Ana Sofia Pereira Gonçalves

Polypharmacology: Drug discovery of the next generation

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Assinatura da Aluna

(Ana Sofia Pereira Gonçalves)

Ana Gonçalves

Assinatura da Aluna

(Ana Sofia Pereira Gonçalves)

Assinatura do Tutor

(Professor Doutor Sérgio Simões)

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"*Intelligence without ambition is a bird without wings*" - *Salvador Dali*

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Abbreviations

CSM – Compound Series Matrix

DDD – Drug Discovery & Development Magazine

FDA – U.S. Food and Drug Administration

NIH – National Institute of Health

NMEs – Number of Molecular Entities

NSAIDs – Non-Steroidal Anti-inflammatory Drugs

OPEN PHACTS – Open Pharmacological Concept Triple Store Open

R&D – Research and Development

RMSD – Root-Mean-Square Deviation

RNAi – RNA interference

SAR – Structure Activity Relationship

SEA – Similarity Ensemble Approach

shRNAs – Short hairpin RNAs

TCM – Traditional Chinese Medicine

T2D-db – Type 2 Diabetes database

Abstract

The traditional drug discovery paradigm is no longer a profitable option. The idea of "one-drug, one-target" is an unreal concept when we are dealing with a biological system and, for that reason, many complex diseases no longer have any effective and safe treatment. A diseased system is like a "battlefield" where complex biological processes are perturbed at more than one point. The polypharmacology approach appeared naturally with the evolution of technology, as the key to solve the "disease" equation. However, the rational design of a multi-target drug is still in its childhood, but when it takes the first steps, our understanding of pharmacological effects in the human body will be improved and new off-targets will be discovered. The purpose of this paper is to review the main concepts underlying polypharmacology, its potential applications and the challenges it has to face to succeed in this paradigm transformation.

Keywords:

Drug discovery; polypharmacology; multi-target drug; drug repurposing; cellular network; prediction methods.

O tradicional paradigma da descoberta de fármacos já não é uma opção rentável. A ideia de "um fármaco, um alvo" é um conceito irreal quando estamos a lidar com um sistema biológico e, por essa razão, muitas doenças complexas ainda não têm nenhum tratamento efetivo e seguro. Uma doença pode ser comparada a um "campo de batalha" onde processos biológicos complexos estão alterados em mais do que um ponto. A abordagem da *polypharmacology* apareceu naturalmente com a evolução da tecnologia, como uma tentativa de resolver este problema. Apesar do *design* racional de um fármaco *multi-target* ainda estar numa fase inicial, os esforços necessários para dar os primeiros passos irão melhorar a nossa compreensão dos efeitos farmacológicos no corpo humano e irão potenciar a descoberta de novos *off-targets*. Neste trabalho irão ser revistos os principais conceitos subjacentes à *polypharmacology*, as suas potenciais aplicações e os desafios que terá de enfrentar para ser bem-sucedido nesta transformação de paradigma.

Palavras-chave:

Descoberta de fármacos; *polypharmacology;* fármaco *multi-target, drug repurposing; network* celular*;* métodos de previsão*;*

1. Introduction

During the past decade, even with scientific improvements and a significant increase of global research, new drugs have often been withdrawn from the market because of low efficacy and/or toxicity. The interaction with multiple targets is an innate property of drug molecules and unintentional drug-target interactions are responsible for causing side effects. Currently, drug design efforts are focused on screening single-target and highly specific compounds, following the old key model proposed by Ehrlich more than a century ago (FISCHER, 1894). This type of drug design is "blind" to many cellular processes and when we consider complex diseases such as cancer, metabolic diseases, cardiovascular diseases and neurological diseases that involve several aspects, fully effective treatments do not exist (CSERMELY et al., 2005; LU, 2012; MEDINA-FRANCO et al., 2013). As stated by Stewart Bates, director of biomarker discovery at GlaxoSmithKline (London) to Drug Discovery & Development Magazine (DDD), "we could be better at preventing that (…) we need to know the biology around the target" (TACHIBANA, 2014). From 2004 to 2013, the total number of new molecular entities (NMEs) approved by the U.S. Food and Drug Administration (FDA) has reached 267 NMEs, which represents an average of 27 NMEs approved per year (FDA, 2014). Considering it takes an average of 12 to 15 years (depending on the therapeutic area) and around 1 billion USD to bring a single drug into the market, this approach is not profitable (CSERMELY et al., 2013). "Business as usual" is no longer an option in the drug industry (BEGLEY et al., 2012).

Over the years, drug discovery paradigm has undergone some modifications moving from the "one target, one drug" model to a multiple-target approach termed as polypharmacology (i.e. a single drug acts on multiple targets of a unique disease pathway or on a complex disease) (REDDY et al., 2013). Lately, the term polypharmacology has appeared in several scientific publications, in a total of 162 from 2004 to 2013 (the data are from PubMed using the query of "polypharmacology" for title and abstract words; see *Figure1* in the Annexes Section). At present, it is evident that the idea of one drug acting on a single receptor is not effective as expected from the reductionism view of the lock and key model (MEDINA-FRANCO et al, 2013). The dream will be the development of a single drug that acts like a drug cocktail, however this dream is not too far from reality. By serendipitous discovery, many drugs with a multi-target profile are currently used in clinical medicine such as non-steroidal anti-inflammatory drugs (NSAIDs), salicylate, metformin, sildenafil and also traditional medical treatments (for example, Traditional Chinese Medicine

- TCM) with multi-component extracts of natural products (CSERMELY et al., 2005). In contrast, to design and predict a polypharmacology drug response, it is necessary to understand the cellular network and the impact that this has on the complete biological system (XIE et al., 2012). Consequently, polypharmacology might be a double-edged sword if the biological mechanisms are not fully understood, causing uncontrolled effects. For example, Staurosporine was excluded from use in clinical practice because it is a potent protein-kinase C inhibitor known to interact with many other kinases (REDDY at al., 2013). One of the actual challenges will be to find the "master keys" that operate a set of several locks to gain access to the desirable clinical effect and, at the same time, avoid unlocking any possible adverse effects (see *Figure 2* in Annexes' Chapter) (MEDINA-FRANCO et al., 2013).

