Send Orders for Reprints to reprints@benthamscience.net

Drug Repurposing: Translational Pharmacology, Chemistry, Computers and the Clinic

Naiem T. Issa[1], Stephen W. Byers[1,2] and Sivanesan Dakshanamurthy[1,2,*]

[1]*Department of Oncology, Georgetown Lombardi Cancer Center* [2]*Department of Biochemistry, Molecular and Cellular Biology, Georgetown University Medical Center, USA*

Abstract: The process of discovering a pharmacological compound that elicits a desired clinical effect with minimal side effects is a challenge. Prior to the advent of high-performance computing and large-scale screening technologies, drug discovery was largely a serendipitous endeavor, as in the case of thalidomide for erythema nodosum leprosum or cancer drugs in general derived from flora located in far-reaching geographic locations. More recently, de novo drug discovery has become a more rationalized process where drug-target-effect hypotheses are formulated on the basis of already known compounds/protein targets and their structures. Although this approach is hypothesis-driven, the actual success has been very low, contributing to the soaring costs of research and development as well as the diminished pharmaceutical pipeline in the United States. In this review, we discuss the evolution in computational pharmacology as the next generation of successful drug discovery and implementation in the clinic where high-performance computing (HPC) is used to generate and validate drug-target-effect hypotheses completely *in silico*. The use of HPC would decrease development time and errors while increasing productivity prior to *in vitro*, animal and human testing. We highlight approaches in chemoinformatics, bioinformatics as well as network biopharmacology to illustrate potential avenues from which to design clinically efficacious drugs. We further discuss the implications of combining these approaches into an integrative methodology for high-accuracy computational predictions within the context of drug repositioning for the efficient streamlining of currently approved drugs back into clinical trials for possible new indications.

Keywords: Drug discovery, network pharmacology, translational pharmacology, chemoinformatics, drug repositioning, drug repurposing, bioinformatics, clinical informatics, phenotypic screening, high throughput screening.

INTRODUCTION

De novo drug discovery faces a number of challenges from the conception of a clinical application to release of a drug into the market [1]. Pharmaceutical research and development suffer from the combination of financial and safety burdens with increasing time to product marketability. These concerns coupled to a very low rate of success have led to drug repositioning initiatives that aim to repurpose currently approved drugs for new indications [1-8]. Over the last decade, particularly since 2010 and the announcement of the NIH's repurposing initiative by the National Center for Advancing Translational Sciences (NCATS), there has been a rapid upward trend in the number of publications addressing drug repurposing (Fig. **1**). Approved drugs have already undergone all four phases of clinical trials and potentially post-market evaluations, and their potencies, efficacies and toxicity profiles in humans are well documented. Consequently drug repurposing is an excellent method for increasing drug development productivity as these compounds can be rapidly evaluated for a new indication directly in phase II clinical trials, thereby reducing time and cost [1].

At present, the methods of choice for drug repurposing are high throughput chemical screening, transcriptome matching, protein-protein interaction assays, and gene activity mapping among other data-driven "biopharmacoinformatic" approaches that aim to link the chemistry to desired biological and clinical outputs [9-10]. However, such large-scale experimental validation poses both technical and financial limitations, and in many cases does not address mechanism of action. To address these concerns, there has been a push to develop and adopt computational-based screening methods. With the advent of high-performance computing, virtual screening may be automated on an extremely large scale, thus driving down resource utilization, expenses and time. The goal of these computational approaches is to maximize the accuracy of drug-protein and drug-disease predictions.

In this review, we discuss computational-based drug repurposing from the perspectives of gene expression, chemocentricity, protein-centricity, network biology approaches, and their integration.

CHEMOINFORMATICS: THE BACKBONE OF DRUG REPURPOSING

The mainstay of most drug repurposing efforts is chemical informatics, with drug chemical properties as the core element. These chemo-centric approaches are driven by the hypothesis that chemically and structurally similar molecules will exhibit similar properties, bind similar protein targets and elicit similar biological effects [11-13]. Advantages of

*Address correspondence to this author at the Department of Oncology, Georgetown Lombardi Cancer Center, USA; Tel: 202-687-2347;
Email: sd233@georgetown.edu

