and 1536-well plates. Scalability can be partially improved using automation and OoC technologies.

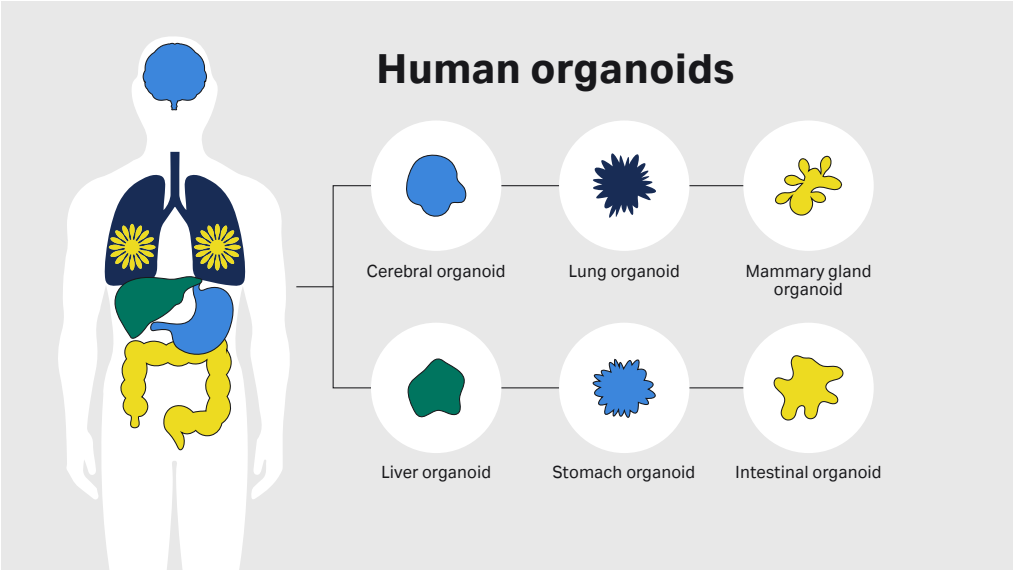

Fig 3. Common human organoids.

2.2. Organ-on-chip

OoC systems are *in vitro* microfluidic models that mimic microstructures, functions, and physicochemical environments of organs on a very small scale (36). Microfluidic chips are designed with tiny channels and chambers, simulating the structures of organs. Cultured cells specific to the organ of interest, such as the liver or heart, are placed on the chip. Physiological conditions like fluid flow, mechanical forces, and electrical signals are mimicked to create a realistic environment. Researchers use OoC systems primarily for drug testing, introducing substances, and observing their effects on the organ model. Sensors and imaging tools collect data on cell behavior and tissue response, which is then analyzed to assess drug efficacy and toxicity.

The first OoC was a lung-on-chip device developed less than 15 years ago (37). Despite being in their early stages, OoCs are increasingly being integrated into drug development pipelines, replacing more traditional models (38,39). Microfluidic technologies like OoCs allow researchers to integrate different technologies in parallel, multiplexed assays, to obtain a comprehensive overview of the impact of therapeutic modulation on an organ. They provide opportunity for scalability and are advantageous in terms of throughput, because of their small volume requirement (40). OoCs are less complex than organoids so the structural information they provide about an organ is limited.

Since their conception, OoCs have been used to replicate functions of specific, single organs, such as the heart, kidney, liver, and lungs, and observing the effects of modulation on these organs, such as in toxicology studies (36). It has become clear that toxicology and other readouts are more accurate when multiple organs are tested concurrently. OoCs that integrate several organs are in development, known as body-on-chip (41). This is likely to be successful at examining toxicity, predicting therapeutic safety, and providing novel insights into disease mechanisms at a whole-body level. Body-on-chip will hopefully aid development of more targeted therapies and overcome the problem examining a single organ in isolation.

Overall, the emergence of fashionable models in drug discovery, such as organoids and OoC systems, signifies a transformative shift in the drug development landscape. These innovations offer more humane and reliable alternatives to traditional animal testing. Organoids provide deep insights into biology, development, and disease modeling, but face challenges related to sample heterogeneity and scalability. OoC systems, despite being relatively new, have gained traction for their ability to mimic organ functions accurately and efficiently, paving the way for multiplexed assays and body-on-chip models. While OoCs may lack the complexity of organoids, they hold promise for toxicity assessment and disease mechanism exploration. Adoption of these models promises to enhance drug discovery by providing more comprehensive and predictive insights, ultimately accelerating the development of safer and more effective pharmaceuticals.

Explore fashionable models in translational science with a specialist today

3. Artificial intelligence

Some of the most significant challenges associated with drug discovery include poor target validation, high costs, and high failure rates. To address these challenges, AI, machine learning (ML), and deep learning (DL) have emerged as transformative tools in the field of drug discovery. These technologies harness vast datasets and computational power to expedite target identification, optimize lead compounds, predict drug interactions, and enhance clinical trial design. By efficiently analyzing complex biological data, AI, ML, and DL approaches not only accelerate the drug discovery process but also have the potential to significantly reduce costs and increase the likelihood of successful drug development, bringing with it a new and improved era of precision medicine.

