Reigniting pharmaceutical innovation through holistic drug targeting

Modern drug discovery approaches take too long, are too expensive, have too many clinical failures and uncertain outcomes. There are many reasons for this unsustainable business model, but primarily, the approaches are not comprehensively holistic. Secondly, none of the pharmaceutical companies openly share the reasons for the failure of their clinical candidates in real time to effectively navigate the 'industry' from committing the same mistakes. It is time for the pharmaceutical industry to embrace, metaphorically speaking, a community-driven 'Wikipedia' or 'Waze'-type shared-knowledge, openly-accessible innovation model to harvest data and create a crowd-sourced path towards a safer and faster road to the discovery and development of life-saving medicines. This may be a bitter pill for Pharma to swallow, but one that ought to be given serious consideration. The time is now for a paradigm shift towards multi-target-network polypharmacology drugs exalting symphonic or concert performance with occasional soloists to reignite pharmaceutical innovation.

From the turn of the 20th century, pharmacognosy and ethnopharmacology combined with anecdotal clinical evidence accumulated over centuries of hands-on knowledge from primordial disease management practices, albeit with uncertain outcomes, formed the basis for the development of drugs. Reverse pharmacology, deep rooted in traditional medicine, laid the foundation for the emergence and evolution of modern drug discovery approaches as a highly formal and regimented science. Advances in genomics, assay and combinatorial chemistry technologies, informatics and robotics led to increased screening operations significantly compared with traditional discovery methods. The pharmaceutical industry-driven high and ultra-high throughput workflows enabled screening of millions of compounds to identify hit candidates for lead development. Despite billions of dollars spent on R&D, only a fraction of the molecules identified from the screening operations find their way into clinical trials. Difficulties in predicting safety profiles, redundancies and efficacies across a genetically-diverse patient population contribute to the high attrition rates in clinical trials. Success in clinical trials was found to be affected, among other reasons, by the poor design of clinical trials, selected clinical end-points unsuited for the desired outcome and lack of evaluation of pharmacogenomics of the patient population selected for the indication. In addition, while a

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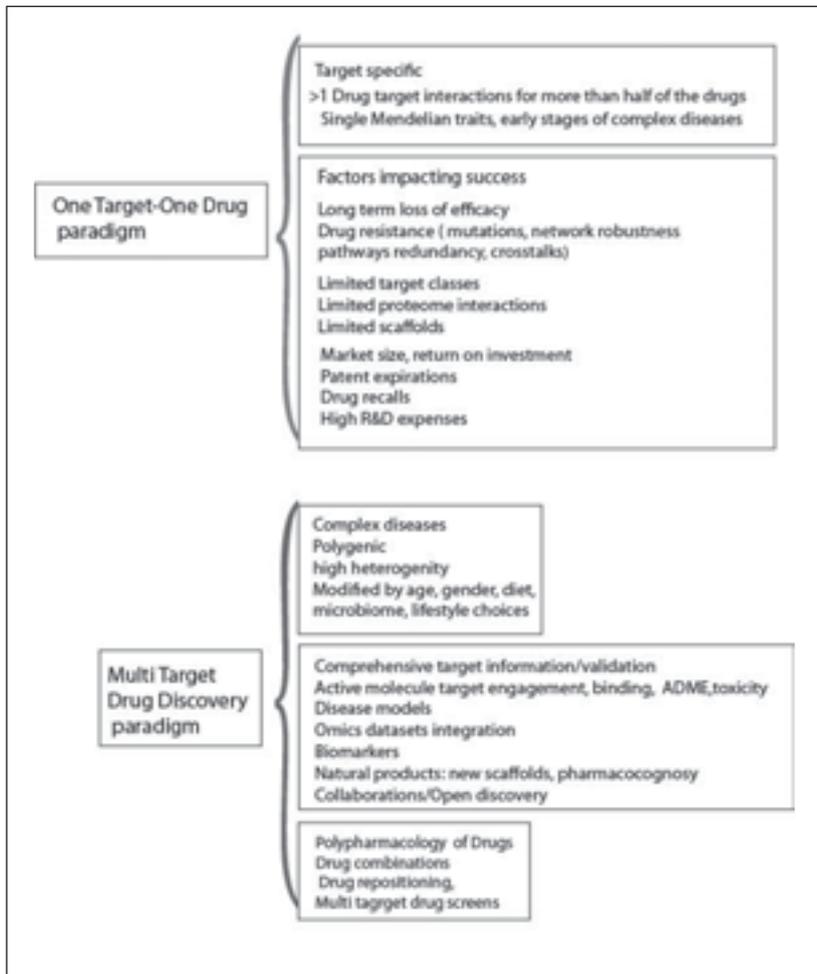
majority of the marketed drugs was approved based on experimental data supporting single target selectivity, recent clinical and basic studies have unambiguously shown that many marketed drugs lack absolute selectivity.