1873-5294/13 $58.00+.00

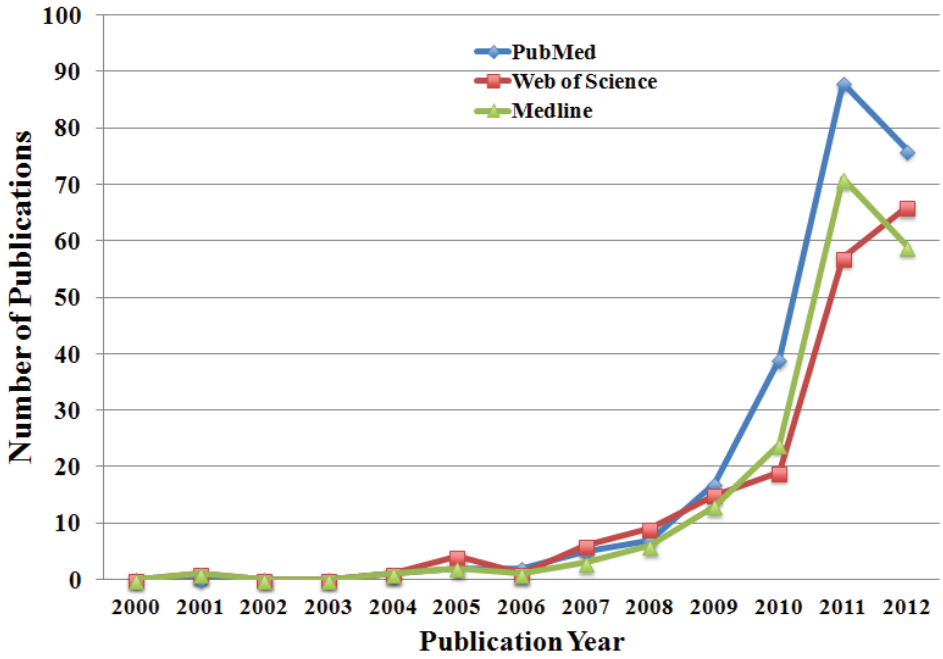

Fig. (1). The number of publications since the year 2000 that have discussed drug repositioning. The following search terms were used in PubMed, Web of Science and Medline: "drug+repurpos*" and "drug+reposition". The spike in publications between 2010 and 2011 correlate with the initiative of the NIH National Center for Advancing Translational Sciences (NCATS) for repurposing currently approved drugs and the availability of the NCATS pharmaceutical collection (published April 27, 2011) of small molecules for public use. With drug repurposing now becoming a more centralized initiative with far-reaching collaborations across a multitude of academic centers, biopharmaceutical companies, as well as corporations in the U.S. and abroad, it is expected that drug repurposing will continue to be an important aspect of the scientific literature.

chemoinformatics, in comparison to the use of classical target-based approaches alone, include: (1) no crystal structure or desired bioactive conformation (i.e. agonist vs. antagonist) is required for the target protein of interest, (2) bypassing the inaccuracies and imprecisions of most ligand-protein docking algorithms and molecular dynamics (MD) simulations, (3) accounting for complex ligand-binding phenomena where bioactive structures of small molecules often occupy only a fraction of the three-dimensional pocket shape of targets, and (4) increasing the ease of use and time efficiency. Furthermore, chemo-centricity provides an explanation for instances where chemically similar drugs bind proteins with dissimilar sequences and structures. In essence, chemo-centric approaches provide a unique vantage point that focuses on properties of the ligands of interest to either circumvent the issues of target-centered approaches or by synergistically contributing to their accuracy.

A variety of chemoinformatics techniques have been developed to measure similarities between small molecules based on a range of metrics (i.e. chemical, structural, topological, bioactivity) and to further map those molecules to pharmacological effects, proteome and genetic associations, as well as binding affinities. Perez-Nueno and Ritchie created a ligand-based shape matching approach for virtual screening from the concept of spherical harmonics-based "consensus shapes", wherein the consensus shape for a set of bioactively-similar ligands that adopt a range of shapes, increases the accuracy of virtual screening performances on difficult targets whose binding sites can accommodate ligands in different ways [14]. Warner *et al.* developed a new approach called Computational Conformer Selection (CCS) in which they created a conformer library of FDA-approved drugs obtained from the ZINC database and used a Tanimoto-combined score, a similarity term that takes into account ligand shape and chemical functional groups. Using CCS and a computational bioactive model of the known MDM2 inhibitor Nutlin-3a they predicted the potential repurposing of S-bepridil, a calcium channel blocker, to MDM2 [15]. Furthermore, Steindl *et al.* utilized pharmacophores, three-dimensional functional and steric models of antiviral compounds for the successful prediction of *in silico* activity profiles [16].

A seminal study that highlights the use of a chemo-centric approach for success in drug repurposing is that of Keiser *et al.* [17]. They formulated the Similarity Ensemble Approach (SEA) for relating receptors based on their ligand's chemical similarity. A database of ~65,000 ligands was parsed into 246 target sets, with an average of 124-289 ligands per target. Similarities across these ligand sets were calculated using the Tanimoto coefficient (Tc) based on standard two-dimensional topological Daylight fingerprints (representations of structural features of ligands). Based on these similarity descriptors, they developed a similarity map relating the ligand targets. The concept is that it is possible to predict or explain a ligand's poly- or mono-pharmacology via the similarity of its known main target to others from this similarity network. A surprising outcome from this study was the prediction and validation of methadone's anti-muscarinic activity via high-affinity binding to M3 receptors. Although methadone is traditionally known to have dual

specificity for NMDA and u-opioid receptors, the similarity map ranked these targets below muscarinics. Additionally, emetine, an anti-parasitic, was unexpectedly predicted and validated to fall in the class of adrenergic alpha 2-blockers, thus raising the possibility of repurposing emetine for human autonomic modulation.

