Perspective on systems pharmacology

when multi-targeting is advantageous

The safety and efficacy of therapeutic drugs still requires improvement. In part this is due to the promiscuity of individual drugs, which on average can interact with an estimated 6-28 other off-target moieties. However, the advent of both systems biology and precision medicine has stimulated a rethink on the process of therapeutic drug design and polypharmacology. More recently, the definition of polypharmacology has morphed to represent therapeutic drugs that have been designed deliberately for multi-targeting that affords beneficial effects to the patient. This emerging effort has been labelled 'Systems Pharmacology' and the products are referred to as multi-target or systems pharmacology drugs.

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The current Drug Discovery and Development (DDD) paradigm was conceived in the early 1960s and has remained relatively unchanged over the past ~60 years. We, and others, have argued that this continues to be a risk-laden, slow, costly and inefficient process, as well as delivering products of questionable value in terms of safety/toxicity and efficacy[1-5]. For example, significant cumulative risk is associated with any effort to bring a candidate drug to market. The initial screening of compound libraries (10^4-10^6 candidates), leads to a single lead compound that has only an ~8% chance of successfully traversing the clinical trials gauntlet[6]. In addition, the failure rate of a drug candidate at each clinical trial phase is reported to be 46% (Phase I), 66% (Phase II) and 30% (Phase III)[4]. The average time required from drug discovery to product launch remains an eye-watering 12-15 years[5]. In addition, the total capitalised cost of bringing a new drug to market was recently estimated at a staggering $2.87 billion[7].

The metrics associated with the DDD process are clearly problematic. There is also a concern about the safety and efficacy value proposition of current marketed therapeutic drug products produced by the current DDD process. In part this is due to:

i) Drug safety: Not all approved drugs stand the test of market pressures due to the scrutiny of pharmacovigiliance and post-market surveillance. In some cases approved drugs can be removed from the market because they manifest safety, effectiveness or economic problems. For example from 1994-2015, the USA Food and Drug Administration (FDA) issued 215 'Withdrawal of Application' notices[8]. During that same time period the FDA actually recalled 26 drugs from the US market predicated primarily on safety concerns[9].

ii) Drug effectiveness: There is now a significant body of evidence that indicates individual patients