Paradigm shift

Despite high failure rates of compounds in clinical settings, target-based drug discovery has contributed to drugs approved for several indications. An overall evaluation of the FDA-approved new molecular entities reveal that the majority of drugs clustered into previously known classes, and encompasses a limited number of molecular targets and diseases. A recent analysis of the DrugBank database identified 435 targets modulated by 989 drugs and include GPCRs, ligand gated ion-channels, receptor tyrosine kinases and certain other classes of enzymes[1]. The analysis also highlighted the fact that only half of the marketed drugs show higher order and number of drug-target interactions within a very limited proteome-scape. The

vast majority of FDA approved drugs target diseases affecting large populations such as cancers, infectious diseases or cardiovascular diseases, which promises a good return on R&D investment; and hence pursued extensively by big Pharma. Rare diseases (which affect small populations) or neglected diseases (more prevalent in developing countries) have largely been ignored because of low profitability for the pharmaceutical industry and low affordability for the poor. Market size decisions as well as business portfolios are also known to influence indication selection, population sizes and end-points in clinical trials. The last decade witnessed patent expirations, drug recalls and toxicity-driven withdrawals, all of which negatively impacted pharma R&D productivity. Despite significant advances in our knowledge, safety from prolonged use of drugs in the post-FDA approval period remains uncertain. Treatment of complex diseases with single or small combination therapies, while effective in the short term or at early stages of a disease, continues to be insufficient in mitigating advanced or recurrent disease progression. Loss of responsiveness due to long-term administration of single agents is attributable to the robustness of the redundant molecular functions within biological networks.

Many diseases are complex, heterogeneous and multifactorial, have several phenotypes, variable risk factors and responses that are also influenced by genetic variations, age, gender and environmental factors such as diet, microbiome and lifestyle choices. The ever too often seen resistance to continued use of drugs and loss in treatment efficacy are generally mediated by network robustness (signalling pathway redundancy and crosstalk) causing compensatory or counter-target activities or neutralising action. The overall low productivity and innovation of new drug classes, as well as safety- and efficacy-driven drug recalls, begs for a fundamental paradigm shift in current drug discovery approaches.

Druggability of genome

Of the 19,000 human protein coding genes predicted from human genome sequence analysis, only about 10% are 'druggable' by *in silico* analysis[2]. The majority of the 'products' from genome, such as the proteins or RNA, still remain functionally unclassified and the expansion of functionality may be further influenced by epigenomic events as well as post-transcriptional or post-translational regulation[3]. The challenge lies in generating high quality data for target identification, druggability via siRNA or chemical perturbation in

healthy and disease states, confounded by the existence of functional and physical networks between molecular players in the same or other signalling pathways. The hub proteins such as p53, p27, BRCA1, ubiquitin and calmodulin can have five or more interactions with other proteins, and may either exhibit a single transient interaction at any given time or participate in several interactions simultaneously. The cross-talk and interdependence of biological networks adds another level of complexity in responses to perturbations with chemicals, biologics as well as other factors. Systems analysis of large networks indicates that targeting upstream events, hub proteins or redundancies will potentially be more impactful in quantitative and qualitative efficiency of cellular response. In addition to proteins and networks, targeting RNA druggability is still under-utilised. All known RNA motifs and their small molecule binders, compiled in the Informa database[4], are attractive to interrogate and yield lead molecules with potential to be validated in disease systems, as exemplified by the evidence drawn from the selective binding of 5'-azido-neomycin B to Drosha and Dicer processing sites of miR-525, a microRNA overexpressed in liver cancer[5]. A target-agnostic approach can thus be effectively used to identify *in vivo* modulators of oncogenic and other disease relevant microRNAs.

Comprehensive target biology

Strong correlative evidence between a target or targets and a disease is ever more critical for the success of rationale-driven drug discovery. The success of designing well-tailored assays for primary and secondary drug discovery screens, prediction of toxicity profiles, biomarker profiling and ultimate patient population-specific responses are all dependent on how much information, both accurate and reproducible, is available for the disease-relevant, therapeutic target[6]. It is essential to characterise a target in both normal and diseased states for its functional domain(s) redundancy, alternatively spliced forms, subcellular localisation, tissue-specific protein and RNA expression profiles, RNA/protein half-life, transcriptional/post-translational regulation and effect of dominant negative mutant isoforms. Both genetic and chemical validation helps define druggability and translational potential of a target. The same target may be modulated in several distinct cancer types or in several seemingly unrelated disorders, and as a result will help expand the indications or design treatments in clinical trials. An array of tools has become available in recent years for target identification, char-

acterisation and validation. These tools include, but are not limited to, protein and RNA microarrays, bioinformatics-driven protein interaction maps, signalling pathways, phenotypic changes by functional studies using genetic-based technologies (RNA interference, knockdowns, overexpression, genomic mutations using clustered regularly interspaced short palindromic repeats (CRISPR-Cas), and mouse, zebrafish or Caenorhabditis elegans models). The exhaustive information pertaining to the therapeutically-relevant drug target can facilitate efficient design of assays or selection of appropriate panel of cell-lines in targeted or phenotypic drug discovery approaches to identify compounds that can serve as chemical tools to dissect the pathways of interest.