While these studies are just a small fraction of the chemical informatics literature, they highlight prototypical concepts involved in chemo-centric approaches. To reiterate, the goal of these approaches is to reliably relate some ligand-based metric(s) to biological outcomes. These metrics include topological signatures, three-dimensional shapes, functional group characteristics as seen with pharmacophore modeling, as well as a number of physical characteristics related to the ligand such as dipole moments for electrostatic considerations and solvent-accessible surface areas. The key attractiveness of chemo-centric approaches is their independence from target-centric parameters. Thus, these approaches are certainly useful for cases where an appropriate protein target crystal structure is absent or when the target of interest exhibits a binding pocket that is able to accommodate a variety of ligands that are seemingly dissimilar. Furthermore, an interesting notion is that some ligands exhibit effects across targets that are unrelated by sequence. It can be easily seen how this sort of polypharmacology can go unnoticed without the utilization of chemo-centric approaches.

As the evidence for the applicability of chemo-centricity grows along with the quickly-evolving techniques of chemoinformatics [18-25], the implementation of these approaches in drug development is becoming more ubiquitous. Indeed, public databases have begun introducing tools for chemo-centric analytics. Notably, PubChem has developed another layer to their database entitled PubChem3D, which generates three-dimensional conformer models of molecules and can be used to query 3-D similarities [26].

Chemoinformatics as the Bridge to Biology and the Clinic

The goal of any drug development/repurposing effort is the achievement of a desired biological or clinical effect while minimizing off-target interactions that could lead to toxicity. In order to do so, it is important to have global knowledge of the particular "biological state" of interest (i.e. disease versus physiological states), such as gene expression patterns, cell signaling pathways, etc. With a snapshot of this biological state, repurposing efforts can be aimed at enhancing/reducing the expression of certain genes that are highly correlated with the biological state, or discovering new mechanisms of action. In addition, linking such biological data with chemical information may also reveal unexpected associations, such as two structurally unrelated drugs inhibiting the same target or eliciting similar clinical outcomes.

In an effort to establish a primer compendium of biological states, Lamb *et al.* created the Connectivity Map, wherein genomic signatures based on mRNA expression were developed for physiological, disease and drug-induced states [27]. With these signatures, it is possible to query the effect of an FDA approved drug on genomic patterns and see what other drug-gene signatures are closely related. If the search returns related matches, then it may be possible for the query drug to be repurposed within the context of an alternative mechanism of action based on an already-established matching drug-gene signature. As a proof-of-concept, the same group discovered that the mTOR inhibitor sirolimus (rapamycin) can overcome dexamethasone resistance *in vitro* in the lymphoid cell line CEM-c1. While the structural and chemical differences between these two drugs are large, their drug-gene signatures are remarkably connected via the Connectivity Map and there is potential for rapamycin to be repurposed for children exhibiting dexamethasone-resistant acute lymphoblastic leukemia [27]. In fact, there is an ongoing clinical trial to assess the efficacy of rapamycin intervention in conjunction with corticosteroid compared to the corticosteroid alone (ClinicalTrials.gov identifier: NCT00874562).

While using the gene signature Connectivity Map is useful for drug repurposing from the perspective of querying for effects similar to those of other clinically-available bioactive small molecules, it has also been utilized for repurposing via "inverse associations". That is, drugs that induce gene expression changes that are opposite to those induced by a disease are predicted to have repurposing potential for that particular disease. This inverse association method was implemented by Josset *et al.* in their successful effort to identify novel antivirals against the human influenza A virus [28]. Their strategy involved creating a global transcriptional signature of the influenza A1N infection using human pulmonary epithelium A549 cells and then creating a query signature for the Connectivity Map using those genes that were differentially expressed in the disease state. Potential drugs for repurposing exploration were those with gene expression signatures that were negatively correlated to that of the query, whereas positively correlated drugs served as controls. Furthermore, *in vitro* analyses concluded that five molecules of the eight chosen from the search inhibited viral growth, whereas none of the molecules with positive connectivity were able to do so [28].

Although combining chemical informatics with biological data is powerful for generating repurposing hypotheses whose endpoint is clinical outcome, such approaches are also valuable for predicting ligand-protein target associations and rationalizing polypharmacological phenomena. Cheng *et al.* developed a computational approach called the bioactivity profile similarity search (BASS), where they constructed a database of pharmacological bioactivity profiles from the NCI-60 human tumor cell line set for target prediction [29]. Using BASS, compounds from within the NCI-60 set were queried across the entire database to find similar bioactivity profiles. From the resulting profiles, neighbor compounds were identified and their target(s) were predicted to be the known target of the query compound. For example, the anticancer microtubule inhibitor paclitaxel was queried in BASS to find that five of the seven neighbor compounds were structurally related analogues of the drug. Remarkably, they found that one neighbor was the structurally unrelated compound vinblastine, which is also an approved anticancer drug, also targeting microtubules. The importance of this particular finding is two-fold: (1) it provides evidence for the notion that similar molecules elicit similar effects, which would allow for inferences of bioactivity and further target identification for untested compounds on the basis of struc-

tural similarity, and (2) it confirms that structurally dissimilar compounds may bind to the same target. With BASS and similar approaches, both of these modalities can be addressed simultaneously. The Cheng group also found dihydrofolate reductase to be a new target for the query experimental drug metoprine, whose annotated target is Histamine N-methyltransferase. This prediction was validated in the literature and annotation in the Therapeutic Targets Database (TTD). In addition, Lounkine *et al.* adapted the SEA method (discussed above) for large-scale target predictions on the basis of adverse side-effects [30]. Among the many drug-target associations they predicted (with a 54% success rate), and discovered that the synthetic estrogen chlorotrianisene exhibited its side effect of abdominal pain (epigastralgia) by binding to COX-1. This was validated by the greater amount of platelet aggregation inhibited by chlorotrianisene compared to acetylsalicyclic acid. This finding is particularly notable as the other estrogens do not exhibit this effect.