The present work has the purpose of demystifying some premade concepts about multi-target drugs and their adverse effects, joining the key reasons that support this idea and the need for a new outlook in the drug discovery field. This paper is divided into four main sections. In the first section, the potential of a polypharmacology approach in the current clinical medicine and also in drug discovery efforts is discussed. Then, the discussion continues with what defines a multi-target drug and the main features associated with these promiscuous drugs. In the third section, the methods for predicting polypharmacology effects and the identification of new multi-target compounds will be presented. Finally, the last section addresses the future challenges that this approach has to face for its acceptance in the drug discovery community.

2. The potential of therapeutic Polypharmacology

The idea of multi-target attacks is quite old. Perhaps it started with the military strategist Carl Von Clausewitz when his complex approach proved to be an efficient antidote to Napoleon's rationally designed campaigns. He argued that instead of striving for successful single battles, strategy should simultaneously aim at "the enemy's forces, his resources and, his will to fight" (CLAUSEWITZ, 1993). A diseased system is like a battlefield where complex biological processes are perturbed at more than one point. When we are losing, it is necessary to restore the normal function in different strategic points in order to win the battle. From a cellular perspective, a drug needs to interact with different targets to restore the normal function of the cell and change the disease status. However, when a drug binds to more than one target, multiple outcomes may appear, both beneficial and harmful. Aware of the duality, polypharmacology can be divided into two types: therapeutic and adverse polypharmacology. Adverse polypharmacology has been the main obstacle for an earlier implementation of this methodology (BORAN, 2010) but now, with scientific advances in biotechnology and *in silico* technology, it is easier to predict adverse polypharmacology and produce a therapeutic polypharmacology.

The possible future applications will be discussed below. It is noteworthy that, in the end, all applications will improve our understanding of the human body, what makes this approach an interesting investment for knowledge of the human kind.

- **Drug repurposing/repositioning**: It is the primary application of polypharmacology to speed up the drug discovery process by identifying new clinical uses for already existing approved drugs. Traditionally, drug repurposing has occurred by serendipity but the benefits for a systemic drug repurposing has significant commercial value, especially when researchers work with "small" markets such as rare and neglected diseases. For pharmaceutical companies, this can be widely profitable because it extends the markets for just one drug, with the construction of a new molecular entity. On the other hand, there are a lot of available data (such as long-term toxicology studies) that can be presented to regulatory authorities and, consequently, the development of new treatments will be more time- and cost-effective than the traditional drug discovery. However, drug repurposing does not only apply to approved drugs. The drugs originally withdrawn from the markets by regulatory organizations can be resurrected, for

example, thalidomide can be used in the treatment of multiple myeloma because this disease does not usually affect women of child-bearing age. This approach can also be applied to other types of drug libraries, such as TCM database, opening the possibility to search the targets of the active components with computational approaches and to understand the success of this medicine practice (EKINS et al., 2011; MEDINA-FRANCO et al., 2013).

- **Personalized medicine**: In an ideal world, polypharmacology could be fully explored if there was readily available information linking the interaction among the entire chemical and target spaces. Nowadays, this is one of the major challenges. In the pursuit of this goal, chemogenomics has emerged to identify all possible ligands for all possible targets (MEDINA-FRANCO et al., 2013). In common sense, drug efficacy and side effects may strongly depend on individual genetic disposition, for example in warfarin treatment. If a non-synonymous single-nucleotide polymorphism occurs in the binding or allosteric site, the change in drug response may result in a disconnection between the ligand and the target. Thus, it is necessary to simulate the drug-target complex formation to develop a multi-target drug because mutations may enhance the success of several therapies. Combining personalized data from next-generation sequencing with all the efforts to implant the polypharmacology approach, personalized medicine is becoming a real possibility (XIE et al., 2012).

- **Anti-cancer drugs**: Cancer is a multi-genetic disease characterized by an increase of network entropy, so this type of disease should be treated through multiple interventions to restore a normal cell state, rather than knocking out only one or two components. Moreover, there is scientific proof that when inhibitors of a specific signaling pathway are used alone, the cancer cell may strengthen other pathways by the appearance of mutations in the genetic encoding for drug target proteins. In most failures of anti-cancer drugs, unwanted off-target effects and undiscovered feedbacks prevent the desired pharmacological goal. Therapeutic polypharmacology may play an important role to overcome system robustness and provide less side-effect. The probability of a cell developing drug resistance with multi-target drugs acting on unrelated proteins is statistically lower when compared to single-target drugs. The examination of differential networks of cancer stages or networks of drug treated and untreated cells, is one of the first steps to identify multiple targets (BOTTEGONI et al.,2012; MEDINA-FRANCO et al., 2013; CSERMELY et al., 2013).

- **Treatment of infectious diseases**: Over the past few years, antibiotic resistance has been a worldwide health care problem. Many research projects work hard to find a solution, but there are so many mechanisms of antibiotic resistance that it becomes a difficult problem to solve. A well-understood example of therapeutic polypharmacology is resistance to β-lactam antibiotics, often caused by the bacterial enzyme β-lactamase. Combining β-lactamase inhibitors with β-lactam antibiotics overcomes this antibiotic resistance, but this combination is now widely used and it has not become effective for all target strains; an alternative is necessary (BORAN, 2010). In a polypharmacological view, the main strategy is to destroy the network of infectious agents. To reach this goal, different strategies can be used, such as the analysis of integrated bacterial/fungal/parasite and human metabolic network to predict potential drug target efficiency (KIM et al., 2012) or random upstream attack to uncover more influential targets than currently known in directed networks, such as metabolic and signaling networks (LIU et al., 2012). In summary, polypharmacology may be the missing piece of the puzzle.