Hit to lead strategies

Once screen actives or hits are identified, it is essential to experimentally determine the mechanism of action, efficacy and safety of the scaffolds and target on-off occupancy and engagement time[7]. The direct binding of the hit molecules to cellular targets can be determined using mass spectrometry on protein samples from cellular thermal shift assays, protein arrays, or compound-affinity chromatography. The binding affinities to specific and off-targets, binding site architecture, co-crystallography studies also provide invaluable information on understanding the chemical species. From the start scaffolds may be tested in assays quantifying potency, selectivity, specificity, lipophilicity, molecular mass and early ADME (absorption, distribution, metabolism and excretion). Collaborative effort between AstraZeneca, Eli Lilly, GlaxoSmithKline and Pfizer to understand the reasons behind drug candidate attrition[8] indicated a strong statistical significance between the physicochemical properties of compounds and safety failure rates in clinical trials, although no rationale emerged for predicting which compounds will be successful in clinical trials. Viable leads share the properties of high efficacy and potency against a specific target, bind the target directly at the same cellular site where the target is expressed, have low off-target effects, no activity against undesirable players such as hERG channels and acceptable toxicity profiles. Target engagement of time and activity at the target tissue/organ requires designing pharmacokinetics/pharmacodynamics (PK/PD) modelling in both preclinical and clinical models. Preclinical drug development that can accurately predict clinical response of drugs can influence the success rate of lead compounds in late stage drug discovery.
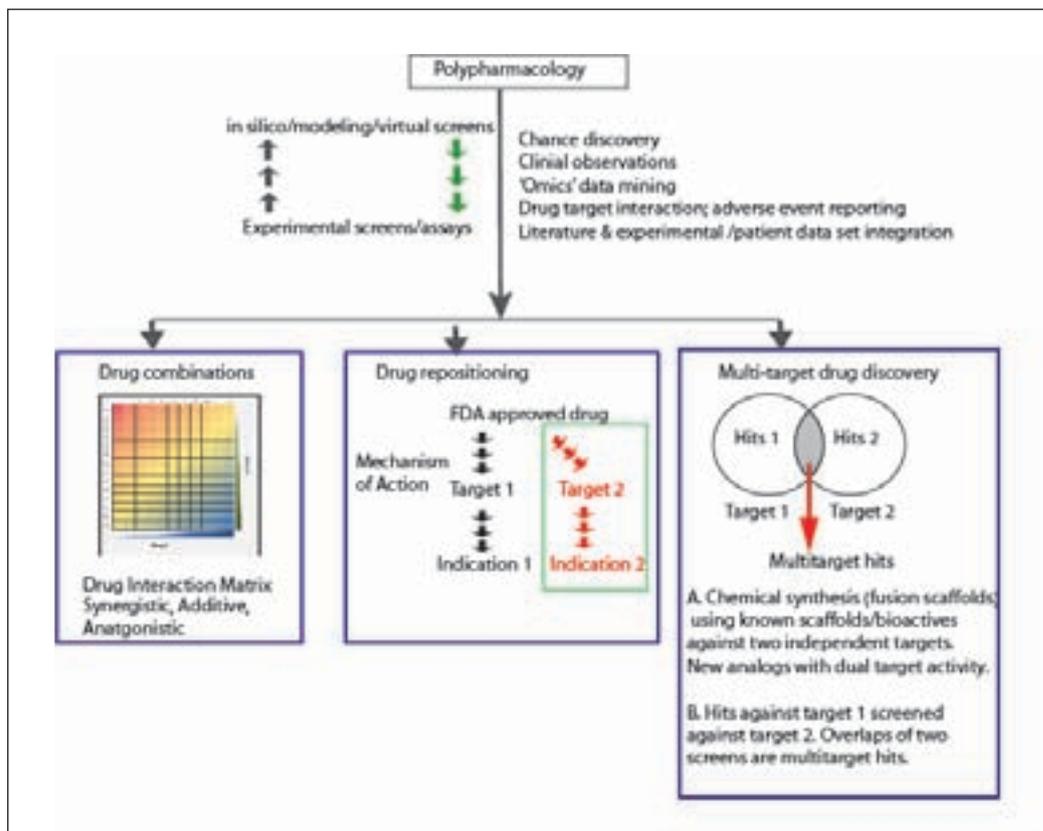
Relevant animal models

There is a growing awareness of the limitations of the true translation potential of clinical research from mouse models; a case in point being that the average rate of successful translation from animal models to clinical cancer trials is less than 8%. Differences in genetics and physiology of mouse and humans add to issues in translational relevance. Cancer models involving mouse subcutaneous xenografts may or may not translate well in later stages of drug discovery. Mouse xenograft models from primary tumour-derived patient cells with distinct driver mutations are often used to validate the clinical translatability of drug candidates, but are inadequate models for tumour initiation and progression. Several potential alternatives include using CRISPR-Cas technology to modify the mouse genome specifically and use of large animal models (rabbits, dogs, pigs, goats, sheep and non-human primates) to closely replicate the human physiology and disease.

Exploiting pan-omics

Cumulative knowledge acquired in recent years has led to an increased emphasis on designing treatments that are more personalised, or clustered into subtypes based on information collated from all sources and databases. The last decade witnessed an explosion in data sets for metabolomics, transcriptomics, genomics, epigenomics and proteomics etc. The high-throughput 'omics' technologies applied to normal and diseased states have generated large volumes of distinct types of omics data. Each type of data has its own properties and requires specific analysis methods and tools. The emerging paradigm for drug discovery underscores the value of integrating the multi-omics data to build a complete model of the processes in biological systems since signalling network cross-talks, functional biological redundancies and protein interactomes clearly influence the drug activity and safety profiles. A more holistic approach, designing drug discovery protocols that integrate the knowledge from traditional medicine to mining omics datasets and designing smarter clinical trials, is certain to improve the drug discovery process by providing new and first-in-class drugs as well as in expanding the repertoire of global diseases targeted.