Given the success of these methods, the concept of using drug-gene signatures, bioactivity profiles and other biological/clinical metrics for chemoinformatics-based target identification and drug repurposing has become a centerpiece for other studies [19, 31-35]. However, it is important to note that reliance on drug-gene signatures also presents a variety of issues: e.g. the handling of multiple transcriptional profiles for a single drug across different cell lines, the choice of differential subset genes considered for the signature, alternative splicing products, post-transcriptional modifications, signaling pathway mechanisms, as well as practical efficiencies such as cost and time for the curation of these signatures, among other limitations. As these limitations are recognized, efforts have focused on building methods from the Connectivity Map and its gene signature foundation. Ji *et al.* constructed a comprehensive drug-protein connectivity map for Alzheimer's Disease through literature mining of the PubMed database and have predicted the potential repurposing of Diltiazam and Quinidine, antihypertensive and anti-arrhythmia agents, respectively, for AD treatment [36]. From the perspective of adequate gene selection, Iorio *et al.* devised the "drug network" MANTRA from the consensus transcriptional responses for individual drugs that summarized their transcriptional effects across different cell lines under a variety of dosages [37]. Using this approach, they unexpectedly predicted that Fasudil, a potent vasodilator, would enhance autophagy, a key process implicated in a range of diseases, and further validated its effect experimentally [37]. Furthermore, to address the issue of signaling pathways, Jin *et al.* created a statistical model that combined cancer signaling pathways and mechanisms with transcriptional responses for the off-target repurposing of cancer therapeutics [38].

Indeed, the focus of drug development/repurposing is biological context and clinical application. While that is true, the concept of drug-biological signatures (i.e. genes, proteins, etc.) provides a unique perspective in chemoinformatics, departing from traditional methods that have relied only on structural, chemical and topological parameters for predicting drug repurposing outcomes. This combined synergy may greatly facilitate bridging basic science and the clinical setting, making repurposing for difficult diseases and biological targets that elude other methods a tangible reality.

PROTEIN-CENTRIC METHODS

Docking

Before the advancements in computational modeling that have led to the momentum of rational drug repurposing, successful repurposing examples have been serendipitous, with that of sildenafil (Viagra) being one of the best known [1]**.** With the ability to crystallize many more protein structures and identify functional binding sites, serendipity has now made way for intentionality. A major contributor to computationally-driven efforts is the concept of molecular docking- the prediction of the free energy of binding of a ligand and its positioning within a defined binding pocket. DesJarlais *et al.* marked one of the earliest efforts to implement docking for drug repurposing, in which they successfully repositioned the antipsychotic haloperidol as a lead drug for HIV-1 protease [39].

In essence, docking is an empirical method that aims to accurately predict ligand-protein binding signatures. However, the approaches taken by the large number of docking programs currently available differ significantly and have been the subject of great debate. Recently, Bohari and Sastry evaluated ligand pose-prediction accuracy in a dataset of 199 FDA-approved drug protein complexes across five popular docking programs- Glide, Gold, FlexX, Cdocker and LigandFit [40]. They found that Glide was most accurate in predicting the top ranked pose, while Cdocker was most accurate in predicting the top Root-Mean-Square-Deviation (RMSD) pose. Interestingly, they found that across all docking protocols except Cdocker, performance decreased as hydrophobic interactions tended to dominate, perhaps indicating that the current understanding of such interactions leaves much to be explored. While further discussion of strategies implemented in docking algorithms and their limitations is beyond the scope of this review, the reader is directed to the following papers [41-46].

At this time, it is generally accepted that docking alone is not a reliable method for drug repurposing until there is a more accurate understanding of the non-covalent interactions that contribute to binding. However, it is imperative to note that protein-centric approaches are not dependent on docking for success. In the following section, we will discuss how structural information of protein target binding sites may lead to successful drug repurposing.

Binding Sites

A common theme in pharmacology is the notion of ligand-binding site complementarity. While this concept of complementarity and its various definitions is heavily discussed in the literature, drug repurposing has more recently focused on its structural aspect (i.e. binding site three-dimensional structure). In this section we will discuss only the ideology and outcomes of this focus, but a thorough review of binding site comparison methods can be found in the review article by Haupt and Schroeder [47]. The key aspect of using binding site structure is that the three-dimensional orientation of residues in space does not necessarily reflect sequence similarities across proteins, and that disparate families and folds of proteins may conserve binding site properties, therefore binding similar molecules.