- **Therapy for metabolic syndrome**: Metabolic syndrome is a multi-genetic disorder related to central obesity, cardiovascular disease, insulin resistance and atherosclerosis. The therapeutic approach involves the control of multiple risk factors using modifications of lifestyle and a targeted approach to control individual risk factors. Pharmacology therapy appears when lifestyle modifications fail to reach the therapeutic goal (KAUR, 2014). To treat these patients a real drug cocktail with anti-obesity drugs, statins and/or fibrates, anti-diabetic medication and anti-hypertension drugs is necessary. More than ever, metabolic syndrome has to be treated like one single complex disease and so it is essential to understand the metabolic network involved. Insulin signaling is the center of the etiology of metabolic diseases. Using a polypharmacology approach, the strategy may be the rewiring of the cellular network from the disease state to the healthy state. This approach includes avoiding network segments which are essential to healthy cells and focusing on specific pathways present in diseased cells, through multiple or indirect targeting (CSERMELY et al., 2013). There are already some projects in this direction, for example T2D-db which is a database of molecular factors involved in type 2 diabetes, providing useful information to reveal new connections between diabetes, obesity, oxidative stress and inflammatory abnormalities (AGRAWAL et al., 2008).

- **Anti-neurodegenerative drugs**: Neurodegenerative diseases (for example, Alzheimer's, Parkinson's and Huntington's disease) are one of the major disorders associated with the aging process and show a complex etiopathology. A good anti-neurodegenerative drug should prevent and slow down deregulated and over-acting signaling pathways, reconfiguring the distorted pathways associated to the disease. The polypharmacology approach may be the key to restore the molecular network of diseased cells, preventing the progression of these disorders (CSERMELY et. al, 2013). One example is the multi-target noncompetitive NMDA receptor antagonists, which were developed for the treatment of Alzheimer's disease, with low affinity for their targets (YOUDIM et al., 2005). Despite some initial efforts in the study of Alzheimer's disease, little attention was dedicated to chemical similarity, metabolic, signaling and drug-target network. A lot of work has to be done to learn more about changes during neurodegenerative progression and probably that effort would be the most important advance for drug discovery in that field (CSERMELY et al., 2013).

- **Anti-aging drugs**: According to some predictions, by 2050 the entire economy of the industrialized world will be consumed by the costs of the sick and elderly people (LIPTON, 2004). Aging is one of the most complex processes of nature. However, elderly organisms show similar warning signals as a result of the loss of network edges during aging, for example: slower recovery of the perturbations and increase of non-specific edges. Furthermore, during the aging process the nuclear pore complexes become more permeable and it is likely that aging increases permeability in others cellular compartments, increasing the number of non-specific edges of the inter-organelle network. Following this idea, most probably the anti-aging drugs of the future will be multi-target drugs, providing an influence on key processes of aging networks. The discovery of these drugs could promote healthy aging and also a new upper limit of human lifespan (CSERMELY et al., 2013).

3. How to design a multi-target drug

Single targets are often inefficient in a complex system because of the compensatory signaling pathways that bypass the inhibition of individual proteins preventing major changes in their outputs, and it is often necessary modulate several nodes simultaneously to affect the disease phenotype. For the past two decades, the pharmaceutical companies have focused on the development of highly selective ligands that interact with individual target proteins with high affinity, but this is an unreal approach (XIE et al., 2012); due the chemical similarities with metabolites, a chemical compound never acts on just one target. In Paolini's work, it was discovered that approximately 35% of their database of 276,122 active compounds had activity for more than one target and around one-quarter of those compounds had demonstrable activity across different gene families of targets (PAOLINI et al., 2006).

A well established practice to multi-target drugs is the combination therapy, i.e. using different drugs with different mechanisms of action. This methodology is currently used in anti-cancer chemotherapy, in the treatment of Alzheimer's disease and in the field of infectious diseases. However, the use of the combination therapy has some drawbacks and the development of a multi-target agent could offer an efficient and cost-effective alternative, solving problems such as drug-drug interactions, uncontrolled pharmacokinetic properties and compliance in age-related diseases (BOTTEGONI et al., 2012).

The development of a multi-target drug is likely to produce a drug that establishes weak interactions with the different targets, amplifying the possibility of indirect effects (KORCSMÁROS et al., 2007). At the molecular level, weak interactions play critical roles in recognition in biological systems such as the "underground" metabolic reactions that use endogenous metabolites as alternative substrates and may have a little impact on the cellular phenotype. Indeed, cooperative weak interactions may have more profound effects on biological systems than a single, strong interaction. An example of those phenotypic changes is the on/off switch of transcription and epigenetic modification (CSERMELY et al., 2005; XIE et al., 2012). Moreover, drug-target interactions *in vivo* are different from *in vitro*. The concentration of the drug in a living organism rarely reaches equilibrium because the concentration of target and the endogenous ligand are constantly changing with time. Consequently, the drug-binding affinity is not an appropriate indicator of drug efficacy *in vivo* (XIE et al., 2012).

Over the past decade, researchers focused only on drug properties to explain drug promiscuity (other term for polypharmacology). Small and hydrophobic molecules usually were the reasons for these multi-target actions, but the results have been contradictory among the studies. When we relate molecular weight with promiscuity, a duality of opinions appears: bigger molecules expose more features to interact with a receptor and thus they can be more promiscuous; on the other hand, the probability of a ligand interacting with a target drops when the selectivity of the ligand increases due to the higher number of chemical features (KORCSMÁROS et al., 2007; HAUPT et al., 2013). In contrast, smaller molecules have less negative features to interact and are easier to accommodate in different shapes than larger ones. Their intrinsic simplicity favors non-selective binding and promotes a multi-target profile (HOPKINS et al, 2006; HU et al., 2013). While disagreeing on the influence of molecular weight, the majority of the studies agree on hydrophobicity as the reason for promiscuity. This result can be a bias because a binding affinity threshold of 10 μM, in the aforementioned studies in Haupt's article, will also reflect unselective bindings as hydrophobic interactions or aggregation. In the pursuit of an elucidation, ligand flexibility and binding site similarities appear to explain drug promiscuity from a structural point of view. If a drug molecule is flexible, it can adapt to different binding sites and if we compare similar binding sites, the drug molecule is capable of sharing the same targets. After an analysis of different studies about this topic, Haupt et al. conclude that the binding site similarity is the main pre-requisite for a polypharmacology drug, leaving the ligand flexibility with minor impact on design of multi-target drugs (KORCSMÁROS et al., 2007; HAUPT et al., 2013).

