IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
February 2022 Vol.:23, Issue:3

Cell-Based Phenotypic Approaches in Drug Discovery

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Manish Kumar Gupta[1], Ketaki Dhane [2]*, Birendra Shrivasttva[3], Supriya Hyam[4], Sujit Nagare[5], Arvind Shinde[6], Anushka Jadhav[7]

[1, 2, 3] *School of Pharmaceutical Sciences, Jaipur National University, Jaipur, India*

[4]*Vijayrao Naik College of Pharmacy, Kankavali, India*

[5, 6, 7] *PSPS, Indira Institute of Pharmacy, Sadavali, India*

Submitted: 25 January 2022
Accepted: 30 January 2022
Published: 28 February 2022

Keywords: Omics, Phenotype, drug discovery

ABSTRACT

Phenotypic drug discovery (PDD) approaches do not rely on knowledge of the identity of a specific drug target or a hypothesis about its role in disease, in contrast to the target-based strategies that have been widely used in the pharmaceutical industry in the past three decades. However, in recent years, there has been resurgence in interest in PDD approaches based on their potential to address the incompletely understood complexity of diseases and their promise of delivering first-in-class drugs, as well as major advances in the tools for cell-based phenotypic screening. Though, phenotypic drug Discovery approaches also have considerable challenges, such as hit validation and target deconvolution. This article focuses on the lessons learned by researchers engaged in PDD in the pharmaceutical industry and considers the impact of 'Omics' knowledge in defining a cellular disease phenotype in the era of precision medicine, introducing the concept of a chain of translatability. We particularly aim to identify features and areas in which Phenotypic drug Discovery can best deliver value to drug discovery portfolios and can contribute to the identification and the development of novel medicines, and to illustrate the challenges and uncertainties that are associated with Phenotypic Drug Discovery to help set realistic expectations concerning its benefits and costs.

INTRODUCTION

Phenotypic is the observable physical properties of an organism; these include the organism's appearance, development, and behavior. An organism's phenotype is determined by its genotype, which is the set of genes the organism carries, and environmental influences upon these genes.

The drug discovery includes

1) Classical pharmacology, also known as phenotypic drug discovery, which is the historical basis of drug discovery, and reverse pharmacology, also designated target-based drug discovery, Under phenotypic profiling many features possible to cover in a single cell to select for robust, meaningful features. These functions were extracted to generate an individual cell profile. (1)

Drug discovery to identify molecules with the ability to alter a cell's phenotype was used in phenotypic screening. Animal models and cell-based assays are both strategies used to identify these molecules. “An image is worth a thousand words is something we hear often and many uses of phenotypic screening(2) strategies in biological research and drug discovery to identify substances such as small molecules, peptides, or RNA that alter the phenotype of a cell or an organism in the required manner.

Phenotypic drug discovery (PDD) implies screening where the molecular mechanism of working is not required the knowledge of the molecular target. As such PDD is comparable to empirical screening, which was historically used in drug discovery before more target-based approaches became popular. The genotype was the genetic contribution to the phenotype. It's phenotypic characteristics as under observable traits, such as height, eye color, and blood type etc. Some of the traits are largely determined by the genotype (3), while other traits are largely determined by an environmental associated factor it allows differential behavior toward previously unmet animals. An animal making a cognitive decision compares the cues presented by the other animal with an internal expectation of which relative looks/smells/sounds like. So phenotypic matching is a very important role.

Review

The Scientists and organizations that worked hard to discover new medicines employ all available knowledge and advance to identify the best starting points and strategies.

Unfortunately, knowledge gaps exist between the understanding of disease and the identification of useful therapeutics. History shows a progression in utilizing new knowledge to reduce the uncertainty and reliance on unplanned fortunate discovery: 'chemotherapy'(4) and 'magic bullets', to Black and Janssen's desire to start with 'pharmacologically active compounds', to Hitchings and Elion's strategy to utilize 'new biochemical understandings', and most recently, the use of genetics and genomics to identify drug targets. Throughout this evolution of knowledge and strategies, the trial-and-error experiment was required to bridge the translational knowledge gap to identify first-in-class compounds. Recently, the reliance upon empiricism was formalized as phenotypic drug discovery (PDD).