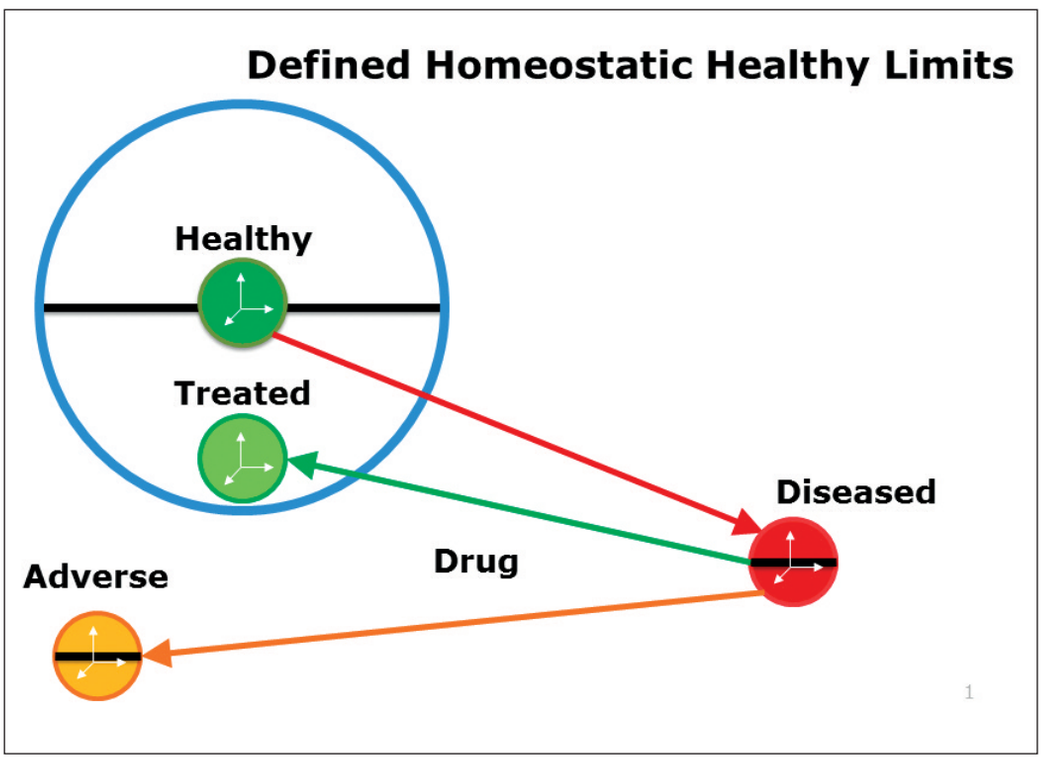

Figure 1
Schematic representation of therapeutic drug effect on individual homeostasis. In the one drug-one target model the health of an individual was determined, in many cases, by a single diagnostic indicator such as blood glucose levels in diabetic patients. A patient was determined to be healthy if the diagnostic value was within a range limit (Healthy – black line represents the range). As disease onset and progression occurred the individual's body was determined to be out of homeostasis (Diseased). However, disease severity was still determined by one diagnostic marker. Application of a therapeutic drug, which theoretically only interacted with a single target, should move the individual back into homeostasis. If the drug interacted with an 'off-target' then the condition of the patient could deteriorate (Adverse). In the case of a one drug-multi-target-pathway/network drug of systems pharmacology, the fundamental homeostatic model still applies, however instead of a one-dimensional diagnostic range and limited efficacy effect, there is a more comprehensive data rich definition of a homeostatic 'circle' Such an approach leads to a more efficacious treatment and minimisation of toxicity, safety and side-effects

diagnosed with the same disease indication respond differently to the same therapeutic drug[10]. For example, Spears and co-workers analysed the effectiveness of a number of different drug classes against major disease indications[11]. They found that most drugs were ~30-75% effective as determined by patient responses. The lowest responders were oncology patients treated with conventional cancer chemotherapy agents (25% of patients responded positively). In contrast, the highest percentage of patient responders resulted from treatment with Cox-2-inhibitors (80%). Therapeutic drugs were reported to be ineffective for Alzheimer's (70%), arthritis (50%), diabetes (43%) and asthma (40%) patients[11].

We have argued in the past that the 'Blockbuster Model' has inadvertently led to the 'wagon-of-woe' for the DDD process[12]. This approach utilised the 'one drug-one target' model that was relatively effective in large, poorly-defined, heterogeneous patient populations. We have suggested the integrated use of more efficient technologies[13], decision-making tools[13], systems biology[14,15] and personalised/precision medicine[16-18] in order to overcome the limitations of such a model. We have presented the concept of systems biology-personalised/precision medicine approaches for the production of more effective and safe therapeutic drugs[17], particularly in the treatment of Alzheimer's disease[19]. In addition, we have suggested that the development of precision medicine drugs[2], as well as an integrated technology platform-DDD approach[3] may also facilitate improved drug pipeline products. In this manuscript we discuss drug polypharmacology, which has usually been equated with therapeutic drug-target promiscuity, resulting in safety/toxicological side-effects and reduced efficacy[20,21]. More recently, the term has been used to describe the benefits of a single drug affecting change at multiple targets (one drug-multi-targets) for more efficacious impact of the drug predicated on systems biology and precision medicine considerations[22]. All this is considered and discussed as well as the emerging paradigm of systems pharmacology[23].

Human complexity, systems biology and precision medicine

The original 'one-drug-one target' model was predicated on a rather simplistic perspective of human anatomy and physiology. The health of an individual was determined by a number of diagnostic markers such as blood glucose levels. When the concentration levels of such a marker changed beyond a certain defined clinical range then the patient was diagnosed with a specific disease indication (see **Figure 1** for a schematic representation

of this process). Administration of a single drug that modulated a specific target changed the concentration of the diagnostic marker back to a normal range value, reverting the individual's pathobiological state to healthy status. However, with the advent of systems biology and a new paradigm driven by precision medicine, a greater understanding of human complexity emerged, concomitant with a re-evaluation of the 'one drug-one target' paradigm[17].

Human complexity and variability

In the past, our understanding and appreciation of human complexity and variability at the cellular, individual and population level has constantly been constrained by lack of adequate analytical, bioinformatic and knowledge management technologies. In addition our comprehension of the dynamic nature of human metabolism and physiology as a function of time was also extremely limited. Furthermore, diagnosis, prognosis and treatment decisions had been driven by a reductionist approach, which led to the development of relatively simple physiological models, as well as a rudimentary and incomplete understanding of complex biological processes occurring in individuals. This has all resulted in a limited ability to make unambiguous and decisive decisions about optimal therapeutic drug treatments for individual patients[17].