The hypothesis that similar binding sites bind similar molecules is the main assumption in a protein target-centric approach to drug repurposing. While there have been successes using this assumption (discussed below), to our knowledge there is no evidence that directly substantiates it. We have devised a rapid computational proteo-chemometric method called "Train, Match, Fit, Streamline" (TMFS) to map a new drug-target interaction space and predict new uses [48]. TMFS incorporates ligand docking and functional contact points, as well as a combination of shape, topology and chemical signatures, to predict ligand-protein signatures with significant accuracy. We further implemented these predicted signatures and we compared the shape and topology of every permutation of protein target pairs via their protomols and residue configurations, respectively. More precisely, we investigated whether an increasing amount of dissimilarity in binding site shape and structural topology correlated with increasing dissimilarity of their predicted ligands. We also performed the same procedure with respect to the protein targets' co-crystallized ligands from their respective PDB entries, which served as our control. Our analysis of over 1 million non-redundant data points yielded the trend that increasingly similar binding sites do in fact bind increasingly structurally similar molecules, a finding we hope will lay down a foundation to pursue future protein-centric drug repurposing.

Several groups have nonetheless succeeded in repurposing drugs for new indications based on this premise of binding site similarity. Kinnings *et al.* predicted and experimentally validated the binding of entacapone to enol-acyl carrier protein reductase, an essential component of fatty acid synthesis in Mycobacterium tuberculosis [49]. This finding is very promising as entacapone, a small-molecule therapeutic for Parkinson's disease through its binding of catechol-O-methyl-transferase, may potentially be used for eliminating multi drug-resistant strains of this bacterium. Di Franchi *et al.* successfully validated the binding of staurosporine, a broad-spectrum kinase inhibitor, to synapsin I, a phosphoprotein involved in synaptogenesis and neurotransmitter release modulation [50]. In addition, Pepin *et al.* demonstrated the feasibility of repurposing eflornithine, an antitumorigenic, for African trypanosomiasis through interaction with parasitic ornithine decarboxylase [51]. While these examples are just a sampling of successes, the exploitation of binding site similarity has become a more prominent protein-centric approach and is being pursued from a variety of perspectives ranging from empirical structural relationships to macroscopic networks and correlations.

NETWORK APPROACHES

While drug repurposing so far has been discussed from the lens of chemo- and proteoinformatics, these approaches remain confined within the boundaries of genetic reductionism- that is, the preconceived notion of "one gene, one disease" wherein drugs are sought-after for their ability to specifically target a single gene's phenotypic products and therefore alter a disease's progression. However, in the past decade with the advent of systems biology, it has become clear that this linear assumption is an oversimplification and that diseases are more likely to be polygenic and polyphenotypic. This biological "robustness" has been revealed in numerous studies ranging from gene knock-outs [52-55] to network analyses of biological pathways and their interactions [56-57]. In essence, disease phenotypes are likely to encompass multiple gene/protein interactions, with multiple diseases displaying some redundancy among each other in those interactions while also retaining those that are unique to them. This is what links seemingly disparate diseases on the basis of common aspects in pathophysiology, and further explains why certain prototypical drugs can be re-applied to diverse pathologies, a utility that until recently would have been incomprehensible. In this context, the aim of drug development/repurposing shifts toward perturbation of robust disease phenotypic networks [58-60]. Examples of elusive diseases for which network pharmacology is currently being applied to include Alzheimer's Disease [61], tinnitus [62], cancers [63], among many others.

Cheng *et al.* used three supervised inference models based on complex network theory to predict drug-target interactions and drug repurposing [64]. Using experimentally-derived drug-target interactions from DrugBank to construct the initial network, they found that the networks-based inference (NBI) model was the most accurate across the benchmarks they proposed. Using the NBI model, they predicted that simvastatin and ketoconazole would target estrogen receptors and further validated their antiproliferative activities via MTT assays on the human breast cancer cell line MDA-MB-231. Interestingly, the NBI model only takes into account network topology whereas the drug- and target-based inference models depend on structural and genomic sequence similarity, respectively. However, the limitation of the NBI model is that it is unable to make predictions unless prior information is available, and therefore can only be used for drug repurposing and not new drug-target interactions. In light of this limitation, there is potential to combine the NBI method with others for the target prediction of a new drug with no prior information.

Capitalizing on this concept of network topology, Campillos *et al.* created a network of drugs predicted to have common protein targets on the basis of their side effects [65]. Out of their 1018 drug-drug relations, 261 were unexpected predictions where drug pairs were chemically dissimilar and came from different therapeutic categories, and *in vitro* analyses further validated the predicted activities. While these verifications are from the perspective of off-target side effects rather than drug repurposing, this study is a seminal cornerstone for future drug repositioning as this sort of drug-target prediction network is novel in successfully using known drug side-effects as the primary means of prediction instead of focusing on drug- or target-based methods. Through validation of side effect-driven drug-target signatures, current drugs can be repurposed as new lead drugs for the off-targets and allow for further optimization. More recently, Lounkine *et al.* also had great success in predicting drug activity by devising a drug-target-adverse reaction network [30].

While these studies provide more of a "proof-of-concept", a few groups have also been successful at using networks for drug repurposing. For example, Zhang *et al.* developed a computational method to predict targets for the compound rhein utilizing a platform that integrates pathway, protein-protein interactions and differential genome expres-

sion [66]. Keiser *et al.* was also successful in constructing a drug-target network representing target similarity on the basis of ligand structural similarities, which was used for the prediction and subsequent validation of 23 novel drug-target associations, such as inhibition of the 5-HT transporter by the ion channel drug Vadilex [67].