Summarizing, to predict the drug response it is vital to identify drug-target interactions on a proteome-wide scale. For this purpose, it is essential to incorporate structural data in drug discovery pipelines and reinforce the efforts in structural genomics as well as in algorithm development for structural bioinformatics (HAUPT et al., 2013). As discussed by Xie et al, the future success of drug discovery lies in an approach whereby we identify the cellular connectivity that simulates the dynamic behavior upon drug perturbation for a given cellular state and link all of the cellular components (such as cell-to-cell communication, genetic and epigenetic variations and others) (XIE et al., 2012). The rational design of a multi-target drug is still in its childhood.

4. Methods for predicting Polypharmacology

The process of predicting polypharmacological effects requires a deep understanding of the structure and function of proteins, as well as of the interaction of proteins with small molecules in the context of biological networks (for example, the determination of the conformational and chemical states upon drug binding through allosteric or orthosteric interactions) (XIE et al., 2012). The enormous molecular data generated in the post-genomic era have appreciably accelerated polypharmacology research, however the organization and also the availability of the information continues to be a big obstacle (REDDY at al., 2013). Bioinformatics and chemoinformatics analysis may help to narrow down the candidates for molecular simulation or to explore the solution space when 3D protein structure is not available. Two important fields are necessary to provide a solid foundation for dynamic analysis: network reconstruction and static analysis. They have developed independently but it is necessary to integrate these disjointed computational techniques into a united framework, as represented in *Figure 3* (XIE et al., 2012).

The current information of molecular pathways, crystal structures, binding experiments, side effects and drug targets are integrated in public and private databases such as DrugBank, BindingDB, ChEMBL, PubChem Bioassay, STITCH, etc. For example, ChemProt version 2.0 server is a resource of chemical-protein interactions, integrating the referred databases and others, including more than 1 100 000 chemicals with biological activity for more than 15 000 proteins, and more than 2 million interactions (ChemProt, 2013). These databases can be used not only to predict the protein targets of a small molecule, but also to design polypharmacological drugs in a rational way (REDDY et al., 2013). If two drugs share similar structures or phenotypes, then their targets may be related and their relationship can be inferred directly using a bioinformatics approaches. The real challenge lies in addressing domain-specific issues (XIE et al., 2012). Recent advances in the integration and mining of chemical databases with biological activity are represented by the Open Pharmacological Concept Triple Store (OPEN PHACTS) project and the PharmaTRek web explorer. These major initiatives aim to create an integrated pharmacological space and represent an effort in making possible open innovations in drug discovery, including multi-target approaches (MEDINA-FRANCO et al., 2013).

In the following sections, the main computational strategies to predict multi-target drugs will be discussed.

4.1- Structure-based prediction

In recent years, the development of 3D ligand binding site characterization has become a tremendous issue of interest. The ligand binding site can be represented as a one-dimensional (1D) fingerprint of atomic spatial distribution (a vector), a 2D graph in which atoms in the structure are nodes and their spatial relationships are encoded as edges, or a cloud of atoms in 3D space. One of the approaches based on this idea is named docking where a panel of tractable targets involved in a disease network is screened against the approved drug molecules by a computational simulation. Although there are several algorithms developed for this analysis, many challenges remain (REDDY at al., 2013; XIE et al., 2012). First of all, the algorithm should be robust to conformational changes in the binding site because proteins are intrinsically flexible, but at the same time, it should be sensitive to different interaction patterns that contribute to binding specificity (CHEN et al., 2010). Second, if the algorithm is to be applied on a proteome-wide scale, it should be tolerant to the uncertainty in homology models. The structural, functional and evolutionary relationships can be inferred from divergent or convergent evolutionary relationships between proteins by detecting sequence and homologous structures, reducing the complexity of the drug-target prediction (POLEKSIC et al., 2009; ARRIAGADA et al., 2013). However, multi-target drugs can exert their activity by binding to proteins, unrelated from the evolutionary point of view, leading to false results (BOTTEGONI et al., 2012). Finally, it would be interesting to extend binding site comparison to protein-protein interaction interfaces, antibody-antigen recognition surfaces and other irregular protein functional sites (XIE et al., 2012). Unlike sequence comparison, in which statistical models have been developed to evaluate similarity, there is no practical mathematical framework for protein structure comparison. To solve this problem, Poleksic's work has been focused on an algorithm that guarantees optimal rigid superposition between protein structures (POLEKSIC, 2009; ARRIAGADA et al., 2013).

Another approach based on structure prediction consists in binding site structure similarities among targets. The definition of a promiscuous drug describes the ability to bind to different receptors due to the similarity of shape and physicochemical properties at the binding site. In a recent study, Haupt et al. established the structural similarity between targets by the alignment of all pairs of proteins. Their structures were aligned using SMAP, a

software and web service for binding pocket similarity search. Since only binding sites of identical promiscuous drugs are aligned with each other, it is possible to use the aligned pair of the proteins to predict the interaction with promiscuous drugs. After removing redundant targets, they only keep sites with a consistent binding site through LigandRMSD, comparing it to a corresponding optimal superposition (HAUPT et al., 2013).

One last example for structure-based prediction was created by Hu et al., named Compound Series Matrix (CSM). This computational methodology was designed for predicting multi-target activity spaces and visualizing promiscuity patterns, with a special focus on related compound series. The CSM represents a methodological extension of the Structure Activity Relationship (SAR), where the changes in promiscuity of a pair of compounds that only differ by defined chemical substitutions (R-groups) are analyzed, under the experimental conditions of the microarray experiment. As a result, CSM statistics establish structural relationships between compounds in multi-target activity space, where promiscuity patterns are captured in structurally related series and provide hypotheses for drug design (HU et al., 2013). For a better comprehension of this methodology, see Figure 4 in the Annexes Section.