It is salutary to consider the dynamic complexity and variability of an individual human patient[17,18]. For example, at the cellular level a single human cell is made up of ~100 trillion water molecules, ~20 billion proteins, ~850 billion fat molecules, ~5 trillion sugars and amino acids, ~1.5 trillion inorganic moieties, ~50 million RNA molecules and 2 meters of DNA within 23 pairs of chromosomes. We estimate, based on the energy requirements of individual cells in the form of adenosine triphosphate (ATP) turnover, there are ~860 billion chemical reactions/interactions performed per day in a single cell! At the individual level, a single human being consists of ~37.2 trillion cells, made up of 210 different cell types, and 78 organs/organ systems. In addition, each one of us hosts ~100-300 trillion microbes, composed of ~10,000 different species that constitute 1-3% of our body weight and contain an estimated 8 million protein-coding genes. These micro-organisms play an intimate and interwoven role in the health and pathobiology of the human host. The molecular machinery of the human body comprises ~19,000 coding genes, ~20,000 gene-coded proteins and 250,000-1 million splice variants and post-translationally modified proteins, more than 100 million antibodies and ~40,000 metabolites. The combined length of DNA in an individual is calculated at approximately 2 x 10^{13} meters, which is the equivalent of 70 round trips between the earth and the sun. We estimate also that the total number of chemical reactions/interactions occurring in a single individual is ~3.2 x 10^{25} per day! This exceedingly large number is actually greater than the number of grains of sand estimated to be present on the entire planet, which has been calculated at 7.5 x 10^{18} grains[18].

A further layer of complexity is that an individual human is obviously not a closed system. On a daily basis each one of us requires inputs as well as outputs. For example, we consume on average ~1.27kg/day of food and drink (if you are following current healthy living advice) ~6-8 litres/day of fluid. It is also thought-provoking to consider that more than 25,000 bioactive food and beverage components have been identified. At any one time in the consumption of a normal meal, an individual may consume several thousand individual bioactive chemicals. In addition we plaster on to our bodies ~100-500 cosmetic ingredients on a daily basis. In terms of output, we lose six litres of fluid/day via urination, which contains ~3,000 active chemical constituents. We also remove on average ~350-500g of solid waste products through defecation on a daily basis and up to six litres of sweat depending on physical exertion. All of this activity is mediated by a transport system consisting of ~100,000 kilometers of arteries, veins and capillaries moving approximately five litres of blood and lymph fluid throughout the body. It is interesting to put all this into context and consider that a modern miracle of technology, the beloved Boeing 747 aircraft, has only six million parts and a mere 285km of wiring or tubing. Is it reasonable to wonder aloud why we struggle with accurate prognosis, diagnosis, and treatment or indeed as to why we actually function at all[18]?

We have previously discussed that it is possible to quantify human complexity[17], but in the case of human variability we are confounded by the range and subtlety of these differences. Such traits can be transitory or permanent and influenced in complex ways by both genetic and/or environmental factors. Sources of human variability include gene mutation (germ-line and somatic), allelic differences, genetic drift, social and cultural influences and nutrition. Common human variations include obvious visible differences such as gender, age and physical appearance. These differences are determined through poorly-understood molecular processes. Such processes are modulated by a wide

Table 1: Comparison of sub-systems in humans. Analysis of the distance (in metres) between individual constituents of each sub-system as well as the average timescale that interactions and reactions occur

SUB-SYSTEM TYPE (HUMAN)	DISTANCE (METRES)	AVERAGE TIMESCALE (SECONDS)
Molecular	~$2x10^{-8}$ - ~$5x10^{-10}$	10^{-6}
Pathway/Network	~$1x10^{-6}$ - ~$3x10^{-8}$	10^{2}
Cell	~$1x10^{-4}$ - ~$8x10^{-6}$	10^{4}
Tissue	~$2x10^{-2}$ - ~$9x10^{-4}$	10^{5}
Organism	~$6x10^{-1}$ - ~(1-2)	10^{6} - 10^{8}

variety of molecular entities and processes that include, but are not restricted to, single nucleotide polymorphisms (SNPs), alternative genesplicing and protein isoforms (eg cytochrome P-450 super family) and epigenetic phenomena. Our basic understanding of these processes have led to the creation of simple semantic descriptors which define such differences and include concepts such as gender, age differentiation (child versus adult) and race. However, such coarse descriptions do not provide adequate insight into the significant and subtle differences that separate us at the molecular level, given that ALL humans are 99.9% genetically the same at the DNA level[18].

Finally, the temporal effect on complexity and variability is an even poorer-understood process. Paradoxically, age is the most obvious manifestation of physical change in the individual. We can all recognise the phenotypical differences between infants versus a young girl/boy versus an elderly woman/man. Also it is 'well-known' that we lose bone density, shrink and our metabolism slows down. However, our understanding of individual or population changes at the molecular and cellular levels is still in its infancy. In the past, our understanding of this staggering, dynamic complexity and variability has been myopic and limited. Hence, how can we produce safe and efficacious therapeutic drugs for individual patients[18]?