The concept of network pharmacology is indeed gaining ground as the assertion of one drug – one target – one disease is no longer dogma. This is exemplified by instances of modern drugs that act promiscuously, such as Nelfinavir, which weakly inhibits multiple kinases to exert its anticancer effect [68]. Multi-faceted networks that take into account a variety of biological and chemical parameters provide a more comprehensive approach to difficult pathologies (i.e. Alzheimer's Disease), and initiatives such as PROMISCUOUS [69] are providing public databases for network-based drug repurposing. Interestingly, while there is a wealth of information relating drugs to disease biological profiles, there is a scarcity of databases containing clinically oriented metrics that could potentially be amendable for chemical systems biology. While drug repurposing has been focused on network parameters such as gene expression patterns, pathway activation, binding affinities and so on, these still remain surrogates to actual clinical significance and outcomes. The ultimate tool that can potentially be utilized for accurate and precise drug repurposing predictions is a direct drug-to-clinical disease outcome comprehensive network that can be further subdivided by patient demographics such ethnic background, age, co-morbidities or even genetic polymorphisms. Clearly, the more clinically-oriented the data, the more likely we would be able to accomplish drug repurposing with confidence and within the framework of pharmacogenomics to tailor it to specific patient populations. As such, we call for a widespread initiative to centralize this data in a public database that could be manipulated for the purpose of drug repositioning.

REPURPOSING IN ACTION: NELFINAVIR

Anti-HIV drugs have been promising targets for repurposing as cancer therapeutics [70]. Of interest is the class of protease inhibitors, which exerts antitumor activity via a non-immune-mediated mechanism. Nelfinavir has received much attention as the most potent broad-spectrum anticancer agent of the protease inhibitors [71].

Nelfinavir's molecular targets in cancer cells have been unknown until recently. Through an integrative computational approach, Xie *et al.* discovered the putative human targets to be multiple members of the protein kinase-like superfamily [68]. Their pipeline consisted of 5,985 PDB binding site characterization and comparison to that of the HIV protease dimer structure, docking of nelfinavir to the filtered targets, molecular dynamics (MD) simulations with binding free energy calculations, and finally a biological network analysis. Their finding of nelfinavir's multi-kinase inhibition complements network studies that indicate polypharmacological multiple node failures as most likely to cause system failure in cancer [72-73]. Interestingly, they conclude that the anticancer effects are a result of weak binding to multiple kinases upstream of the PI3K/Akt pathway.

This outcome not only highlights the importance of an integrated computational approach for drug repurposing, but also provides a different hypothesis for the mechanism of action of a potential antitumor therapeutic. Nelfinavir's polypharmacology may not only contribute to off-target effects, but also to the potential of overcoming previously therapy-resistant cancer types. Furthermore, given that nelfinavir is able to inhibit as the growth of HER2-positive breast cancer cells, at concentrations comparable to doses used among HIV patients [74], nelfinavir stands a good chance of entering the group of antitumorigenics.

FUTURE DIRECTIONS

The ultimate goal of any computational drug repurposing effort is to achieve perfect prediction accuracy with the greatest efficiency. Each of the previously discussed modalities has its strengths and weaknesses, but individually rarely achieves the desired outcome. As processing power, data structures and memory are advancing at an exponential pace, there is a push for a more integrative approach in which computations can be distributed in parallel in order to maintain computational efficiency while obtaining maximal accuracy.

Chemoinformatics has become an increasingly complex field comprised of a wealth of biochemical data that is ripe for mining, especially with the recent increase in public accessibility to chemical databases on the internet. The use of computational and mathematical approaches to build pharmacological and clinical associations provides unique perspectives to the biochemical data that may otherwise go unnoticed. It is widely understood that pharmacodynamics cannot be explained by a single dimension such as complementary binding, and that the sum of all pharmacodynamics modalities are not necessarily additive. To optimize the success of drug repurposing endeavors, it is imperative that this interplay of working parts is elucidated. As previously mentioned, our novel and completely *in silico* TMFS method predicts ligand-protein associations with high accuracy using a combination of metrics across various modalities [48]. With this success, we then used TMFS to predict the repurposing of and experimentally validated, mebendazole, an anti-parasitic that can inhibit to VEGFR2 with anti-cancer properties, and celecoxib, an anti-inflammatory agent that can bind to the adhesion molecule cadherin-11, which is important in rheumatoid arthritis and poor prognosis malignancies.

While the molecular and chemical mechanics are indeed major players in drug repurposing endeavors, the endpoint perspective of disease phenotypes has also been gaining ground. In a large-scale scale study, Gottlieb *et al.* successfully created PREDICT, a method for similarity-based inference of new drug indications based on drug-drug and disease-disease similarities [75]. By implementing gene expression profiles, similar to CMap, PREDICT is able to contribute in the arena of personalized medicine, important in next generation pharmacogenetics and pharmacogenomics. The integration of genetic/phenotypic signatures with biochemical/biophysical as well as network modalities would allow for not just more accurate drug repurposing, but also to a pharmacotherapy that can be fine-tuned to the individual

patient at a resolution far exceeding that previously been known.