Genomics

Advances in next-generation sequencing (NGS) platforms have led to exponential increase in sequencing data from whole genomes (WGS),

DNA coding exomes (WES), DNA variants (SNP, single nucleotide polymorphisms, deletions, insertions), DNS segments interacting with proteins (chromatin immunoprecipitation, Chl-IP), mRNAs (transcriptome), long non-coding RNA (lncRNA) and circular RNA (circRNA). Genome-wide association studies (GWAS) have the potential to identify genetic variations, especially the single nucleotide polymorphisms, associated with a disease. Data on DNA variations is now available for Crohn's disease, Type 2 diabetes, prostate cancer, age-related macular degeneration, obesity and 50 other human diseases. The potential of establishing associations between disease and genetic variations can ultimately influence diagnostics and disease management.

Pharmacogenomics

Precision medicine can improve efficacy scores of FDA-approved drugs or new compounds in clinical trials by integrating information from pharmacogenomics. A genetic profile-based identification of patient population subgroups is helpful in predicting patient response to drug dosage, mechanism of action, minimising drug-related toxicity and adverse events. Pharmacogenomics data provides information on genetic marker-driven effects on drug response, refractiveness of drug treatments, as well as functional effects of genomic variants. The concept of pharmaco-metabolomics-aided pharmacogenomics helps combine the roles of environment and gut microbiome interactions in interpretation of especially immune mediated disorders.

Epigenomics and transcriptomics

Epigenome encompasses all epigenetic mechanisms that regulate chromatin via DNA methylation and histone modifications (acetylation, methylation, phosphorylation, sumoylation, ubiquitination and proline isomerisation). Epigenetics mediates differential gene expression, which may be development-, tissue- or cell-specific, or dysregulated in pathogenesis. Numerous technologies, old and new, encompassing high throughput DNA sequencing and chromatin immunoprecipitation (ChIP), DNA microarrays (ChIP-chip) are used to profile DNA/histone modifications. Genome sequencing studies have identified epigenome hotspots in DNA from different cells and tissues. Epigenomics data from Encyclopedia of DNA Elements (ENCODE) and The NIH Roadmap Epigenomics projects, has helped define functional DNA segments that either possess specific chromatin structures or have protein binding sites or directly code for proteins or non-

Biomarkers, challenges and opportunities

Biomarkers are functional, reliable and measurable biochemical indicators in health and disease. In recent years, biomarkers research has played a critical role in pharmaceutical R&D for creating groundbreaking therapies and companion products. Biomarkers use in such discoveries helped in the recognition, validation and therapeutic intervention in diseases that were considered insurmountable just a few decades earlier; development of breast cancer drug Herceptin is such an example. Similarly, their widespread clinical use has enabled, along with high technology, development of ultra-sensitive detection devices and new instrumentation. In the last three decades, research for ever more complex drugs and biotechnology-derived products has led to significant adaptation of ultrahigh-throughput automation, miniaturisation and data analytics with advanced computing. Coupling biomarkers research to drug discovery albeit presents a much higher complexity. Technically challenging detection modalities, needed to keep pace with emerging biology and chemistries, present immense challenges and contrasting opportunities. As most biomarkers work is carried out at the interface of basic biology, clinical and instrumentation research, transitioning the laboratory-derived information to clinical application adds another dimension to the problem. However, potential exists to leverage expertise from diverse disciplines and adapt techniques and methods to enhance productivity, reduce timelines and achieve compelling results. There is a constant need for innovation, with ever increasing cost pressures on pharmaceutical discovery in a globally challenging healthcare environment. An integrated approach from the outset thus adds a much-needed aspect to strengthening the traditional, linear drug discovery for better clinical outcomes.

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coding RNAs in normal and diseased human tissues and cells[9]. The epigenomic signature data from various sources are accessible through IHEC (International Human Epigenome Consortium); and increasingly, more comprehensive global or locus-specific genomic aberrations are being reported for many diseases including hypertension, asthma and pain. Application of epigenomics can positively impact clinical therapeutics in multifactorial diseases and improve early cancer detection, prognosis and prediction of treatment responses (pharmaco-epigenetics).

Biomarkers

Genomic, imaging, proteomic and metabolomics technologies have unravelled a plethora of novel genetic/proteins which can serve as biomarkers that can help predict disease susceptibility, disease subtypes and disease progression, as well as predict response to a drug treatment[10]. If validated reproducibly in disease, the biomarkers can be incorporated in preclinical and clinical workflows

Natural products as drug leads: to be or not to be?

Natural products (NPs), evolutionarily optimised by the living organisms for serving different biological functions, and thus, with inherent diversity and complexity, are a good source of drug leads. However, NP-based drug discovery is rather neglected by the pharmaceutical industry:

• Typical high throughput screening platforms are not readily suitable for bioactivity detection of plant extracts.