Systems biology

The emergence of systems biology (also known as pathway, network or integrative biology) was predicted on an attempt to address and embrace human complexity and variability in human metabolism, physiology and pathobiology. The development of systems biology is still in its nascent ascendency. In its first generational incarnation (1940s-50s), a systems approach to biology was predicated on theoretical considerations of complex systems analyses. Second generation systems biology (late 1990s-early 2000s) has its roots in high throughput analytical omic measurements, bioinformatics, bioengineering, computational sciences and mathematics. It is an attempt to establish a more integrated and hierarchical paradigm that facilitates the creation of new biological pathways and networks at the molecular and cellular level[15]. This provides a framework for understanding the holistic system of genetic, genomic, transcriptomic, protein, metabolite and cellular events that are in constant flux and interdependent. In order to facilitate such efforts, two distinct approaches have evolved, namely computational modelling-based systems biology and data-driven systems biology. The former relies primarily on computational modelling and simulation tools. While there has been some confusion in the past about terminology it is also now referred to 'bottom-up' systems biology. The latter approach predominantly utilises analytical datasets that are mined in a discovery manner for new knowledge using a variety of bioinformatics and knowledge assembly tools and is now categorised as 'top-down' systems biology[17].

Implementation of Systems biology in DDD

We opined back in 2004 that systems biology could "…provide a new dynamic to invigorate pharmaceutical companies… predicated on a more complete understanding of problems associated with the DDD process"[15]. A consensus emerged that systems biology had the potential to impact the entire DDD process by identifying biological subsystems and how they interact to produce complex molecular, pathway/network, cellular, tissue and organism behaviour[23,24]. Such claims appear extraordinary when you consider the complexity and variability of both individuals as well as differ-

ent human sub-populations (see section above). In addition the difference in molecular, pathway/network, cellular, tissue and organism reaction timeframes and proximal distances is dramatic (see **Table 1**) and attempts to integrate all such processes appears exceedingly difficult. However, in spite of these daunting obstacles there has been a great deal of activity in the application of systems biology to the DDD process, and a number of books have been written on the subject[25]. But, to date, the effect of systems biology on DDD has been somewhat underwhelming because of interpretation and utilisation difficulties with the data and information obtained on specific biological sub-system or system perturbations.

Due to the aforementioned limitations, systems biology has only provided notable insights into: i) drug-target networks; ii) predictions of drug-target interactions; iii) adverse drug effects of drugs; iv) drug repositioning; and v) predictions of drug combination[25,26]. There are continued efforts to broaden the applicability of systems biology to the DDD process. For example, recently we and others have suggested a systems biology approach to provide an understanding of causal onset, progression and effective treatment of any disease, including complex disease states such as Type II diabetes[27] and Alzheimer's Disease[19,28]. We have proposed the following broad-based systems biology approach to drug discovery[19]:

i) Network biology discovery: Multi-omic analysis at the gene, protein and metabolite level.

ii) Identification of potential targets: The network biology analysis should provide a prioritised list of target genes and/or proteins.

iii) Functional validation: Utilisation of RNAi screens to either overexpress or knock down each of the selected genes in the system under investigation.

iv) Drug candidate screen: Selected, prioritised molecular targets that are expressed in the tissue or organ under investigation.

v) Target selection evaluation: Any target must be expressed in the pathobiological tissue or organ and causal onset, progression and dynamic (temporal) elements must be demonstrated.

Clearly, there is much to do before systems biology can adequately demonstrate its routine and practical usefulness in DDD, but the trends discussed here, albeit briefly, provide encouragement for the near future. In comparison, systems biology has had a much greater impact on the evolution of precision medicine over the past decade[17-19,27].

Precision medicine

We described recently, in some detail, the advent of precision medicine drugs[2]. The development of such therapeutic agents is a continuing and realistic attempt to improve the efficacy of therapeutic drugs by treating targeted patient sub-populations. The term 'precision medicine' was first coined by Clayton Christensen in his book the *Innovator's Prescription* published in 2009[29]. However, the descriptor 'precision medicine' did not gain wide acceptance and usage until a report entitled 'Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease' was published by the US National Research Council (NRC) in 2011[30]. The report stated: "Precision medicine is the tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient, but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology and/or prognosis of those diseases they may develop, or in their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side-effects for those who will not"[30]. This approach utilises individuals and defined (sub)-population-based cohorts that have a common network of disease taxonomy. In addition, it requires an integrated molecular and clinical profile of both the individual as well as the sub-population-based cohort. We have argued that precision medicine uses a '1-in-N' model (in contrast to the 'N-of-1' personalised medicine model)[18]. This is predicated on widely-used biostatistical data analysis and 'big data' analytical tools, and forms the basis of precision medicine drug development[2].