At the core of PDD(5) is an unbiased selection of drug candidates without prior assumptions as to how the candidate will work. PDD is evolving to a more formalized strategy to help address the uncertainty and risk associated with using empiricism to bridge mechanistic knowledge gaps.

The Discovery of first-in-class medicines is a great achievement. To be of value, medicines must be both effective and safe; not doing more harm than the disease. What makes their discovery extremely challenging is the difficulty of identifying potential therapeutic compounds that will trigger mechanisms to specifically and safely change a disease phenotype.

Medicine	Year	Indication	Activity identified	Compound source	Strategy
Salvarsan	1909	Antiparasitic	Rabbits infected with syphilis	Arsenic analog	Empirical screening
Prontosil	1935	Antibacterial	Infected mice	Azo dyes	Empirical screening
Penicillin	1943	Antibacterial	Bacterial culture	Mold	Scientific opportunism
Warfarin	1954	Anticoagulant	Cows bleeding to death	Moldy hay	Scientific opportunism
Metformin	1957	Antidiabetic	Herbal remedy	Plant	Analog lead activity
Azathioprine	1961	Immunosuppression	Microbial growth assay	Antimetabolites	Chemical hypothesis
Propanolol	1964	Blood pressure	Tissue assay	Hormone analog	Chemical hypothesis
Omeprazole	1972	GERD[b]	Dogs	Active toxic compound	Empirical screening
Cyclosporine	1976	Immunosuppression	Hemagglutination	Scientific opportunism	Empirical screening
Tamoxifen	1973	Breast cancer	Animal models of disease	Hormone analog	Empirical screening
AZT	1987	HIV	Antiviral	Nucleosides	Repurpose
Artemisinin	2006	Malaria	Parasites	Plant	Empirical screening

At the core of this challenge is identification (6) of the dynamic molecular actions that trigger the change in phenotype: the right key (medicine) to unlock the right lock (target) that triggers the right phenotypic change (disease).

The goal for this phenotypic drug discovery is to describe easily the discovery of a chronological sample of important medicines by considering the role of empiricism in their discovery. The brief descriptions that follow identify the source of the modality, how the disease-modifying activity was identified, and the type of knowledge and strategy used as a starting point. (7)

The descriptions focused on the initial findings while acknowledging that a significant contributor to success was the characteristics of the individuals and organizations that addressed the uncertainties and risks associated with validation of the initial findings and progression to products.

As the understanding of medical science has evolved, so did the desire for a more rational approach to invent new medicines, before the era of genetic revolution, medicines were identified primarily by a 'compound-first' approach. Pioneers in this era include Paul Ehrlich who invented the first 'magic bullet', salvarsan for syphilis and African Trypanosomiasis from chemical dyes, and Sir James Black and Dr. Paul Janssen who emphasized to initiate discovery efforts with a 'pharmacologically active compound'(8). Among Janssen's discoveries were loperamide, an antispasmodic, and fentanyl, an analgesic; these were derived from Meperidine. George H. Hitchings Jr, working with Gertrude Elion, emphasized the power of empirical, phenotypic screens when he stated in his 1988 Nobel lecture entitled 'Selective Inhibitors of Dihydrofolate Reductase' that 'Those early, untargeted studies led to the development of useful drugs for a wide variety of diseases and has justified our belief that this approach to drug discovery is more fruitful than narrow'.

They show interest in empirical drug discovery(9) and its formalization under the name PDD came after an analysis of the discovery strategies for new molecular entities approved by the US Food and Drug Administration (FDA) between 1999 and 2008. This analysis determined that most first-in-class small-molecule drugs were discovered empirically, whereas the majority of those that followed were discovered using target-based drug discovery (TDD)(10).

This report concluded that the mechanistic knowledge available when a program is initiated is often insufficient to provide a blueprint for the discovery of first-in-class medicines. The formalization of Phenotypic Drug Discovery to a strategy or discipline was subsequently suggested by Eder and coworkers in the analysis of first-in-class new molecular entities from 1999 to 2013. In this work phenotypic screening was defined as the testing of a large number of (in most cases randomly selected) compounds in a system-based approach using a mechanistic agnostic assay. Using this different definition Eder and co-workers found that PDD contributed to much fewer discoveries (11). Phenotypic Drug Discovery has the potential to be much more than random screening in complex systems, as defined by Eder and co-workers. Phenotypic Drug Discovery has the opportunity to create further value by

providing a strategy to address mechanistic knowledge gaps at any level. This was the thinking and rationale behind the earlier analysis by Swinney and Anthony.