• NPs are complex structures with multiple stereocentres. Isolation and unambiguous structure elucidation are time-consuming, and labour and cost-intensive.

• Resupply of NPs is critical for hit-to-lead and preclinical studies. Total synthesis of NPs with multiple chiral centres is rather challenging.

• NPs are not necessarily 'drug-like', requiring structural modification for improved solubility and bioavailability.

• NPs are not directly patentable and need significant pharma investment to modify the structure and claim that the invention (therapeutic use) is 'significantly different' from the natural source.

The Western reductionist approach follows the 'one target-one compound' paradigm, however, such entities are not quite effective for multi-factorial diseases. The concept of polypharmacology and multi-targeted drugs has only recently begun to gain recognition. This paradigm shift has reopened the doors towards holistic medication, and turned the attention towards the herbal universe. Such 'natural' drugs have a complex chemical composition in a variety of quantities that allows synergism between the active substances. Much integrated effort encompassing metabolomics, chemical genomics, proteomics and network pharmacology is required for target identification and elucidating the mechanisms of multi-component/multi-target herbal medicines.

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to address toxicity, safety, efficacy and patient selection. The safety and efficacy of investigation drugs can be further improved by including genetic profiling of polymorphisms and drug-metabolising enzymes.

Pharmacognosy

It is a study of ethnobiology, taxonomy, sample collection, extraction, isolation and defining chemical and biological properties of naturally occurring, medicinally-active substances obtained from plants, microbes and animals, etc. Various herbal and mixed formulations containing minerals and metals have been documented historically in folk medicines. The natural products may be whole organisms, parts of a plant, animal, secondary metabolites or extracts. The rich biodiversity in terrestrial and marine organisms promises to offer novel cell permeable chemical scaffolds and novel bioactive compounds. Known bioactive natural products include drugs against cancer, bacterial, protozoan and fungal infection and inflammation. The bioactivity evaluation of natural extracts is both labour and time-intensive, requiring several types of extract preparation, reiterative bioassay guided fractionations and isolation of active scaffold (HPLC, Mass spec), target specificity and mechanism of action. In case of low active ingredient yields from a source, chromatographic fingerprinting is often performed to identify richer sources of the active compound from other related sources. The safety of publically-consumed botanicals, herbal and dietary supplements has been addressed by EU hERGscreen network (http://hergscreen.univie.ac.at/), a database for cardiotoxic risk assessment based on ligand-based human Ether-à-go-go Related Gene (hERG) channel 3D pharmacophore models.

Natural products, the phoenix

From the turn of the 20th century, natural products have been a trusted source of a vast majority of anti-cancer drugs and anti-infectious agents, and by far the richest source of novel molecular scaffolds. Some famous examples derived from natural products over the past 30 years include artemisinin (malaria), colchicine (gout), galanthamine (dementia) and paclitaxel (cancer). Curcumin is the most celebrated example and is currently in 26 clinical trials for a variety of indications. The therapeutic effects of traditional medicine or formulations have been documented in various civilisations and continue to be utilised for treatment of several disorders in many countries. Natural product discovery has largely been deprioritised due to complexities in large-scale production, either natural sources or via chemical synthesis, among others. Reverse pharmacology (clinic to laboratory) aims to expand the drug discovery approaches to include lead identification from traditional formulations and extracts. Reverse pharmacology initiates from documented clinical and experimental observations, utilises modern preclinical and clinical approaches for active principal identification and isolation, mechanism of action or target identification as well as formal safety and toxicology clinical study to identify viable leads.

Biologics revolution

Recombinant protein based therapies (monoclonal antibodies, growth factors, hormones, blood factors, enzymes, vaccines, anticoagulants, fusion proteins) have been developed for various diseases. The recombinant drugs are produced in bacteria, yeast as well as mammalian cells. Biologics that stimulate immune response against tumours such

as oncolytic viruses, antibodies and vaccines, as well as T-cell mediated therapies hold great promise for tumour immunotherapy. Biologics discovery helps to diversify a company portfolio, shortens the path to discovery and gains from restricted competition from biosimilars and generics. The increased pharma interest in biologics discovery is reflected in the 2015 FDA approval of several first-in-class antibodies, proteins and hormones[11]. Though target specific, treatment with biologics for chronic disorders are also known to result in adverse events involving immune reactions and disorders, infections and tumours. The long-term treatment with biologics (eg Humira) is also far more costly than the small molecule drugs. Unlike the generic small molecule drugs, which bring down the treatment cost to patients, the biosimilar drugs are still associated with high production costs and may not be as efficacious and safe as the original biologic.

Stem cell therapy

The hype and hope offered by stem cell therapy are undeniable. Stem cells are being used in the treatment of blood and immune disorders, skin grafts or corneal damage repair (www.eurostemcell.org). Despite high optimism in the therapeutic potential of the stem cells, the field faces numerous challenges as developing new stem cell treatments requires in-depth understanding of human cell lineages, cell markers, niche-dependent potency and processes controlling cell proliferation, differentiation and functional specialisation. Investigational drugs can be tested in stem cell models for their ability to repair damage in appropriate animal models under good laboratory practice, though the progress has been rather slow. Cellular reprogramming efforts by genetic factors to generate induced pluripotent- and lineage-specific stem cells from somatic cell types hold great promise for basic research as well as in clinical applications. A complementary, alternate approach that has been equally successful involves the use of small molecules to maintain the self-renewal potential of stem cells and thus target specific epigenetic processes, signalling pathways and the associated cellular processes. This approach is quite attractive, and holds promise for manipulating cell fate to a desired outcome.