4.2- Ligand-based prediction

The concept underlying ligand-based prediction states that two ligands with similar chemical structures may exhibit similar bioactivities. A notable advance in this field was the development of the Similarity Ensemble Approach (SEA). SEA technology is a statistical method that relates proteins (targets) according to their pharmacology properties, aggregating many small similarity signals among the ligands (SEAChange Pharmaceuticals, 2014). It can be used to quickly search large compound databases to build cross-target similarity maps because this method focuses only on meaningful connections that reflect underlying similarities between pharmacological profiles, predicting unexpected targets accurately (XIE et al., 2012). Keiser and colleagues used this approach to predict the activity of 656 marketed drugs on 73 unintended "side-effect" targets. Half of the predictions were confirmed by proprietary databases unknown to the method or by new experimental data, with affinities for these new off-targets ranged from 1 nM to 30 μM (LOUKINE et al., 2012). One of the main disadvantages of this modern chemogenomic method is the actual development of a multi-target candidate because it still depends on the possibility of creating a molecule that can physically interact with multiple proteins. There is still a lot work to be

done and considerable efforts have been made to develop 3D representations of molecules and pharmacophore models to predict off-targets (BOTTEGONI et al., 2012).

4.3- Phenotype-based prediction

Drugs and their targets can be related by their phenotypic responses, although similar drug phenotypes are not always associated with similar drug action. One of the main computational challenges in using genomic and proteomic studies to generate molecular signatures is to separate essential targets or proteins markers from the confusion resulting from cell variations, doses and other effects (XIE et al., 2012). In spite of this obstacle, the following studies used this approach to determine off-targets. Campillos et al. used phenotypic side-effect similarities to deduce whether two drugs share a target using text mining tools to relate the drug side effects with its unknown targets (CAMPILLOS, 2008). Chen et al. constructed a bioassay network using PubChem data creating a map for a drug-target network, a protein-protein interaction network and biological pathways through similar nodes to identify specific drug profiles and important target pairs (CHEN et al., 2009).

Advances in RNAi technology have introduced a powerful approach for searching the cellular mechanisms of drug actions in higher organisms. With this methodology, Jiang et al. characterized small-molecule function in mammalian cells. The phenotype of cells expressing short hairpin RNAs (shRNAs) for diverse selected chemotherapeutics generated a functional shRNA signature that allows establishing biochemical modes of action (JIANG et al., 2010). It is expected that additional drug-target and pathway-disease associations will arise from future phenotype screening experiments (XIE et al., 2012).

4.4- Other predictions

- **Systems biology/pharmacology approaches**: Polypharmacology is an important area of integration between systems biology and drug discovery, which suggests not only that drugs act on multiple targets but also that they are often involved with multiple diseases. Systems biology/pharmacology approaches use experimental and computational techniques to attain the systems-level understanding of diseases and the mechanisms of drug actions. (REDDY et al., 2013) Mapping the polypharmacology network onto the human disease-gene network would reveal important drug targets involved with multiple diseases. This method may be applied to TCM to enhance our understanding in herbal

medicine and to discover new drugs from plants. Liu et al. proposed an integrated model, combining oral bioavailability prediction, blood-brain barrier permeation prediction, multi-drug target prediction (by docking) and network pharmacology techniques for licorice, one of the oldest and most popular herbal medicines in the world used as a cough reliever, anti-inflammatory, immunomodulatory, anti-platelet, antiviral (hepatitis) and detoxifying agent. They generated a drug-target-disease network where they linked the candidate compounds with all their potential targets and consequently their diseases information (LIU et al., 2013). One example of drug-target-disease network is represented in Figure 5 (see Annexes Section).

- **Fragment-based approaches**: The rational design of multi-target ligands can be extremely challenging. A key challenge in multi-target drug discovery focuses on the affinity balance for different target proteins and, at the same time, the equilibrium of target residence to achieve the desired *in vivo* efficacy profile. Because of what was previously cited, the multi-target approach continues to get some resistance from the drug discovery community. One possible way to overcome this limitation is designing a dual-target design strategy. Morphy and Rankovic were the first researchers that suggested this idea. The first step is the selection of two pharmacologically relevant targets located on complementary pathological pathways, based on chemical and pharmacological considerations. Secondly, researchers must question whether or not modulating the two selected targets could lead to additive effects or synergistic potentiating. Then, the pharmacophoric functions responsible for binding to targets must be identified. Finally, the key pharmacophoric functions can be combined in one dual-target compound to obtain hybrid, fused or chimeric compounds. This decision of hybrid generation will be driven by the nature of the targets, the availability of reference compounds and chemical viability (MORPHY et al., 2005; BOTTEGONI et al., 2012).

- **Protein-Ligand-Based Pharmacophores:** Knowledge on protein-ligand data is increasing at great speed thanks to public initiatives. On the target side, the Protein Data Bank stores around 101.050 structures of proteins in 3D and protein–ligand complexes, of which about 17,270 relate to druggable proteins and their ligands (PDB, 2014). On the ligand side, ChEMBL is a repository of more than ten million bioactivity data gathered from literature and addressing about 1.5 million ligands and 9,414 molecular targets (ChEMBL, 2013). In spite of this vast matrix of experimental data, pharmacophores have been widely used in many areas of computational drug design but

rarely in target fishing applications. The idea to identify potential targets based on protein-ligand-derived-pharmacophores was applied by Langer et al. in a series of screenings focused on small-protein-ligand matrices. Two of the reasons that explain why pharmacophore-based target identification has not yet become a standard *in silico* ligand profiling method are the absence of an exhaustive collection of protein-ligand based and (or) ligand-based pharmacophore database and also the lack of clear benchmarks comparing with ligand similarity or docking strategy. In an attempt to change this, Meslamani et al. suggest a hybrid profiling method using the best possible approach to determine the function of ligand and binding site properties. They presented the PharmDB, a collection of structure-based pharmacophores (68,056 entries) from 8,166 protein-ligand X-ray structures. Several ligands were profiled using screening protocols on the entire pharmacophore collection, generating a pharmacophore mapping which was compared to docking and similarity approaches. Simplifying the procedure, the pharmacophore features of the ligand are identified based on six standard features: hydrogen bond acceptor/donor properties, positive/negative ionizability; hydrophobicity and ring aromaticity. The algorithm discards all features that do not match the protein-ligand interactions using adjustable topological rules and only the top 10 models are selected. However, there are still two options for adding steric constraints to the pharmacophores: shape or excluded volumes. This methodology is recommend for profiling targets when a 3D structure is available, with the exception of profiling polar ligands to small, polar and buried activity sites for which molecular docking is preferable because this methodology is better suited to molecules with these features (MESLAMANI et al, 2012).