Precision medicine drugs

Precision medicine drugs are defined as "those therapeutic products for which the label includes reference to specific biological marker(s), identified by diagnostic tools, that help guide decisions and/or procedures for their use in individual patients"[2]. It is important to note that the physician utilises the biological biomarker(s) listed on the drug label in prescribing the precision medicine drug. Last year the Center for Drug Evaluation and Research (CDER) at the FDA approved a record high 59 new drugs. However,

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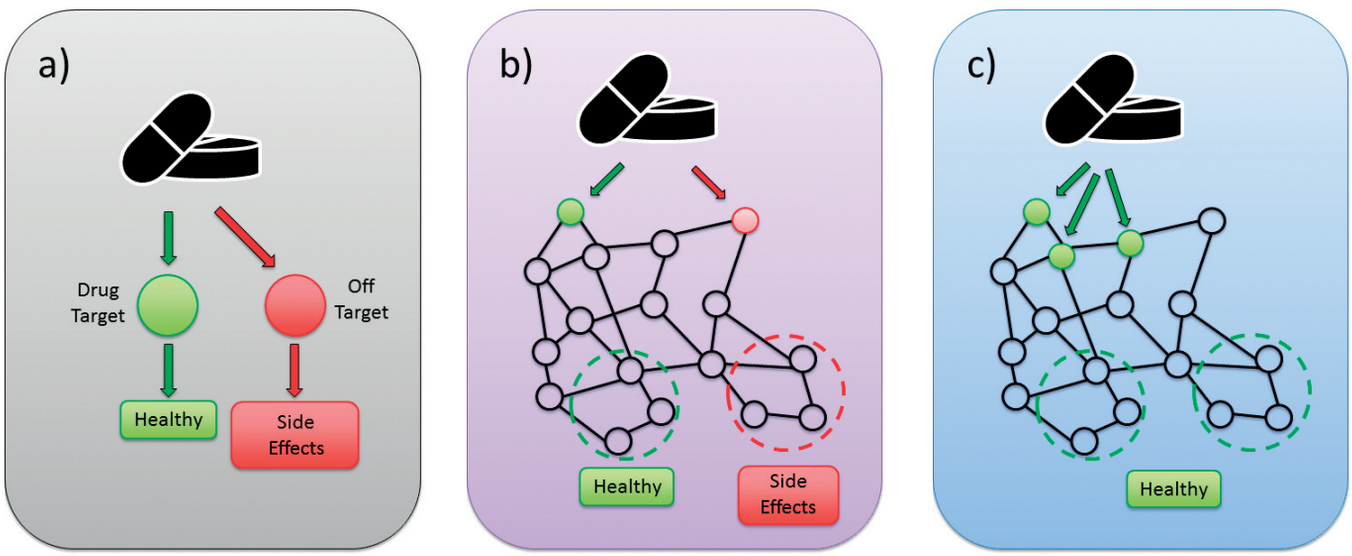

Figure 2: Comparison of the classic v systems biology v systems pharmacology approach. a) Representation of the one drug-one target model. b) Representation of a systems biology perspective of therapeutic drug effects . c) Representation of the one drug-multi-target-pathway/network approach of systems pharmacology. *Note that the figure was adapted and modified from the work of Mikel Elorriaga (https://www.slideshare.net/mikeltxopiteaelorria/advanced-systems-biology-methods-in-drug-discovery)*

24 (40%) of them were classified as 'molecularly targeted' representing a new annual record for precision medicine drugs approved[31]. This continues the trend from 2017 where of the 46 new drugs approved 16 of them (~35%) were classified as precision medicine drugs2. The use of precision medicine drugs is another example being employed by the pharmaceutical sector to improve the quality of therapeutic drugs provided to individual patients. These advances are predicated on the advent of precision medicine and its focus on the grouping and identification of sub-populations (1-in-N model). In turn, precision medicine was conceptualised from our more refined and detailed understanding of human pathobiology and pathophysiology brought about by the development of systems biology tools, technologies and insights[17-19,24,27].

Systems pharmacology

The old model of 'one drug-one target' was developed on the assumption that specific drug treatments would be superior due to the absence of off-target side-effects. However, the promiscuity of drugs that bind to numerous targets is now a well-characterised phenomenon[20-22]. Recently it was estimated that promiscuity rates varied from 6-28 individual targets per therapeutic drug[21]! It is noteworthy that armed with hindsight, most Blockbuster Drugs (one drug-one target) are pleiotrophic in nature. In other words the drug has both numerous beneficial and adverse effects on the patient. A well-known example of such a drug is aspirin. The drug manifests effects in the treatment of inflammation, pain, fever, assorted cancers, stroke and cardiovascular disease, but is also associated with gastritis and bleeding. Aspirin was recently reported to have an estimated 23 different putative targets, possibly explaining the pleiotrophic properties of the drug[32]. A 21st-century example of another widely-used pleiotrophic drug(s) is the statin family members. In both cases the one drug-one target model was used originally in the development of such therapies, without the insights of drug promiscuity.