In many cases, the difference between Phenotypic Drug Discovery and target-based drug discovery is painted as black and white. In reality, there is generally some level of mechanistic knowledge with target agnostic strategies; however, this knowledge does not involve a specific molecular interaction, and for TDD there is a hypothesis regarding the molecular interaction but the mechanistic knowledge is incomplete as it does not include the exact molecular dynamics that will trigger the change in phenotype (as noted earlier).

Phenotypic Drug Discovery relies on the translatability of the phenotypic marker in the screening assay while target-based drug discovery relies on the translatability of the target to the disease.

Overall, the target-based drug discovery strategy is a linear process with well-defined and tractable technical milestones. Typically, targets representing known ‘drugs able’ proteins are selected by their association or ‘validation’ with a particular therapeutic indication. Enablement of primary screens is typically low risk and based on previous industrial experience with members of the molecular target class. target-based drug discovery flow schemes are principally concerned with enhancing primary target potency/efficacy, achieving biochemical selectivity, and demonstrating cell-based activity upon target engagement, tasks that are informed and generalizable from prior experience with the target class. With these assets in place, a drug discovery team can optimize lead compounds for biopharmaceutics properties and safety to provide a drug candidate. Although challenging, discovery and optimization of advanced leads/clinical candidates by TDD follows a process, with predefined milestones and established cycle times. This process is strongly dependent on the validity of the target and is most effective for followers/best in class and monogenetic diseases. (13).

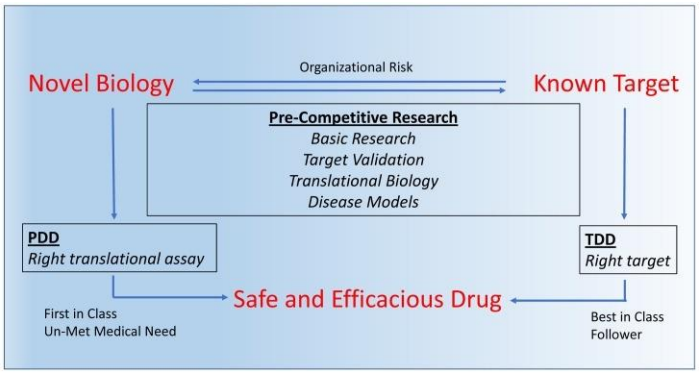

Fig.1 Phenotypic drug discovery (PDD) complements target-based drug discovery (TDD).

PDD uses empirical, target-agnostic lead generation to identify pharmacologically active molecules and novel therapeutics which work through unprecedented drug mechanisms. A significant barrier in the decision to implement PDD is balancing the potential impact of a novel mechanism of drug action with an under-defined scientific path forward, with the desire to provide infrastructure and metrics to optimize return on investment, which a known mechanism provides. A means to address this knowledge gap in the future is to empower precompetitive research utilizing the empirical concepts of PDD to identify new mechanisms and pharmacologically active compounds to explore disease biology and de-risk pharmaceutical R&D.

The development of the physiologically relevant in vitro disease models are foundational to PDD. As a result, PDD assays are frequently very complex multifactorial cellular systems will be unique to the disease model and contrast significantly with TDD assays, which are generally more standardized and process-friendly.

Phenotypic drug discovery flow scheme development is frequently dynamic and utilizes the results of pilot screens and project progression to reveal unwanted cellular processes and signaling pathways, which in turn requires modification of flow schemes to identify undesirable phenotypic mechanisms.

Phenotypic actives representing distinct chemical clusters can, in principle, be working through diverse mechanisms; as a result, in vivo proof-of-concept data are frequently desired early in the lead optimization phase to confirm/establish linkage between the in vitro and in vivo systems. This article focuses on the lessons learned by researchers engaged in phenotypic drug discovery process in the pharmaceutical industry and considers the impact

of ‘omics’ knowledge in defining a cellular disease phenotype in the era of precision medicine, introducing the concept of a chain of translatability.