5. Future Challenges

The major limitation of the polypharmacology approach is the incomplete information of the pathways/mechanisms of many diseases at the molecular level and the lack of ability to support quantitative data analysis (REDDY et al., 2013; XIE et al., 2012). A lot of protein structure information was locked up in pharmaceutical companies because of intellectual property protection and that is why current business models are one of the major obstacles for the implementation of this idea. The transformation of open innovation R&D models and even calls for open-access sharing of data could make the difference because, in the end, polypharmacology is an interdisciplinary field (TACHIBANA, 2014). It is still difficult to continually access/update the quantity of information provided by different databases. No synchronization is done and the lack of a unique central project is missing. In addition, more precise mining techniques and mapping methodologies are needed to analyze the complex information (REDDY et al., 2013). The databases suffer from many uncertainties because ligand-target interaction networks have a large number of false-positive entries. Although an average probability of the particular interaction is given, this does not take into account if two proteins are expressed at the same time in the same compartment, or the healthy status of the cell (KORCSMAROS, 2007).

In polypharmacology, an effect does not lead to a linear combination of independent events involving the same ligand and several targets, instead, target-ligand associations often affect each other, which are complicated to understand let alone predict (BOTTEGONI et al., 2012). The future success depends on a new generation of computational tools to identify the correct multiple targets, their multi-fitting and low-affinity drug candidates (CSERMELY et al., 2005; XIE et al., 2012). The rapid development of biotechnology systems such as genomics, proteomics, metabonomics and others can be a great contribution to our understanding of the nature of the disease, effective identification of the targets and also the elucidation of mechanisms of action, leading this novel drug design paradigm to success (LU, 2012; MEDINA-FRANCO et al., 2013). The most important strategy starts with finding the multi-target key that is associated with a desired clinical effect and the calculation of free-energy landscapes in the association and dissociation of protein-ligand complexes for efficient drug profile (MEDINA-FRANCO et al., 2013).

Another relevant challenge is the construction of the right structure to produce the designed drugs and mechanism of identification using advanced modern technologies (LU, 2012). It is important to integrate ligand- and phenotype-based approaches with target-based

methodologies and this is only possible with the integration of several tools from chemoinformatics, bioinformatics, molecular modeling, systems biology as well as heterogeneous omic data (XIE et al., 2012). Some advances have been made in this field with fragment-based approaches, but they are still at the beginning.

Concluding, an enormous investment from pharmaceutical companies is essential to start using the polypharmacology concept, but after the optimization of the process, this approach will probably be very rewarding. They have to bet on innovative computational technologies to cut the high costs associated with it and maybe, in first attempts, produce more efficient *in vivo* tests for a correct validation of the computational methods or, who knows, the creation of an anticipated human whole body model (silicon or virtual human) to help in the development of such network-targeting drugs (KOLODKIN et al., 2012). This frightening approach is just a call for collaboration between researchers and industry, where together they will improve the success of drug discovery.

6. Conclusion

Polypharmacology can be considered a double-edged sword, also a curse and a blessing if side effects are not fully understood. The fact is that the toxicities or targets of many phenotype drugs are either largely unknown or insufficiently understood in most cases. Drug discovery, as we know it, has to assume a new position and forget the secure model of "one-target, one-drug" because this model is leaving the industry with a serious innovation deficit, despite the technological advancements. We are dealing with nature and it can be more unpredictable than we think. Our only defense is to understand the full spectrum of pharmacological actions of a drug in the human body and polypharmacology could be the "Holy Grail" of the drug discovery. We can use known drug properties to understand the underlying mechanisms, however, it is too expensive to conduct these studies experimentally and that is why we need more predictive algorithms and increased integration of available data. The full understanding of this emerging paradigm could lead to new multi-target treatments, side-effect predictions and the identification of new targets (drug repurposing). As a matter of fact, the NIH and FDA have launched programs to identify new uses for existing agents developed by pharmaceutical companies (ALLISON, 2012).

Drug discovery for the next-generation is already on the move. A modification of mindsets from pharmaceutical companies is urgent in order to put all efforts on this hard task called polypharmacology.

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8. Annexes

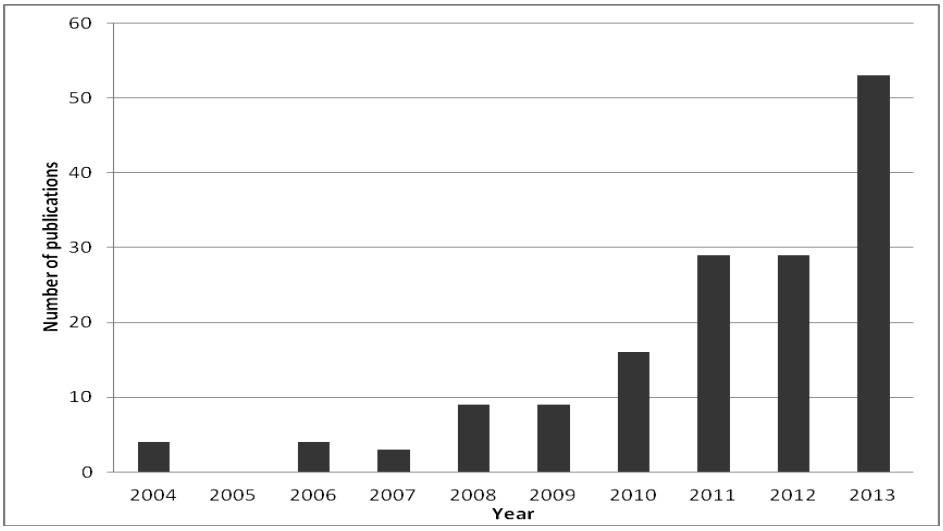

Figure 1 – Number of publications per year with the key word "polypharmacology" mentioned in titles/abstract (resource: PubMed, 2014 June)