Definitions

The growing body of evidence from drug promiscuity and systems biology studies indicated that the 'magic bullet', one drug-one target model was simplistic. In part, this is due to the compensatory mechanisms and redundant functions present in biological systems, facilitating a resilience to single-target drug perturbations. Currently, it is understood that many drugs bind to multiple targets that in turn participate in, and are associated with, multiple biological processes. This property is referred to as drug polypharmacology[22]. The term was applied originally to off-target adverse effects of the drug. More recently, the definition has morphed to reflect the exploitation of these characteristics in a more beneficial manner. Polypharmacology is now

defined as the design or use of a drug to either: i) simultaneously interact with multiple functionally-related targets that act together, or ii) inhibit targets that differ functionally from the primary target of the drug to produce additional advantageous effects for the individual patient[22].

Our understanding of human complexity delineated by system biology studies combined with recognition of drug promiscuity and polypharmacology led to the advent of 'systems pharmacology'. This latter approach requires a comprehensive molecular/cellular profile of the healthy individual/population (systems biological profile), the diseased individual/population (systems pathobiological profile) and the corresponding response of the individual/population to a drug (systems pharmacological profile). The resultant comparative datasets from systems biology versus systems pathobiology analyses and the corresponding drug effects profile constitutes a systems pharmacology approach. In addition the differences between these various states of the patient/population are referred to as 'systems response profiles'[23]. A schematic representation is captured in **Figure 1**. As can be seen the fundamental principles for the one-drug-one target model are essentially the same as a systems pharmacology approach. However at the systems level a multitude of targets, biomarkers and diagnostic components are all considered. The resultant effect is a more comprehensively-defined health/disease/adverse framework of the individual /population as well as a safer, more efficacious drug.

Systems pharmacology is predicated on the polypharmacology of therapeutic drugs. So what is the difference between the two terms? They are both used as descriptors to describe efforts to overcome the one drug-one target model. Polypharmacology refers to a one drug-multi-target concept as utilised in drug repurposing[1]. However, systems pharmacology refers to a broader concept best captured as one drug-multi targets associated with pathways and networks. Thus systems pharmacology is based on the rational design of drug therapies using information based on molecular, cellular and physiological complexity. This type of approach produces systems pharmacology drugs rooted in molecular interactions between a single drug and multi-targets present in defined pathways/networks.

Combination therapy versus systems pharmacology drugs

We have discussed above the limitations of the one drug-one target compound that includes safety/toxicity and side-effect issues as well as limited efficacy. In the case of multi drug-multi-targets (ie combination therapy) such an approach is designed to elicit synergistic effects brought about by different mechanisms of action by more than a single drug. In this context, systems pharmacology is a one drug-multi-target model combined with pathway/network analysis that should significantly enhance efficacy and reduce safety/toxicity effects. Anighoro et al have suggested that while there is "…highly significant therapeutic relevance of combination therapies, [there are] potential advantages of a targeted therapy based on a single drug that modulates the activity of multiple targets over single-targeted or combination therapy"[33]. Some of the advantages of systems pharmacology drugs over combination therapies include:

i A single systems pharmacology drug with multi-target activity may have a more predictable, therefore superior pharmacokinetic (PK) profile compared to a number of individual drugs administered in combination.
ii Acute toxicity may be enhanced in more non-selective combination therapies.
iii Adverse synergistic effects may be more pronounced in combination therapies.
iv The probability of developing target-based resistance to multi-target drugs is statistically lower than is the probability of developing resistance against single-target drugs.
v The administration of a single systems pharmacology drug results in a more consistent and predictable ADME profile.
vi Drug-drug interactions do not exist in a systems pharmacology regime.
vii A single agent binding to multiple targets might be easier to develop given that the regulatory requirements showing safety/efficacy of a drug combination.

For all these reasons, the development of single drugs with a desired multi-target profile defined by pathway/network analysis do offer a compelling, cost-effective alternative to drug combinations. Historically, drug combination therapy has been more extensively explored in the clinic than the use of single multi-target drugs. However, both approaches are likely to yield single drugs or drug combinations with improved safety and efficacy profiles. In either case, key to success will likely be the informed selection of suitable biological targets and molecular pathways/networks that need to be modulated with drug molecule(s)[33].

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Table 2: Select examples of FDA-approved commercially-available systems pharmacology drugs