Phenotypic drug discovery (PDD) implies screening where the molecular mechanism of the action is not assumed and does not require knowledge of the molecular target. As such phenotypic drug discovery is comparable to empirical screening, which was historically used in drug discovery before more target-based approaches became popular, Currently, there is a resurgence in interest in phenotypic drug discovery, driven by many factors, not least the limited success of target-based drug discovery (15).

One of the key features of current phenotypic approaches is the biological relevance of the assay systems deployed and in this regards, the commercial availability of unlimited quantities of pure human cell types, particularly those derived from induced pluripotent stem cells, is having an impact.

Stem cells are power of the development of many new disease models and with high levels of translation to human biology and diseases, these phenotypic assays are increasingly being used in early toxicity testing. Phenotypic drug discovery content imaging systems, facilitates the rapid analysis of increasingly complex multi-parametric measurements of cellular phenotypes or biomarkers.

PDD looks set to be with us for the foreseeable future, with a role alongside target-based and other approaches to drug discovery that is phenotypic drug discovery- The Drugs typically act by engaging a molecular target; however, prior knowledge of that target is not essential.

In the case of phenotypic drug discovery, a ‘physiologically relevant biological system or cellular signaling pathway is directly interrogated by chemical matter to identify biologically active compounds (16).

This target-agnostic approach is the underlying attribute that differentiates PDD from hypothesis-driven TDD. These target-agnostic and empirical aspects of PDD are consistent with its description and usage by scientists in academia and industry Most drug discovery projects that are based on a molecular target hypothesis also test active compounds in phenotypic cellular assays. Although these are not the phenotypic or empirical drug discovery examples, novel and therapeutically.

Important MoAs that differentiate targeted drugs can be discovered phenotypically. An example of this 'molecularly informed phenotypic discovery' paradigm was the empirical observation that the oestrogen receptor (ER) antagonist fulvestrant displayed greater than expected efficacy, leading to the clarification of its ER-degrading mechanism.

The unique promise of PDD is its ability to exploit a disease phenotype to discover novel treatments for diseases for which the root cause is unknown, complex, or it may be multifactorial, and for which scientific understanding is insufficient to provide valid molecular targets. However, PDD should not be regarded simply as an alternative screening technology or as an easy fix to the challenges of clinical attrition rates y(17).

We referred above to Phenotypic Drug Discovery as being at risk of undergoing a hype cycle. We intended to constructively minimize overly optimistic expectations for 'quick wins' from PDD, but also to provide advice and encouragement to ameliorate the potential for a trough of disillusionment that may arise when organizations are not frequently rewarded with first-in-class or best-in-class drugs or even with tractable leads from phenotypic screens.

The safety of new drugs is critically important to regulators, pharmaceutical researchers, and patients, Even though there are unexpected toxicities still account for 20–30% of clinical trial failures, in part due to the persistence of animal testing as the primary approach for de-risking new drugs, improved methods for safety attrition that incorporate human-relevant biology are needed. This recognition has spurred interest in non-animal alternatives or new approach methodologies (NAMs) including in vitro models that utilize advances in the culture of human cell types to provide greater clinical relevance for assessing risk(20). These phenotypic assay systems use human primary and induced pluripotent stem cell-derived cells in various formats, including co-cultures and advanced cellular systems such as organoids, bioprinted tissues, and organs-on-a-chip. Despite the promise of these human-based phenotypic approaches, the adoption of these platforms into drug discovery programs for reducing safety-related attrition has been slow. Here we discuss the value of large-scale human cell-based phenotypic profiling for incorporating human-specific biology into the de-risking process. We describe learnings from our experiences with human primary cell-based assays and analysis of clinically relevant reference datasets in developing in vitro-based toxicity signatures. We also describe how Adverse Outcome Pathway (AOP) frameworks can be used to integrate results from diverse platforms congruent with weight-of-evidence approaches from risk assessment to improve safety-related decisions in early discovery.

Phenotypic drug discovery (PDD):-

Phenotypic discovery is a mechanistic agnostic strategy, and the newly identified therapeutics are used to identify the mechanism of action. The identification of active therapeutics is accomplished through empirical trial and error. The therapeutics are identified in which disease-relevant phenotypes provide a chain of translation between the observation and clinical response.1,2 Phenotypic drug discovery includes follow-up of observations in clinical trials and mechanistically agnostic screens in physiologically relevant models of diseases (cells)(21).