Just as the original idea that one gene corresponds to one phenotype has been reshaped, so is the old axiom of "one drug, one protein". The effects that therapeutic drugs have on the human body are interconnected on various levels, starting from the most basic level of a single protein to the vast phenotypic network resulting from the interplay of multiple pathways, functions and expression patterns that occur in precise timing. In moving forward, drug repurposing efforts of the future must keep not only this, but also the individual patient, within sight.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This project has been funded in part with federal funds from the National Institutes of Health Grants NIH R01-CA129813, NIH R01-CA170653 NIH P01CA130821, NIH HHSN261200800001E, and NIH UL1TR000101. We acknowledge the Georgetown-Lombardi Cancer Center support grant P30CA51008.

REFERENCES

[1] Ashburn, T. T.; Thor, K. B. Drug repositioning: identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.*, **2004**, *3*, 673-683.

[2] Paul, S. M.; Mytelka, D. S.; Dunwiddie, C. T.; Persinger, C. C.; Munos, B. H.; Lindborg, S. R.; Schacht, A. L. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.*, **2010**, *9*, 203-214.

[3] Huang, R.; Southall, N.; Wang, Y.; Yasgar, A.; Shinn, P.; Jadhav, A.; Nguyen, D. T.; Austin, C. P. The NCGC pharmaceutical collection: a comprehensive resource of clinically approved drugs enabling repurposing and chemical genomics. *Sci. Transl. Med.*, **2011**, *3*, 80ps16.

[4] Collins, F. S. Mining for therapeutic gold. *Nat. Rev. Drug Discov.*, **2011**, *10*, 397.

[5] DiMasi, J. A.; Hansen, R. W.; Grabowski, H. G. The price of innovation: new estimates of drug development costs. *J. Health Econ.*, **2003**, *22*, 151-185.

[6] Hughes, B., 2007 FDA drug approvals: a year of flux. *Nat. Rev. Drug Discov.*, **2008**, *7*, 107-109.

[7] Lawrence, S. Drug output slows in, 2006. *Nat. Biotechnol.*, **2007**, *25*, 1078.

[8] Buehler, L.K. Advancing drug discovery – beyond design. *Pharmacogenomics*, **2004**, *4*, 24-26.

[9] Wagner, B.; Kitami, T.; Gilbert, T.; Peck, D.; Ramanathan, A.; Schreiber, S.; Golub, T.; Mootha, V. Large-scale chemical dissection of mitochondrial function. *Nat. Biotechnol.*, **2008**, *26*, 343-351.

[10] Bajorath, J. Computational analysis of ligand relationships within target families. *Curr. Opin. Chem. Biol.*, **2008**, *12*, 352-358.

[11] Johnson, M.A.; Maggiora, G.M. *Concepts and applications of molecular similarity*; Wiley:New York,, **19**90.

[12] Matter, H. Selecting optimally diverse compounds from structure databases: a validation study of two-dimensional and three-dimensional molecular descriptors. *J. Med. Chem.*, **1997**, *40*, 1219-1229.

[13] Whittle, M.; Gillet, V. J.; Willett, P.; Alex, A.; Loesel, J. Enhancing the effectiveness of virtual screening by fusing nearest neighbor lists: a comparison of similarity coefficients. *J. Chem. Inf. Comput. Sci.*, **2004**, *44*, 1840-1848.

[14] Perez-Nueno, V. I.; Ritchie, D. W. Using consensus-shape clustering to identify promiscuous ligands and protein targets and to choose the right query for shape-based virtual screening. *J. Chem. Inf. Model*, **2011**, *51*, 1233-1248.

[15] Warner, W. A.; Sanchez, R.; Dawoodian, A.; Li, E.; Momand, J. Identification of FDA-approved Drugs that Computationally Bind to MDM2. *Chem. Biol. Drug Des.*, **2012**, *80*, 631-637.

[16] Steindl, T. M.; Schuster, D.; Laggner, C.; Langer, T. Parallel screening: a novel concept in pharmacophore modeling and virtual screening. *J. Chem. Inf. Model.*, **2006**, *46*, 2146-2157.

[17] Keiser, M. J.; Roth, B. L.; Armbruster, B. N.; Ernsberger, P.; Irwin, J. J.; Shoichet, B. K. Relating protein pharmacology by ligand chemistry. *Nat. Biotechnol.*, **2007**, *25*, 197-206.

[18] Willett, P. Searching techniques for databases of two- and three-dimensional chemical structures. *J. Med. Chem.*, **2005**, *48*, 4183-4199.

[19] Paolini, G. V.; Shapland, R. H.; van Hoorn, W. P.; Mason, J. S.; Hopkins, A. L. Global mapping of pharmacological space. *Nat. Biotechnol.*, **2006**, *24*, 805-815.

[20] Vieth, M.; Higgs, R. E.; Robertson, D. H.; Shapiro, M.; Gragg, E. A.; Hemmerle, H. Kinomics-structural biology and chemogenomics of kinase inhibitors and targets. *Biochim. Biophys. Acta*, **2004**, *1697*, 243-257.

[21] Izrailev, S.; Farnum, M. A. Enzyme classification by ligand binding. *Proteins.*, **2004**, *57*, 711-724.