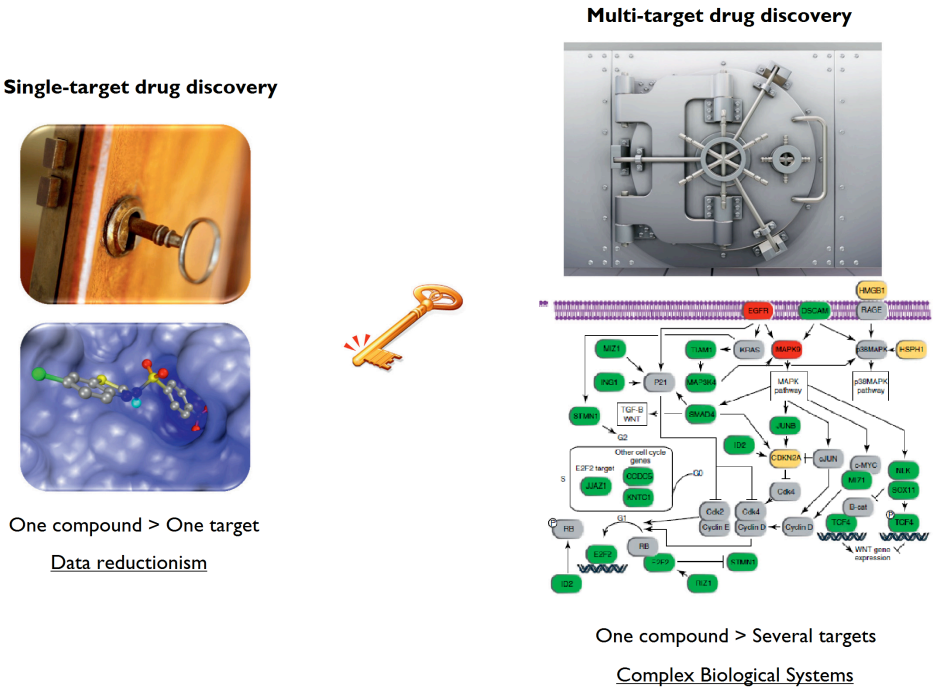

Figure 2 – Schematic representation of the different drug discovery approaches. Diseases are associated with a complex process with multiple targets, which are more difficult to "unlock" in just a single target. (Based on MEDINA-FRANCO et al., 2013)

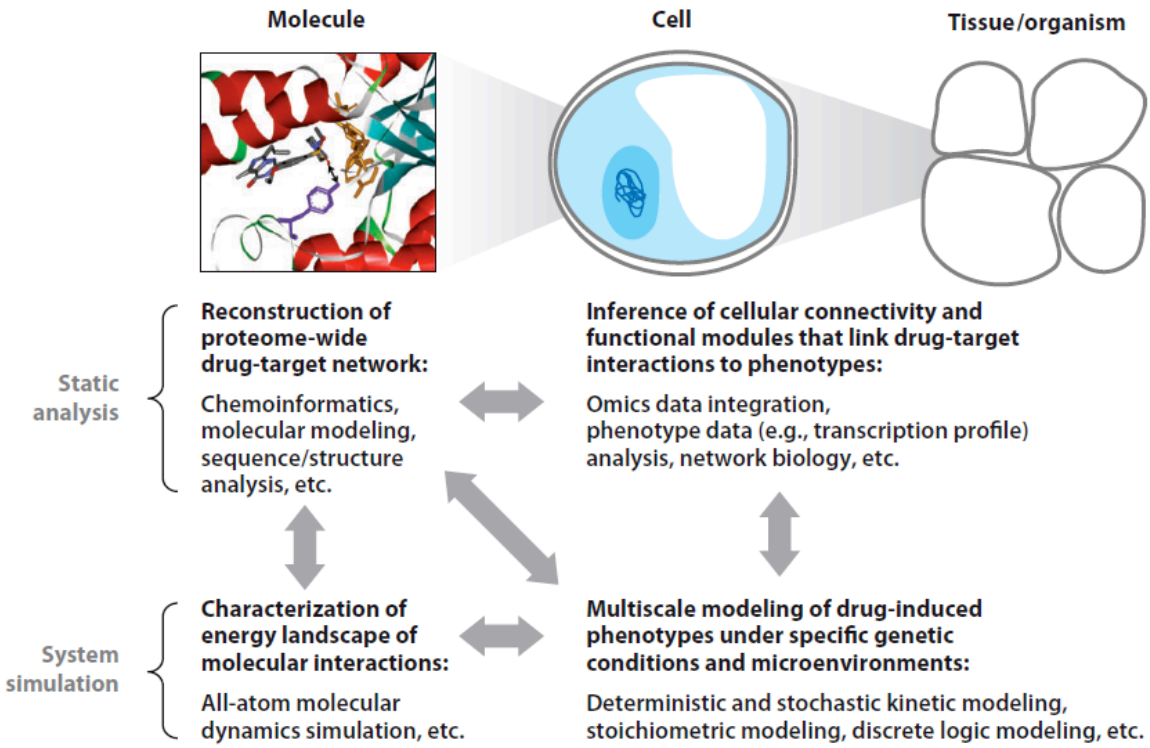

Figure 3 – The definition of polypharmacological effects requires multi-scale modeling, from atomic details up to the organism levels (XIE et al, 2012).