DISEASE INDICATION	SYSTEMS PHARMACOLOGY DRUG	MULTI-TARGETS	REFS
CNS-Neurological			
Schizophrenia, Adjuvent-	Brexpiprazole (Rexulti)	$D_{2,3}$: 5H-$T_{1A, 2A, 2B, 7}$: $Alpha_{1A, 1B, 1D, 2C}$	35
Parkinson's Disease	Safinamde (Xadago)	MAO-B, Dopamine-RI, Blocks Na^+/Ca^{2+} Channels	35
Bipolar Mania	Cariprazine (Vraylar)	$D_{2C, 2S, 3}$, 5H-$T_{1A, 2A, 2B, 2C, 7}$: $Alpha_{1A}$: H_1 : mACh	35
Acute Mania	Asenapine (Saphris)	D_{1-4} : 5H-$T_{1A, 1B, 2A-C, 5A, 6, 7}$: $Alpha_{2A-C}$: $H_{1,2}$	34
Oncology			
Thyroid Cancer	Lenvatinib (Lenvima)	$VEGFR_{1, 2, 3}$ Kinases	35
Breast Cancer- Her2(+)	Neratinib (Nerlynx)	Her2, EGFR Kinases	35
Breast Cancer	Ribociclib (Kisqall)	Cyclin D1 : $CDK_{4, 6}$	35
AML- FLT3(+)	Midostaurin (Rydapt)	FLT3-mut : c-kit : PDGFR : src : VEGFR	35
Cardiovascular			
Cardiac Arrhythmia	Dronedarone (Mutaq)	Na^+ & K^+, L-type Ca^{2+} channels	37
Infection			
Hepatitis B, Adjuvent-HIV	Tenofovir (Viread)	Viral Reverse Transcriptase, DNA and Mitochondrial DNA Polymerase	38

Implementation of systems pharmacology

Talevi has discussed systems pharmacology drugs in the context of the substrate-enzyme 'lock and key' model. He has suggested that a multi-target drug could be conceived as a "skeleton or master key capable of [simultaneously] unlocking several locks". However, he opined subsequently that such a model was simplistic and did not adequately describe the effect of a drug on multi-targets over a prolonged period of time due to significant up-and-down regulation of gene signatures[34]. A detailed comparative systems analysis (ie the systems response profile consisting of healthy versus pathobiological versus drug effect systems biology analyses) is required to produce systems pharmacologic drugs[23], but how to efficiently go about both rationale target selection and drug candidate design in such a complex system? Recently, Ramsey and colleagues discussed these issues and made the following salient points presented below[35]:

i) Target selection: It is necessary to have a comprehensive understanding of target-disease associations, pathway-target-drug-disease relationships, as well as adverse events profiling capability. In addition it should be understood that target selection is either additive (targets on the same pathway) or synergistic (targets on functionally complementary pathways) in nature.

ii) Drug candidate design/selection: The typical approach is to integrate a different combination of pharmacophores (a molecular recognition feature of a substrate/ligand necessary to bind to a biological macromolecule such as a target protein) into the systems pharmacology drug candidate. The design of the candidate drug is typically defined by the nature of the targets, the availability of starting frameworks and the chemical tractability. Beyond that, the essential requirement of multi-target compounds is that each pharmacophore retains the ability to interact with its specific target.

These challenging tasks involve consideration of structure-activity relationships that determine

substrate-target interactions. In addition, establishing the same degree of affinity for each target and minimising off-target, adverse effects are critical factors to be addressed. Ramsey has argued that "...the rational design of multi-target compounds is far from being an easy task, dealing with the crucial issues of selecting the right target combination, achieving a balanced activity towards them and excluding activity at the undesired target(s), while at the same time retaining drug-like properties"[35]. In order to achieve such a difficult task a variety of approaches have been employed and include[22,33-35]:

i) **Computational ligand discovery:** A variety of tools are used including chemoinformatics, virtual screening, pharmacophore development, molecular docking and dynamics studies.

ii) **Quantitative Structure Activity Relationships (QSAR):** Used in lead optimisation to correlate molecular structure with pharmacological and biological activity.

iii) **Biological validation of leads:** Use of high throughput compound assessment and identification of pharmacophores.

iv) **Receptor biology and binding affinities:** Screening compounds against specific targets.

v) **Cell-based assays:** Used as a preliminary screening tool.

A number of the tools and technologies used in conventional DDD are also used in systems pharmacology drug discovery. However, the advent of the systems biology toolbox facilitated the construction of pathways/networks to afford the one drug-multi-target-pathway/network paradigm necessary for the creation of systems pharmacology drugs.

Systems pharmacology approaches

Two different brief descriptions of specific target selection and drug candidate design/selection examples will serve to highlight the approaches used in the implementation of systems pharmacology. In the former case the identification of individual targets and synergistic combinations of targets is critical to the systems pharmacology discovery process. Most target proteins consist of more than one structural domain, but there is a "limited repertoire of domain types". Since protein domains mediate drug-protein interactions, Moya-Garcia and colleagues used this principle to guide the design of systems pharmacology drugs[22]. In their study they associated multi-target drugs with CATH functional families. (CATH is a database of 95 million protein domains classified into 6119 super families). They concluded that a small fraction of a specific functional family of proteins (CATH-FunFams) were druggable and showed using structural analyses that the domains in these families have the potential to be the druggable entities within drug targets[22].