Collaborative and open innovation

Partnerships and collaborations across pharmaceutical companies and academia can help improve the drug development process by reducing redundancy and a more judicious allocation of resources[12]. Open innovation where ideas, data, failures, reagents and tools are shared between collaborating partners can accelerate the early drug discovery research. Innovation from global collaborations will support expansion of the drug discovery landscape. Open innovation has led to a boost in the research of neglected and rare diseases. Merck opened up its data and analysis tools to enable complex model building under SAGE bionetworks (http://sagebase.org/). In another form of compound collaboration, the Eli Lilly-PD2 Initiative (https://pd2.lilly.com) seeks to test molecules and promising compounds originating from academic research in the Eli Lilly phenotypic discovery platform. The profiling data and the secondary assay information are shared with the academic researchers. Again, we reinforce that it is time for the pharmaceutical industry to adopt a community-driven 'Wikipedia' or 'Waze'-type shared-knowledge, openly accessible innovation model to harvest data and create a crowd-sourced path towards a safer and faster road to the discovery and development of life saving medicines.

Approaches for holistic drug targeting

Comprehensive disease models can be structured to define the possible role of individual genetic loci, network interactions or post-transcriptional events that may contribute to complex disease development and progression. With the advent of personalised medicine, theoretically the sequence information from a patient can be compared within the large datasets available from genome sequencing, transcriptome analysis, epigenomics, proteomics and metabolomics to create working disease models. The addition of phenome-wide association studies (PheWAS), which links diseases to genetic variants, is a good starting point for identifying new drug-disease pairs. The personalised model can be used to define a treatment regimen based on all available FDA-approved drugs, known targets or pathways. In practice, there are limitations in establishing a direct and specific causal relationship between the experimental data sets and clinical disease progression. While there is an exponential increase in computational algorithms and deep machine learning protocols for mining the databases, there is still not enough data validating the bioinformatics datasets with the animal model/clinical data.

Biological systems are complex, circuitous and labyrinthine. Diseases are even more complex. A disease may more likely be an eventual phenotypic outcome of a misguided pathway or network. Developing successful treatment strategies to

Precision medicine

The landmark Precision Medicine Initiative, announced by US President Obama in 2015, heralds an unprecedented paradigm shift in how the stakeholders – researchers, physicians, payers and patients – work together towards an individualised approach for disease treatment and prevention that takes into account individual variability in genes, environment and lifestyle for each person. It brings an unprecedented improvement in our understanding of underlying disease mechanisms and the detection and treatment options. The cohort programme's goal is to extend precision medicine to all diseases, and in its current form it is US-centric. Precision medicine is truly translational in scope and integrates all allied biomedical disciplines including global clinical trials in an individualised, patient-centric way. It does not mean, however, the discovery and development of drugs or medical devices, unique to any given patient. That would be exceedingly cost prohibitive and unrealistic to expect. The power of precision medicine is to bring forth the uniqueness of each patient for a particular disease at the molecular level and integrates the precise treatment needed from the outset. Biomarkers and diagnostics play a critical role. The convergence of panomics and systems biology are integral to link all the interacting pathways in addressing the heterogeneity of disease etiology and develop an individualised, true combination drug therapy, much like the principle behind the herbal treatments. Routine practice of precision medicine in a clinical setting for all diseases and all patients may or may not be realised in our lifetime, but it is a worthy approach and a lofty goal for all nations to embrace at least in some form and work collectively together to eradicate diseases that ravage mankind. This is the true holistic approach from 'bench to bedside'.

ward-off complex diseases requires a comprehensive understanding of systems biology to integrate all available knowledge and fill gaps as needed. Expecting single drugs to cure diseases is therefore rather simplistic and/or unrealistic.

Polypharmacology

Polypharmacology is the property, norm rather than exception, of bioactive small molecules, natural products and other chemical species to interact with multiple targets/pathways in biological systems. More than half of 'single-target' drugs were shown to interact with more than five targets. The polypharmacology of molecules has wide implications ranging from predicting harmful off-target effects to designing safe and efficacious drug combinations or repositioning drugs for new indications. Shared functional domains and binding sites across target families, binding promiscuity of minimal scaffolds/fragments of chemical species are some of the factors underlying polypharmacology. Identification of all possible interactions between a chemical compound and the biological targets is a challenging task and predictions usually employ integrated approaches utilising bioinformatics mining of 'omics' datasets, molecular docking using x-

ray crystal structures or models, ligand-based quantitative structure activity relationship (QSAR) similarity prediction of two- or three dimensional fingerprints of small molecules, binding pocket similarity of proteins. The predictive power of virtual methods has limited utility unless validated by experimental science in physiologically relevant systems where for instance, the protein domains are not inflexible and in binding sites/folds change with conditions. Polypharmacology-driven drug discovery has the potential for selecting molecules that have higher efficacy, lower toxicity and less potential for synergistic effects compared with multiple prescriptions administered simultaneously. Polypharmacology that does not involve toxic promiscuity of drugs is predicted to be beneficial for the treatment of complex diseases and is of great value in multi-target drug discovery. The drugs with adverse side-effects should be deprioritised early in drug discovery while identifying those exhibiting potentiation of therapeutic activity by hitting multiple targets followed by characterisation of pharmacological profiles.

