THE FUTURE OF DRUG DISCOVERY

cutting-edge technologies meet traditional paradigms in assay development

With more than 1,500 drugs approved by the Food and Drug Administration (FDA) on the market, the easiest disease targets are already covered[1]. Today's scientists are challenged to find new compounds, acting alone or in combination, on intractable targets to treat multicausal diseases including cancers, metabolic and psychiatric disorders. The technologies and assays coming to the market enable new avenues in drug discovery creating opportunities to study complex disease mechanisms and find cures for the indications mentioned above.

By Dr Roger Bossé

From the ancient Egypt Pharmacopeia described in Ebers Papyrus until the emergence of high throughput screening (HTS) in the early 1990s, drug discoveries mostly resulted from serendipity, intuition and trial-and-error efforts (forward pharmacology) or from first-generation rational drug design (reverse pharmacology). The discovery of penicillin from mould secretions reported by Fleming originated from serendipity and intuition[2]. The H2 blocker and antacid cimetidine (Tagamet), derived from histamine which is known to promote gastric acid release, is a representative example of a drug developed using reverse pharmacology[3].

The traditional drug discovery approaches used until the end of the 1980s revealed too time-consuming, low-throughput and cost-ineffective by the industry which – relying on automation and second-generation (computer-assisted) rational drug design – started to perform high-throughput screening campaigns of large combichem libraries on purified biological targets. Despite the increasing complexity of the disease targets to address, HTS campaigns contributed to deliver close to 50% of the total drugs approved by the FDA since 1930 (**Figure 1**)[1]. However, the drug attrition rate observed during clinical trials remains high in all therapeutic areas mainly due to a lack of efficacy

Figure 1

observed during Phase II-III studies, offsetting some of the HTS benefits[4].

Changing the drug discovery paradigm

The overall efficiency of pharmaceutical research and development (R&D), defined as the ratio of the New Molecular Entities (NMEs) and Biologic License Applications (BLAs) launched as a function of their associated R&D investments, has declined over the last decade[5]. To improve the situation, numerous advances were brought to the drug discovery process.

For instance, predictive toxicology concepts were introduced during absorption, distribution, metabolism, elimination and toxicity (ADMET) to fail drug candidates more rapidly and reduce the drug attrition rate related to safety. While toxicity was traditionally assessed using animal models during the late stage of drug development, predictive toxicology required the development of *in silico* and *in vitro* methods executable at large scale and sooner in the drug discovery workflow. *In silico* methods essentially involve quantitative structure activity relationships (QSAR) where potentially toxic compounds are filtered out from libraries based on their structure. *In vitro* assays rely on specialised cell lines (eg hepatocytes for liver toxicity), microscale physiological systems (eg organ-on-a-chip) and small animal models (eg zebrafish, *C.elegans*, etc). It is worth mentioning that both European Union and North American agencies spearheaded large scale predictive toxicology programmes, such as EU-ToxRisk and EPA-ToxCast/Tox21 respectively. To further decrease the compound attrition rate due to lack of efficacy, scientists moved from a target-based approach to a phenotypic strategy where assays take advantage of more biologically-relevant models, including mono- or co-cultured cells, organoids and small animal models such as those described previously. HTS campaigns are also performed using smaller and more focused libraries composed of a few hundred thousand compounds pre-assembled using QSAR. Chemistry efforts to expand the actual druggable chemical space are under way to better address biological target structural diversity. It is thought that, based on Lipinski's Rule of Five, up to 10^{60} druggable structures could be synthesised[6]. Just over 10^{7} different drug structures, natural or synthetic, are reported in the Beilstein database of organic compounds, indicating that less than one trillionth of the total druggable chemical space is exploited to date[7].

For different reasons including costs, dwindling pipelines and productivity issues, the pharmaceutical industry needed to rethink its R&D strategies and the way drug discovery should be performed. A short-term tactic used by most major pharmaceutical companies was to acquire new drug pipeline content through merger and acquisition activities. Longer-term, several companies opted to decentralise their research programmes via collaborative efforts with external partners. Several academic-

industry collaborations were then initiated during the last decade, allowing partners to share expertise and knowledge on disease mechanisms, novel drug targets and assay technologies. While academic collaborators can profit from industrial know-how in development and specialised resources including medicinal chemistry, HTS and preclinical study set-ups, industrial partners can access new discoveries and ideas from academia to enhance their innovation potential. Substantiating the early benefits of academic-industry collaborations and to further facilitate the flow of respective knowledge and competences, several pharmaceutical companies decided to relocate their R&D sites to be near world-renowned academic hubs[6]. Additionally, translational medicine contributes to improve the drug discovery paradigm. With the aim of bringing new drugs to the market more rapidly and safely, translational medicine relies on a workflow involving multiple feedbacks from clinicians, including pathologists, at the different stages of drug development (**Figure 2**). Quantitative pathology, otherwise known as digital pathology, emerged as a new discipline playing a pivotal role in translational medicine-based drug discovery.