There have been increased efforts to develop improved disease-relevant assays and to identify new medicines with novel mechanisms of action. A recent report by Haasen and co-workers documents the lessons from 5 years of phenotypic screening at Novartis from 2011 to 2015, detailing a dramatic increase in the percentage of phenotypic screens. Among the many lessons and trends was an increase in more disease-related models using induced stem cells and primary human cells and the use of small-scale screens unchanged lead discovery strategies.

Among the strengths of PDD is repurposing to rapidly identify new therapies. This can be particularly important for emerging pathogens, such as COVID-19. Wang and co-workers reported using Vero E6 cells infected with nCoV-2019Beta CoV/Wuhan/WIV04/2019 to screen compounds with broad-spectrum antiviral activity. In this screen, remdesivir and chloroquine were identified as candidate medicines (22).

Advances in machine learning and artificial intelligence (AI) are poised to make a large impact on PDD. AI will provide new biomarkers and more precise phenotypes. Phenotypic endpoints which translate to the clinic, the chain of translatability,1 are essential; however, not all disease states are associated with defined sets of disease markers. In these situations, high-dimensional profiles composed of gene expression profiles or cellular morphology features are envisioned to define surrogate disease phenotypes where reversion of the ‘disease state’ profile to a ‘wild type’ or ‘normal’ representation is an indication of therapeutic efficacy.

Cell Painting is a surrogate phenotype approach where cellular morphology is measured by fluorescent labeling of eight cellular compartments and subsequent automated high-content imaging analysis of ∼1500 features per cell. The resulting cellular morphological profile

reflects general changes in the cellular state following chemical or genetic perturbation. Morphological changes in cell state can cluster structurally similar compounds or can identify functionally similar but structurally distinct molecules and has been used to deconvolute the mode of action of phenotypic actives working through non-protein targets.

Molecular Mechanism of Action:-

The molecular mechanism of action of drug is the connection of the molecular interactions between the therapy and the biological target (e.g. receptor, enzyme, etc.) that yields the physiological response. They include conformational changes of the receptor and binding kinetics.

PDD has the potential to be much more than random screening in complex systems, as defined by Eder and co-workers.PDD has the opportunity to create further value by providing a strategy to address mechanistic knowledge gaps at any level. (23).

The major distinction between these two strategies is in how they address incomplete mechanistic knowledge. PDD relies on the translatability of the phenotypic marker in the screening assay while TDD relies on the translatability of the target to the disease. In both case there is still the risk and uncertainty associated with the identification of a specific MMOA that will be therapeutically useful, and this must be derisked in a clinical experiment.

CONCLUSION

Phenotypic drug discovery PDD offers an unbiased evaluation setup in the process of drug discovery. The opportunities and identification of new drug targets are under the study of therapeutic importance. The identity of providing an approximate solution to a problem that cannot be solved precisely. Phenotypic drug discovery screening also allows engagements of multiple targets and the physiological mechanism that synergistically participate, resulting in the phenotype. The recent advances in automated screening, endpoint quantification programs allow us to identify and document precise patterns of morphological perturbations, and including identification of similarities and differences in these patterns allows us to characterize compounds and diseases/phenotypes. The together, phenotypic drug discovery screens greatly contribute to allowing us to create tailor-made assays to identify drugs for our unmet medical needs novel drug discovery, PDD can be seen as a complementary approach that can together increase the discovery and developing drugs with novels molecular MoAs.

The target identification of proceeds on the mechanism of action Phenotypic drug discovery is a challenging drug discovery strategy on multiple levels, but it has a successful track record of delivering first-in-class drugs. It is a powerful approach providing a route to enhance innovation in the pharmaceutical industry and to deliver truly novel therapeutics for unmet medical need.