Drug repositioning

Drug repositioning or repurposing is one of several endpoints of polypharmacological concept that involves identification of new indications for FDA-approved drugs. Developing an approved drug for novel indication is both time and cost-effective as the safety, toxicity profiles, formulations and pharmacology of marketed drugs are already established and found satisfactory by the regulatory agencies including the FDA. Drug repositioning is a financially viable approach that can help resurrect not only the bioactive compounds that previously failed clinical endpoints but could help revive expired patents. Approximately 9,000 approved or registered drugs have been reported to be available for repurposing projects[13]. Drug repurposing has historically been anecdotal, based on clinical observations and chance findings. The most cited example is the serendipitous repurposing of sildenafil for erectile dysfunction, a selective phosphodiesterase 5 inhibitor, originally developed as an anti-hypertensive drug. In addition to chance discovery of new uses for old drugs, other strategies for drug repurposing include experimental and *in silico*-based approaches. In the phenotypic screening approach, FDA-approved drugs are tested for activity in assays for cell viability, migration, caspase activity, physiologically-relevant muscle cell contractions, electrophysiology, etc. The activity of drugs is compared across a panel of cell lines representative of various stages of a disease or across

cells derived from various cancers. The drugs active in a selective cell or with pan-panel activity are further optimised for activity in combination screens against a set of standard of care drugs used in clinical settings. The synergistic pair is tested in animal models before it is advanced for further clinical investigation. *In silico* drug repositioning utilises bioinformatics tools to define interactions between drugs, targets, text and literature mining of public databases. The PheWAS-driven association between genetic variants and disease can also serve as a starting hypothesis for defining new drug-disease linkages. NIH lists 6,500 rare and neglected diseases (R&N) for which only 250 treatments are available (www.ncats.nih.gov/trnd). The drug repositioning approach is a low-hanging fruit that can be exploited effectively to address the low returns on investment and market-size issues of neglected and rare diseases. With the increase in information on genetics and molecular pathways as well as availability of appropriate animal models for rare and neglected diseases, the last decade has seen an increase in the number of repurposed drug approvals for R&N disorders. Examples include: mitefosine, an anti-cancer alkylphospho-choline used for topical treatment of breast cancer metastasis repurposed for the treatment of Leishmaniasis; arbaclofen, for cerebral palsy management, is recommended for GABAergic treatment of Fragile X syndrome; losartan, an anti-hypertensive drug repurposed to alleviate the symptoms in subset of population with Marfan syndrome, a connective tissue disorder which affects 1 in 5,000, and difluoromethylornithine (eflornithine, DFMO), an ornithine decarboxylase inhibitor and anti-cancer drug, is being proposed for the treatment of facial hirsuitism, neuroblastoma as well as sleeping sickness. The drug repurposing programmes are being accelerated through effective collaborations and partnerships across academia and industry. AstraZeneca, while preserving its patent rights, has provided access of its deprioritised compounds to researchers at MRC (UK) under the Mechanism of Disease Initiative programme. The NCATS (NIH) in its Therapeutic Discovery Pilot programme screens selected projects using 58 compounds from eight top pharmaceutical partnerships in an effort to reveal new mechanisms of action and potential indications. The progress in repositioning is mitigated by complex regulatory, legal, pricing and patenting issues.

Combination therapy
Prescribing combinations of drugs for disease management is a widely employed clinical practice, and combinations are known to include two or more active ingredients against the same indication or a formulation of multiple distinct active ingredients against distinct targets. The combinations could target crosstalk between pathways that are activated or repressed in disease settings. While clinical observations define combination prescriptions, experimental quantitative determination of drug combination is more complex as the net effects are mathematically grouped under additive, synergistic or antagonistic based on fractional effects at various concentrations of the two drugs[14]. The *in vitro* profiling is further complicated by translation of the effects in pharmacokinetic models where the two drugs may potentiate or reduce therapeutic efficacy via modulation of individual ADME characteristics. A large number of drug combinations curated from 140,000 clinical studies have been compiled into a Drug Combination database (DCDB; http://www.cls.zju.edu.cn/dcdb/). As with other approaches, profiling drug activity and side-effects, network crosstalk and modulation help design effective new drug combinations. A large combination study called the Comprehensive Undermining of Survival Paths or (CUSP9) was rationally designed for the treatment of recurrent glioblastoma (GBM)[15]. The drug combination includes nine FDA approved drugs that block the activity of 17 distinct targets: artesuanate (PI3 kinase, AKT, TLR2, TNF), disulfiram (ALDH), captopril (ACE, AT1, MMP); celecoxib (carbonic anhydrases, COX), aprecipitant (NK-1 receptors), auranofin (thioreductase, STAT3, cathepsin B), itraconzole (P-gp efflux pump, BCRP, Hedgehog, 5 Lipooxygease), ritonavir (AKT, mTOR, cyclin D3, proteasome) and sertraline (TCTP, AKT, mTOR). The side-effects and concentrations of individual drugs were also carefully evaluated to minimise risks of side-effects while at the same time targeting multiple effectors of the disease. The HIV/AIDS treatment with HAART (Highly Active Anti-Retroviral Therapy) effectively combines two-nucleoside reverse transcriptase inhibitors lamivudine and zidovudine in presence of a protease inhibitor[16]. This combination was based on clinical data showing that lamivudine treatment alone triggered resistance due to Met 184 Val (M184V) mutation in viral reverse transcriptase (RT) but the M184V mutant was still sensitive to zidovudine and a combination of the two RT inhibitors effectively suppressed the catalytic activity required for HIV replication. While several combination treatments are FDA approved, combination therapies add to the healthcare costs, require strict adherence to regimens and the possibility of developing new