Cutting-edge technologies to the rescue

In the early times of modern drug discovery, the main technological arsenal available to scientists was comprised of microscopes, pipettes, test tubes and primitive immunoassays, such as radioimmunoassays (RIAs). With the emergence of industrialised target-based drug discovery, microscopes were relegated to a second-string role while pipettes, test tubes and immunoassays underwent significant improvements leading to automated liquid handlers, microplates, ultrasensitive non-radiometric immunoassays and associated multilabel detectors. Ironically, scientists were replaced by robots during large-scale assay execution (HTS). The needs for changing the drug discovery workflow and the consequent resurgence of phenotypic assays necessitated substantial adjustments, including improvement of time-proven/relegated technologies such as microscopy.

Advances in microscopy

Novel microscopy-based technologies such as high-content screening (HCS) and high-content image analysis (HCIA) have proven to be very valuable in the most recent drug discovery efforts. Not only does generating high quality images allow for detection of a specific target signal, it enables recording of holistic phenotypic changes happening in the whole cell, organoid or small organism analysed as well. However, literature shows that most of the high content data published so far only relied on a few image-based features measured from all samples tested, limiting access to more complete valuable phenotypic information available[8]. The lack of advanced technologies allowing for the multiparametric analysis of all collectable data was originally hypothesised as being one of the major impediments limiting the potential of HCS and HCIA. Artificial intelligence including machine learning is used to alleviate these limitations. Supervised machine learning (SML) software helps to perform automatic phenotypic classification. Such software is considered essential for high content data analysis – even if it comes at a cost – as expert pre-identified references are required to set biologically-relevant predictive models. To overcome this bottleneck, development of enabling methods to decipher high-content screening results, unbiased from existing control phenotypes, is currently under way. These methods are based on modelling data issued from unsupervised multiparametric analysis that create self-organising maps (SOMs), which eventually helps grouping treatments that generate similar phenotypical responses[9]. That approach, referred to as active learning, has the potential to identify novel chemotypes and cellular phenotypes while confirming expected hits on already identified targets. Results obtained to date show that active learning significantly reduces the time and costs to reveal the same phenotypic targets identified using SML[10].

HCS and HCIA are both based on classical optical microscopy with a resolution limited by diffraction to approximately 200nm. Stimulated emission depletion (STED) microscopy technique, whose inventors received the 2014 Nobel prize in chemistry, overcomes the diffraction-limited resolution barrier by using a pair of lasers to control the excitation state of fluorescent molecules in a targeted manner allowing resolution of 50nm or less[1]. STED is primarily a point-scanning technique where the fluorescence spot produced by a first laser is sharpened by stimulated emission induced by the second laser. STED provides much sharper images compared to classical microscopy allowing visualisation of individually-labelled biomolecules even in a complex environment. For instance, nanoscale STED imaging of green fluorescent protein-labelled neurons was demonstrated in living brain slices[12].

Advances in cellular models

Improvements in cell imaging technologies discussed above are complemented by the development of

Figure 2

enhanced cellular models mirroring *in vivo* systems. For instance, *in vitro* cell-based assays help in reproducing the complexity of biological environment and are generally predictive of how a compound behaves *in vivo*. Immortalised cell lines, primary cell cultures and, more recently, human pluripotent stem cells (hPSCs) have shown to be invaluable phenotypic models for basic research and drug discovery efforts.

Immortalised cell lines, such as CHO-K1 and HEK-293, are still very popular in several laboratories. The fact that they proliferate indefinitely and are easy to maintain in culture at low cost makes them very convenient, namely for executing HTS campaigns. However, immortalised cell lines have a very limited biological relevance, especially when they are used to overexpress exogeneous proteins. That is why a growing number of cell biologists rely on primary cells since they closely resemble the function of the organ or tissue they compose. Primary cells require limited handling or manipulation to preserve their original characteristics and functions which somehow limits their use at large scale. Stem cells, namely inducible pluripotent stem cells (iPSCs), have found their way in fundamental and applied research. They can be used at all stages of the drug discovery workflow: from target identification to ADMET studies. Since stem cells are capable of unlimited self-renewal, they represent reliable sources of physiologically relevant cells upon controlled differentiation. These two properties make stem cells very suitable alternatives to recombinant/immortalised cell lines and primary cells. However, a more general use of stem cells is hampered by challenges related to directed differentiation and associated cost of maintenance[13].

Scientists need to pay significant attention to cell culture conditions since they can drastically alter cellular phenotype and biological functions. Whereas cells are traditionally grown and used either in suspension or as adherent monolayer monocultures, cells sometimes need to be co-cultured with other cell types found in their natural environment to better adapt and react with biological relevance. This is namely the case of neurons co-cultured with either astrocytes or microglial cells[14]. Furthermore, numerous reports show that cells differ in structure and function when used in suspension or as adherent forms. Similar observations were reported with cells grown on 2D surface-coated support compared to 3D scaffolds. It is worth noting the strong interest in growing cells in 3D since such cultures, namely co-cultures, spheroids and organoids, are closer to *in vivo* natural systems providing more biologically relevant information than single cell populations[15]. Organoids are specialised 3D cell cultures, derived from pluripotent stem cells such as hPSCs or other progenitors, generated to replicate functions of a specific organ. The possibility to obtain any cell type composing the human body through hPSCs together with the potential of generating patient-specific tissues using human-induced pluripotent stem cells (hiPSCs), allows for significant breakthroughs in personalised medicine[16]. Recently-reported 3D matrix and cell printing capabilities should facilitate the latter efforts in a foreseeable future[17].