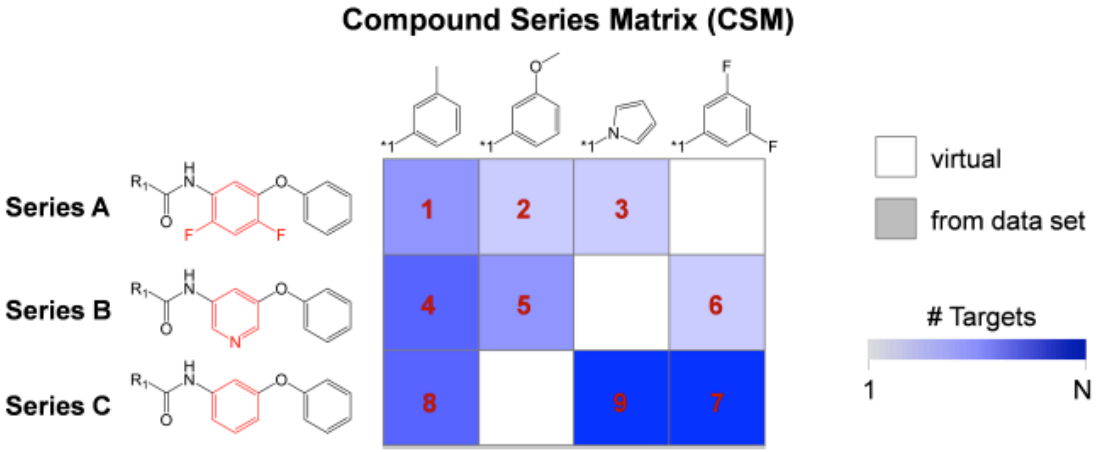

Figure 4 – The CSM is generated by combining structurally analogous series with similar core structure (bottom left) and different small substituents. Each combination defines a real (filled cell) or virtual (empty cell) compound. Cells are colored according to the number of targets that compounds are active against, which reflects the degree of compound promiscuity (HU et al., 2013).

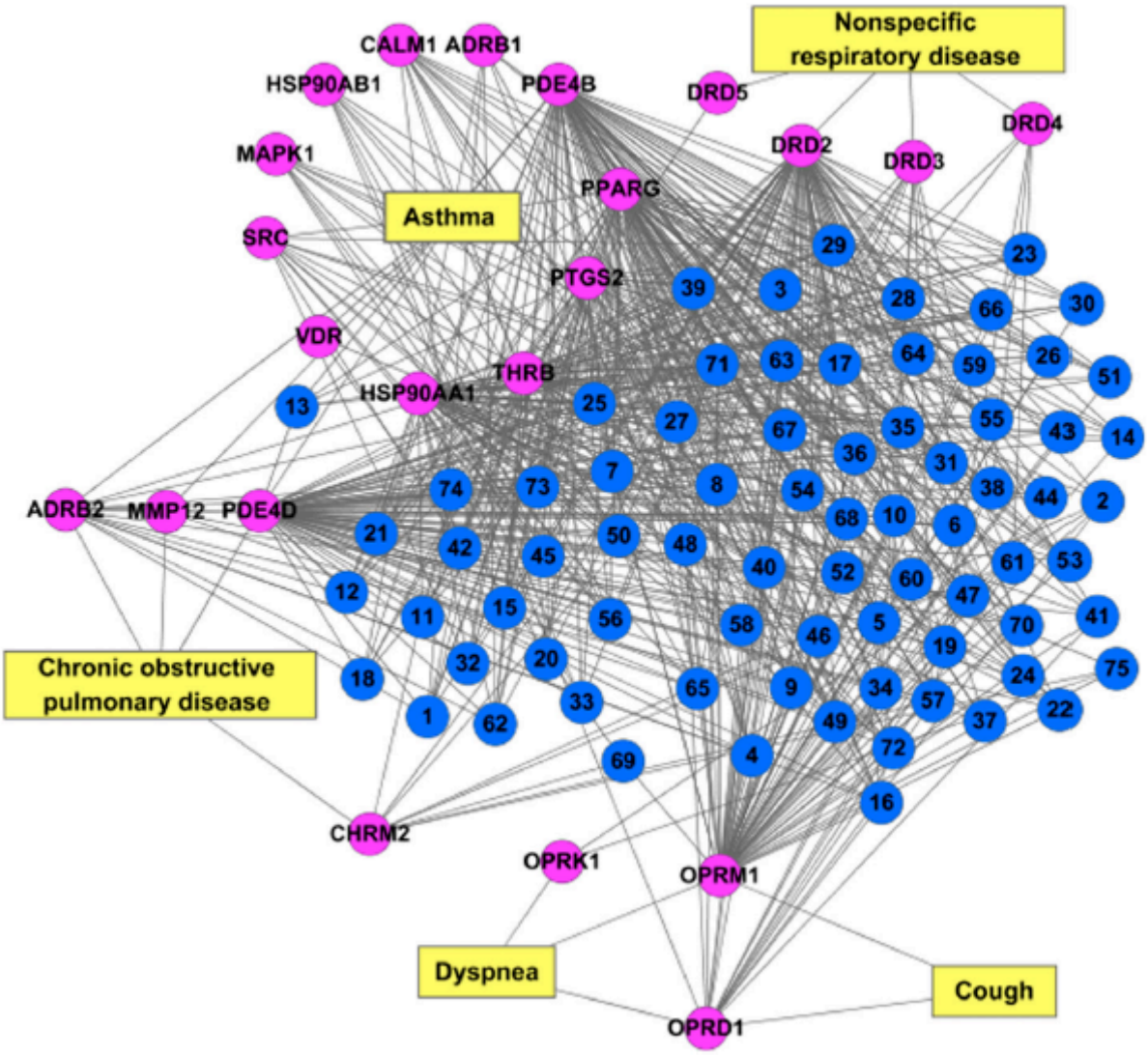

Figure 5 – An example of a drug-target-disease network of the respiratory system (LIU et al., 2013).