3.1. Using AI to predict protein structure

Traditionally, determining protein structures was slow, expensive, and often yielded low-resolution results. AI-based protein structure prediction has transformed this process and was recognized as Science's breakthrough of the year in 2021 (42). AI-based technologies employ ML and DL algorithms to predict protein structures with remarkable accuracy. By using approaches such as homology modeling, *de novo* structure prediction, and molecular dynamics simulations, AI provides structural insights into challenging or experimentally inaccessible proteins.

One remarkable AI innovation, AlphaFold, and its updated version AlphaFold2 demonstrate exceptional accuracy in predicting 3D protein structures (43). Developed by DeepMind, AlphaFold leverages a database of known protein structures to predict unknown structures precisely and rapidly. AlphaFold can significantly expedite target identification and validation, reducing the timeline from months or years to hours or days.

Another AI company, RoseTTAFold, introduced innovative methods for predicting protein structures. RoseTTAFold simultaneously considers protein sequences, amino acid interactions, and 3D structure. It predicts protein structures with a resolution close to that of AlphaFold but has lower computational demands and quicker turnaround times (44,45). The accessibility to protein structure prediction is poised to streamline drug discovery efforts further. AI's role in predicting protein structures is a transformative force in advancing drug discovery. It offers unprecedented speed, accuracy, and potential for developing vital therapies, exemplified by its contributions to understanding and combating the COVID-19 pandemic (46).

3.2. AI in molecular modelling and clinical prediction

AI also plays a pivotal role in clinical prediction. In drug design, AI algorithms are harnessed for molecular modeling. They provide precise predictions of molecular behavior and offer valuable insights toward enhancing drug efficacy (47). AI's ability to swiftly screen thousands of compounds aids in predicting their potential effectiveness as drugs. Through the utilization of vast data resources and ML techniques, AI streamlines the systematic identification of promising drug candidates, mitigating risks associated with drug development and significantly reducing the time and costs (48).

AI can also be used to personalize medicine by analyzing extensive genomic datasets, characterizing patients' genomic profiles, and mining electronic health records for insights into their medical history, treatment responses, and risk factors (49). The integration of this data facilitates improved decision making regarding the most safe and effective treatments for each patient. AI's predictive capabilities can improve prognosis predictions and inform precise treatment decisions by assessing disease risk, progression, treatment responses, and discovery of novel biomarkers (50).

3.3. The role of AI in clinical trial design

Looking toward the later stages of drug development, AI is proving to be a game-changer in the realm of clinical trial design and management, addressing the persistent challenges that often plague these trials. Clinical trials are known for their time, cost, and high failure rates. AI's data-driven approach combined with ML streamlines critical aspects of clinical trial design, such as selecting and refining endpoints, predicting sample sizes, and ensuring the validity of endpoints, ultimately saving valuable time and resources (51,52). AI enhances patient cohort selection, recruitment, and retention by leveraging innovative models to generate insights into favorable patient populations, a process that traditionally involved laborious manual efforts (53,54).

AI also optimizes the assessment schedule, eliminating non-essential procedures and ensuring relevant ones are included. This reduces participant burden and enhances recruitment and retention rates (55). It also aids in safety data monitoring, by creating comprehensive risk profiles, and efficiently documenting safety measures (56). AI provides valuable tools for research and analysis, simplifying data sourcing and integration into study design. This comprehensive approach improves productivity, minimizes human error, and reduces the risk of unconscious bias, leading to more successful and efficient clinical trials.

AI is a transformative tool for drug discovery and development because it addresses challenges such as poor target validation and high costs. By leveraging vast datasets and computational power, AI accelerates target identification, streamlines clinical trial design, and significantly reduces the time and costs associated with drug development. It revolutionizes protein structure prediction with remarkable accuracy and enhances personalized medicine, enabling tailored treatment decisions and better patient outcomes. AI optimizes clinical trial design, improving patient recruitment and retention, assessment schedules, and safety data monitoring. While AI offers tremendous benefits, concerns remain surrounding data privacy, regulatory guidelines. Safeguards must be put in place to protect patient data (57,58).

Explore accelerating your drug to market with AI, with our translational medicine specialists

4. Paint-by-cell

Paint-by-cell is the term used to describe an unbiased, phenotypic drug discovery approach involving high-content image-based screening assays that employ predictive model systems to evaluate therapeutic candidates in terms of bioactivity, toxicity, and mechanisms of action. Paint-by-cell has existed for several years but has recently been integrated with AI- and ML-based approaches and is gaining new traction in drug discovery.

Paint-by-cell, also commonly referred to as a high-content screening (HCS) or high-throughput screening (HTS), is a powerful tool that solves problems related to the efficiency, speed, and cost-effectiveness of biological and pharmaceutical research. Image-based cell profiling assays have shown great promise in enhancing understanding of disease mechanisms, exploring target efficacy and small molecule toxicity, and enabling more personalized medicine (59).