Label-free technologies for unbiased assay development and biomarker quantitation

Most assay technologies commonly used to characterise molecular mechanism of action (MOA) of drugs rely on labelled biosensors derived from endogenous molecules known to interact with biological target(s) of interest. Labels used to modify those biosensors include either radiometric (eg ^{125}I, ^{35}S) or non-radiometric markers such as fluors. Labelling biomolecules is always at risk of modifying their biochemical properties and pharmacological profiles that could lead to biased analyses. Label-free platforms were developed to alleviate the molecular biases inherent to using labelled reporter molecules so one could produce more relevant information.

Two of the most popular label-free technologies used to date rely on either optical biosensors based on the detection of dynamic mass redistribution (DMR) or impedance biosensors relying on cellular dielectric spectroscopy (CDS). Both techniques can measure changes of live cell morphology in real time[18]. Since the modulation of signal transduction pathways affects cell morphology, DMR and CDS

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technologies are well-suited to measure holistic cell responses in an unbiased and pathway-specific manner.

DMR biosensors are composed of a resonant waveguide grating surface capable of reflecting an incoming white light source at different wavelengths. Magnitude of the reflected light wavelength recorded in DMR is proportional to the amount of matter (eg cytoskeleton elements, proteins) brought into proximity of the biosensor. When cells spread or grow on the biosensor after drug treatment, there is a positive shift of the reflected wavelength since additional cytoskeleton elements are brought to proximity to the sensor. The opposite phenomenon is observed when cells contract or die. Further to measuring cellular morphological changes through the modulation of cell surface receptors (eg GPCR and RTKs) or intracellular enzyme (eg kinase, phosphatase, etc), DMR can also measure several types of interactions involving purified molecules such as small ligand binding to proteins and protein-protein interactions. It is worth mentioning that, contrasting with CDS, DMR can measure phenotypic changes occurring with cells used at various confluency states.

CDS biosensors consist of electrode arrays where confluent cells form an 'isolating interface' generating impedance. When voltage is applied on biosensors, electrodes produce electrical currents flowing around and between cells (eg extracellular current) and through cells (eg transcellular current). When cells contract or die after drug treatment, gaps between cells appear attenuating the isolating interface and a decrease in impedance is recorded. Conversely, an increase in impedance is recorded when cells spread or grow. Contrasting with DMR, CDS requires cell confluency and cannot be applied to measure molecular binding events involving purified components.

On a different note, mass spectrometry is now growing in popularity as a label-free biomarker detection method in bioscience research. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF MS) was shown to have great potential for imaging on tissue sections[19]. When coupled to liquid handling to manage compounds and liquid chromatography to desalt samples prior to injection, MALDI TOF MS can perform HTS with speed, sensitivity and accuracy comparable to label-based technologies[20].

The rise of gene-editing technologies

Gene-editing technologies are undoubtedly among the most impactful scientific breakthroughs of the last century. These technologies allow scientists to add, delete or modify genetic material at specific loci in the genome. The CRISPR-Cas9 method has a lot of popularity in the scientific community since it was shown to be more accurate and efficient than other gene editing systems, while being fast and cheap to execute. Gene editing technologies are used all along the drug discovery workflow from target identification to the generation of new models and therapies. High resolution CRISPR screens of gRNA libraries allow identification and validation of new disease targets, namely in oncology[21]. CRISPR was further applied in studies of drug absorption, distribution, metabolism and excretion (ADME) and for ADME model generation[22]. One of the most spectacular use of CRISPR-Cas9 is in immuno-oncology where researchers engineer T-cells to treat either hematological or solid tumours[23]. The engineered T-cells can be thus (re)injected to cancer patients during autologous or allogenic grafting. Under co-ordinated translational oncology efforts, an increasing number of clinical investigations involving CRISPR-Cas9 engineered T-cells are under way while specialised organisations started offering T-cell engineering and grafting services to cancer patients.

Summary

Today's drug discovery scientists are increasingly facing challenges inherent to the complexity of diseases and the need to bring safer drugs to patients faster and at a lower cost. The drug discovery workflow, developed a few decades ago for HTS, is evolving under the guidance of multidisciplinary teams, including clinicians. Researchers benefit from new powerful technologies allowing for a better identification and validation of new disease targets. Technological advances also allow scientists to set more biologically-relevant disease models and develop unbiased assays to find more efficient and safer therapies based on either small or large molecules. DDW

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