In the second example Thiel and co-workers outlined a detailed quantitative systems pharmacology (QSP) approach to determine systems pharmacology drug properties through "a mechanistic consideration of processes underlying drug absorption, distribution, metabolism and excretion (ADME) as well as the resulting drug action itself", as well as "a detailed description of drug pharmacokinetics (PK) and, simultaneously, drug pharmacodynamics (PD)"[36]. In this study, a QSP approach was applied to quantify the drug efficacy of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) inhibitors by coupling physiologically-based PK models, at the whole-body level, with affected biological networks at the cellular scale. They presented results that revealed insights about drug-induced modulation of cellular networks in a whole-body context, thereby describing complex pharmacokinetic/pharmacodynamic behaviour of COX-2 and 5-LOX inhibitors in therapeutic situations. The results demonstrated the clinical benefit of using QSP to predict drug efficacy and, hence, its value in helping to design optimal systems pharmacology drugs[36].

Systems pharmacology drugs

The concept of systems pharmacology drugs was first suggested in 2000. In the intervening years the academic and pharmaceutical communities have enthusiastically developed this paradigm and now a significant number of such drugs are available on the market today. Lin and colleagues noted that from 2000-15 the FDA approved a total of 361 New Molecular Entity (NME) drugs. They estimated that ~43% of those approved drugs had two or more targets, although to be clear such entities were not necessarily designed as systems pharmacology drugs[37]. Subsequently, Ramsey and colleagues did a similar study (2015-17) and noted that of the 101 NME drugs approved by the FDA, 34% were single target drugs, 21% were multi-target drugs and 10% were combination therapies. They concluded that these numbers "...unequivocally supports the attractiveness..." of systems pharmacology drug strategies[35].

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The systems pharmacology drugs approved by the FDA between 2015-17 were predominantly anti-neoplastic agents constituting 12 of the 21 (57%) approved NMEs. An additional four approved systems pharmacology drugs were therapies for CNS indications, with the remaining five drugs approved for anti-infective, musculoskeletal, genitourinary and alimentary tract /metabolism disorders[35]. Systems pharmacology drugs are most relevant for disease states involving large target networks and pathways, as is the case with most cancers. Cancer cells are characterised by a transformed phenotype associated with uncontrolled proliferation and immortality. This abnormal cell activity is sustained by significant protein deregulation. For example, the protein kinase family consists of more than 500 different proteins and they are involved in multiple cellular pathways and networks. The original one drug-one target model produced highly-selective kinase inhibitors. However, it has become increasingly clear that more effective targeted therapies based on kinase inhibitors should target the disease at multiple target nodes in the network, which explains the significant efforts to create systems pharmacology-based kinase inhibitors in the treatment of several forms of cancer. A representative sample of FDA approved marketed systems pharmacology drugs in oncology are shown in **Table 2**.

We concur with Talevi that there are three more general applications where systems pharmacology drugs can be expected to impact in the future. Firstly, and as noted above, complex disease conditions such as cancer and CNS disease are a clear focus of current effort and development (**Table 2**). Another area of applicability is in drug resistance. The ability to simultaneously modulate different targets could be advantageous to individuals expressing intrinsic or induced variability in drug response due to modifications in key disease-relevant biological pathways and activation of compensatory mechanisms[34]. Apart from the obvious applications in the field of antimicrobial chemotherapy (it is less probable to develop resistance linked to single-point mutations against multi-target than single-target agents) this strategy could also be pertinent to treat non-infectious conditions characterised by high incidence of the drug resistance phenomena, eg epilepsy. One final application for consideration is in prospective Drug Repositioning. We have previously highlighted the resurgence of Drug Repurposing and Repositioning (DRPx)[1]. However, most such cases have occurred by serendipity or exploitation of the original mechanism of action, using a retrospective DRPx approach. Talevi has argued that in contrast prospective DRPx should explore drug-repositioning possibilities much earlier in the drug discovery process. This would entail the design of multi-purpose drugs to treat different indications; such as co-morbidity disorders (eg diabetes and cardiac disease).

Conclusions

The reality of the one drug-multi-target-pathway/network systems pharmacology drug appears to afford considerable advantages over the one drug-one target or combination drug-multi target approaches. However, the tremendous therapeutic potential of multi-target drugs, their rational discovery and their development still represent a formidable challenge. In addition the necessity of balancing the beneficial polypharmacology versus the harmful promiscuity of such drugs still needs to be evaluated as more of these drugs are brought to market. Nonetheless, building on the accumulated evidence, the concept of systems pharmacology drugs has made rapid and spectacular progress from being an emerging paradigm when first enunciated at the beginning of 2000 to one of the hottest topics in drug discovery and development in 2017-18. Does the merging of systems biology, precision medicine, precision medicine drugs and now systems pharmacology drugs provide the harbinger of more safe and efficacious drugs? Initial indications appear to suggest that there is indeed a new dawn breaking for patients and the products they rely on to keep them healthy and alive. DDW

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