HTS technologies allow for the rapid testing of thousands of compounds against specific biological target molecules (60). A typical workflow involves seeding cells in multiwell plates (384- or 1536-well plates), followed by treatment with compound libraries. This is usually done using automated or semi-automated methods such as liquid handling robots and automated cell culture systems. Cells can then be stained, and high-throughput imaging is performed using automated widefield or confocal microscopes. The data analysis pipeline and output of the experiment depends on the purpose of the assay. Screening experiments are designed to pinpoint compounds that produce a pre-defined phenotype while profiling experiments profile the phenotypic changes associated with modulation using an unbiased approach. Both approaches integrate AI- and ML-based bioinformatics pipelines which accurately and comprehensively classify cell phenotypes and facilitate the rapid identification of potential drug candidates (59).

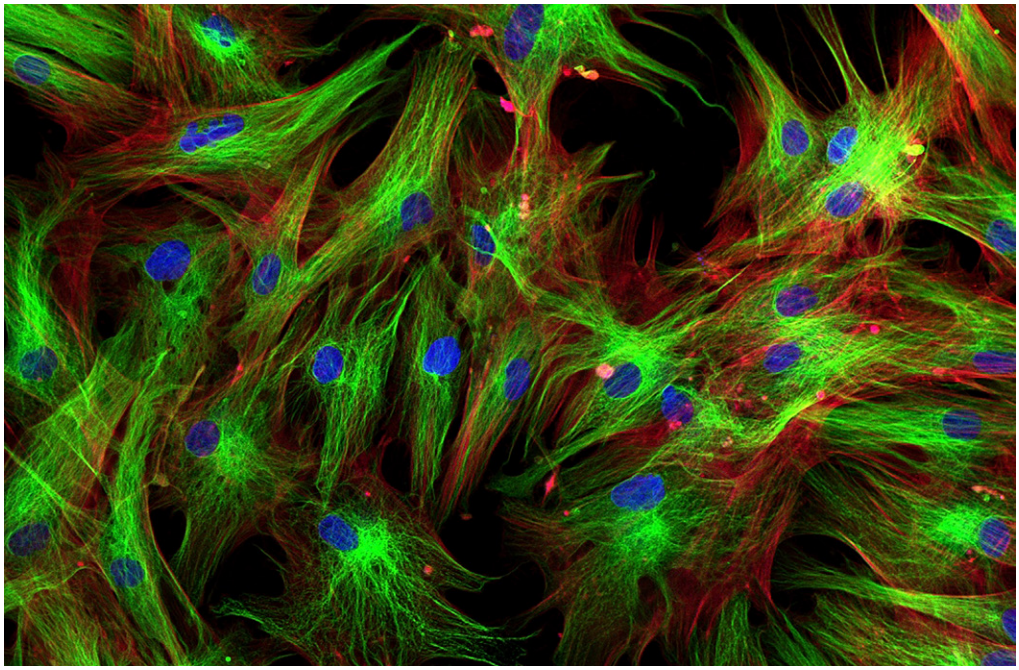

Fig 4. Fibroblasts (skin cells) labeled with fluorescent dyes.

A key benefit of paint-by-cell techniques lies in their ability to identify potential compound leads where the precise mechanism of action is unknown (60). If one compound is found to be effective against a disease, compounds that cause similar phenotypes (as identified by their original HCS assays) can be tested without the need to set up a new screen, and their mechanisms of action can be inferred (61) Aside from identifying novel compounds and deciphering their efficacy, cell painting can provide early insights into compound-induced cytotoxicity in a high-throughput manner minimizing wasted time and resources (60).

Although cell painting and other HTS-based assays have the capacity to provide insights into potential compounds of interest, mechanisms of action, and toxicity effects, these assays generate vast volumes of data (62). The exhaustive amount of data generated can be challenging to analyze and interpret. The integration of AI- and ML-based algorithms for data management, analysis, interpretation, and insight generation give researchers the capability to use cell painting-based assays.

Owing to the substantial benefits of applying automated, information-rich cell-based assays to drug discovery and development pipelines, paint-by-cell is regularly implemented throughout the entire process of drug development and can be considered a mainstream technology within the pharmaceutical and biotech industries (62). It is being implemented to meet the needs of large-scale biology within academic research and translational settings, where it can enable rapid, high-quality insight generation in the study of complex systems such as stem cell biology and regenerative medicine (63).

Talk to our experts about ways to deliver high-throughput screening in translational medicine

5. Conclusion and future perspectives

The constantly evolving landscape of drug discovery means that novel technologies and approaches are required to address current challenges, ultimately leading to more targeted therapeutics and successful trials. We discussed the pivotal role of four trends: Targeting undruggables to expand the horizons of therapeutically modifiable biological targets, leveraging new models towards the replacement of animal testing for therapeutics, harnessing AI for predictive and analytical purposes, and adopting HTS technologies such as paint-by-cell – in advancing drug discovery. There are new challenges that arise with applying new technologies to translational research, drug discovery, and drug development pipelines, but the future is promising. An increased focus on precision medicine, personalized treatments, and interdisciplinary collaborations aim to bring safer and more effective therapies to patients.

Interested in learning more about the ever changing landscape of drug discovery and how Cytiva can help support your journey from discovery to delivery?

Get in touch today to continue the conversation with one of our translational medicine specialists.

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