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adverse side-effects with time. Regulatory compliance also complicates combination synergistic therapies as well as drug repurposing especially if two competing pharmaceutical sources are involved.

Multi-target drug discovery

There has been a recent shift in the drug-discovery paradigm from single target-single drug to a multi-target drug discovery approach (MTDD) for the identification of single compounds with activity against two or more targets. The MTDD approach has several advantages over combination drug therapies: (A) the pharmacokinetics and safety profiles for single multi-target drugs are easier to evaluate than predicting potential long term adverse effects developing with combination drugs, and (B) a multi-target drug is guaranteed to interact with its targets in the same tissue/cell compartment and, unlike the combination drugs, will not have adverse drug-drug interactions. Both experimental screening as well as virtual *in silico* approaches deliberately design the screens to identify compounds that are active against multiple targets of interest. Several rational-based designs, computational-based docking and virtual screening approaches are available for identifying drugs with multiple functions. Several recent examples attest to the attractiveness of this approach, including: (i) successful linking of the ABL kinase inhibitory domain of imatinib with the hydroxamic acid/benzamide (zinc binding) for anti HDAC activity to design a fusion molecule inhibiting both ABL and HDACs[17], and (ii) identification of a dual kinase/bromodomain inhibitor through virtual mining of >six million compounds in the E-molecules, wherein, of the 908 predicted EGFR inhibitors, 108 also docked to BRD4 co-crystal structures, eight experimentally inhibited the BRD4 bromodomain-activity and one that inhibited both BRD4 binding as well as EGFR kinase activity[18].

Holistic insights

Science drives technology. Invention leads to innovation. Both scenarios are inherently and fundamentally intertwined. For the betterment of humanity, it is imperative upon us, the guardians, to see that science-driven inventions ultimately lead to technology-based innovations. The era of modern drug discovery has gone through revolutionary advances, as outlined throughout this discussion, and it is time to coalesce around a broadly defined holistic approach, and to enthusiastically adopt a community-driven 'Wikipedia' or Waze'-type open innovation model to create a crowd-sourced path

towards a safer and faster road to the discovery and development of life-saving medicines.

An overall analysis of drugs identified from target-based drug discovery has revealed limitations in drug innovation and proteome targeting. Despite high R&D expenditure, there is a lack of significant improvement in therapeutic outcomes and overall patient survival. One of the main reasons for limited therapeutic efficacy of current therapies is partial understanding of the disease pathophysiology and overall deficiency in developing therapeutics targeting overlapping dysregulated pathways. Complex heterogeneous polygenic diseases require more genetics-guided, finely-tailored treatment regimens to maximise effectiveness. The panomics databases serve as valuable tools for extracting diverse underlying disease mechanisms, identifying genetic loci in disease and ultimately revealing linkages between drugs and targets that can serve as starting points for designing holistic approaches. The evolution of a highly integrated approach with minimal false positives requires quality control of data being deposited in public databases, optimisation of standard operating protocols for data-mining, integration with patient electronic medical records, supportive regulatory protocols and collaborative efforts of patient groups, academia and industry in sharing negative/positive datasets. With an increase in the number of studies identifying new and rare variants in a wide spectrum of diseases, the process of inquiry and data extraction is bound to evolve into a highly optimised science with increased deliverables. The selection of the right combination of targets for a disease of interest is critical in multi-target drug discovery and requires good understanding of target-disease associations, pathway-target-drug-disease relationships and adverse events profiling. Systematic repurposing of approved drugs has the potential to bring a transformational therapy to regional breakouts of diseases such as Zika and Ebola in quick order. Finally, drug discovery will be even more holistic if it addresses the global health crisis, enabling patient access to medicines and patient care for the treatment and management of chronic, infectious, rare and neglected diseases. With the global resources reforming health education, drug production costs, intellectual/regulatory systems, basic science and innovation and a renewed appreciation of traditional medicines as a rich source of novel scaffolds, we must embrace and empower a drug discovery paradigm that will truly be holistic[19]. The time is now for the drug discovery community to focus efforts towards the discovery and

development of multi-target-network polypharmacology drugs exalting symphonic or concert performance with occasional soloists to reignite pharmaceutical innovation.

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