REFERENCES

1. Hughes JP, Rees SS, Kalindjian SB, Philpott KL. Principles of early drug discovery. Vol. 162, British Journal of Pharmacology. 2011. p. 1239–49.
2. Zheng W, Thorne N, McKew JC. Phenotypic screens as a renewed approach for drug discovery. Vol. 18, Drug Discovery Today. 2013. p. 1067–73.
3. Hubaud A, Singh AP. Genetics in Drug Discovery. Vol. 37, Trends in Genetics. Elsevier Ltd; 2021. p. 603–5.
4. Huang CY, Ju DT, Chang CF, Muralidhar Reddy P, Velmurugan BK. A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. Vol. 7, BioMedicine (France). EDP Sciences; 2017. p. 12–23.
5. Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M. Opportunities and challenges in phenotypic drug discovery: An industry perspective. Vol. 16, Nature Reviews Drug Discovery. Nature Publishing Group; 2017. p. 531–43.
6. Donelli G, Vuotto C, Mastromarino P. Phenotyping and genotyping are both essential to identify and classify a probiotic microorganism. Microbial Ecology in Health & Disease. 2013 Mar 11;24(0).
7. Varytė G, Zakarevičienė J, Ramašauskaitė D, Laužikienė D, Arlauskienė A. Pregnancy and Multiple Sclerosis: An Update on the Disease-Modifying Treatment Strategy and a Review of Pregnancy's Impact on Disease Activity. Vol. 56, Medicina (Kaunas, Lithuania). NLM (Medline); 2020.
8. Black SJ. A personal perspective on Dr. Paul Janssen. Vol. 48, Journal of Medicinal Chemistry. 2005. p. 1687–8.
9. Decker S, Sausville EA. Drug Discovery. 2007.
10. Brown D. Unfinished business: target-based drug discovery. Vol. 12, Drug Discovery Today. 2007. p. 1007–12.
11. Swinney DC, Anthony J. How were new medicines discovered? Nature Reviews Drug Discovery. 2011 Jul;10(7):507–19.
12. Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M. Opportunities and challenges in phenotypic drug discovery: An industry perspective. Vol. 16, Nature Reviews Drug Discovery. Nature Publishing Group; 2017. p. 531–43.
13. Szabo M, Akusjärvi SS, Saxena A, Liu J, Chandrasekar G, Kitambi SS. Cell and small animal models for phenotypic drug discovery. Vol. 11, Drug Design, Development and Therapy. Dove Medical Press Ltd.; 2017. p. 1957–67.
14. O'mahony A, Gupta P, Denker SP. Phenotypic Profiling Identifies Biomarkers and Mechanisms of Toxicity.
15. Scannell JW, Bosley J. When quality beats quantity: Decision theory, drug discovery, and the reproducibility crisis. PLoS ONE. 2016 Feb 1;11(2).
16. Yum K, Hong SG, Healy KE, Lee LP. Physiologically relevant organs on chips. Biotechnology Journal. 2014 Jan;9(1):16–27.
17. Kannt A, Wieland T. Managing risks in drug discovery: Reproducibility of published findings. Vol. 389, Naunyn-Schmiedeberg's Archives of Pharmacology. Springer Verlag; 2016. p. 353–60.
18. Li H, Xie W, Gore ER, Montoute MN, Bee WT, Zappacosta F, et al. Development of phenotypic screening assays for γ-globin induction using primary human bone marrow day 7 erythroid progenitor cells. Journal of Biomolecular Screening. 2013 Dec;18(10):1212–22.
19. Joseph LM, Chibale K, Caira MR. Preparation and Physicochemical Characterization of an Inclusion Complex Between Dimethylated β-Cyclodextrin and a Drug Lead From a New Class of Orally Active Antimalarial 3,5-Diaryl-2-Aminopyridines. Journal of Pharmaceutical Sciences. 2016 Nov 1;105(11):3344–50.

20. Armijo-Olivo S. The importance of determining the clinical significance of research results in physical therapy clinical research. Brazilian Journal of Physical Therapy. 2018 May 1;22(3):175–6.
21. Zheng W, Thorne N, McKew JC. Phenotypic screens as a renewed approach for drug discovery. Vol. 18, Drug Discovery Today. 2013. p. 1067–73.
22. KeshavarziArshadi A, Webb J, Salem M, Cruz E, Calad-Thomson S, Ghadirian N, et al. Artificial Intelligence for COVID-19 Drug Discovery and Vaccine Development. Vol. 3, Frontiers in Artificial Intelligence. Frontiers Media S.A.; 2020.
23. Swinney DC. 2 Drug Discoveries and Molecular Mechanism of Action. 2015.