[22] Bender, A.; Jenkins, J. L.; Glick, M.; Deng, Z.; Nettles, J. H.; Davies, J. W. "Bayes affinity fingerprints" improve retrieval rates in virtual screening and define orthogonal bioactivity space: when are multitarget drugs a feasible concept? *J. Chem. Inf. Model*, **2006**, *46*, 2445-2456.

[23] Nidhi; Glick, M.; Davies, J. W.; Jenkins, J. L. Prediction of biological targets for compounds using multiple-category Bayesian models trained on chemogenomics databases. *J. Chem. Inf. Model.*, **2006**, *46*, 1124-1133.

[24] Schuffenhauer, A.; Floersheim, P.; Acklin, P.; Jacoby, E. Similarity metrics for ligands reflecting the similarity of the target proteins. *J. Chem. Inf. Comput. Sci.*, **2003**, *43*, 391-405.

[25] Horvath, D.; Jeandenans, C. Neighborhood behavior of in silico structural spaces with respect to *in vitro* activity spaces-a novel understanding of the molecular similarity principle in the context of multiple receptor binding profiles. *J. Chem. Inf. Comput. Sci.*, **2003**, *43*, 680-690.

[26] Bolton, E. E.; Chen, J.; Kim, S.; Han, L.; He, S.; Shi, W.; Simonyan, V.; Sun, Y.; Thiessen, P. A.; Wang, J.; Yu, B.; Zhang, J.; Bryant, S. H. PubChem3D: a new resource for scientists. *J. Cheminform.*, **2011**, *3*, 32.

[27] Lamb, J.; Crawford, E. D.; Peck, D.; Modell, J. W.; Blat, I. C.; Wrobel, M. J.; Lerner, J.; Brunet, J. P.; Subramanian, A.; Ross, K. N.; Reich, M.; Hieronymus, H.; Wei, G.; Armstrong, S. A.; Haggarty, S. J.; Clemons, P. A.; Wei, R.; Carr, S. A.; Lander, E. S.; Golub, T. R. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science.*, **2006**, *313*,, **19**29-1935.

[28] Josset, L.; Textoris, J.; Loriod, B.; Ferraris, O.; Moules, V.; Lina, B.; N'guyen, C.; Diaz, J. J.; Rosa-Calatrava, M. Gene expression signature-based screening identifies new broadly effective influenza a antivirals. *PLoS One*, **2010**, *5*, e13169.

[29] Cheng, T.; Li, Q.; Wang, Y.; Bryant, S. H. Identifying compound-target associations by combining bioactivity profile similarity search and public databases mining. *J. Chem. Inf. Model*, **2011**, *51*, 2440-2448.

[30] Lounkine, E.; Keiser, M. J.; Whitebread, S.; Mikhailov, D.; Hamon, J.; Jenkins, J. L.; Lavan, P.; Weber, E.; Doak, A. K.; Cote, S.; Shoichet, B. K.; Urban, L. Large-scale prediction and testing of drug activity on side-effect targets. *Nature.*, **2012**, *486*, 361-367.

[31] Chen, M. H.; Yang, W. L.; Lin, K. T.; Liu, C. H.; Liu, Y. W.; Huang, K. W.; Chang, P. M.; Lai, J. M.; Hsu, C. N.; Chao, K. M.; Kao, C. Y.; Huang, C. Y. Gene expression-based chemical genomics identifies potential therapeutic drugs in hepatocellular carcinoma. *PLoS One*, **2011**, *6*, e27186.

[32] Sirota, M.; Dudley, J. T.; Kim, J.; Chiang, A. P.; Morgan, A. A.; Sweet-Cordero, A.; Sage, J.; Butte, A. J. Discovery and preclinical validation of drug indications using compendia of public gene expression data. *Sci. Transl. Med.*, **2011**, *3*, 96ra77.

[33] Oprea, T. I.; Nielsen, S. K.; Ursu, O.; Yang, J. J.; Taboureau, O.; Mathias, S. L.; Kouskoumvekaki, L.; Sklar, L. A.; Bologa, C. G. Associating Drugs, Targets and Clinical Outcomes into an Inte-

grated Network Affords a New Platform for Computer-Aided Drug Repurposing. *Mol. Inform.*, **2011**, *30*, 100-111.

[34] Scheiber, J.; Jenkins, J. L.; Sukuru, S. C.; Bender, A.; Mikhailov, D.; Milik, M.; Azzaoui, K.; Whitebread, S.; Hamon, J.; Urban, L.; Glick, M.; Davies, J. W. Mapping adverse drug reactions in chemical space. *J. Med. Chem.*, **2009**, *52*, 3103-3107.

[35] Laggner, C.; Kokel, D.; Setola, V.; Tolia, A.; Lin, H.; Irwin, J. J.; Keiser, M. J.; Cheung, C. Y.; Minor, D. L.,Jr; Roth, B. L.; Peterson, R. T.; Shoichet, B. K. Chemical informatics and target identification in a zebrafish phenotypic screen. *Nat. Chem. Biol.*, **2011**, *8*, 144-146.

[36] Li, J.; Zhu, X.; Chen, J. Y. Building disease-specific drug-protein connectivity maps from molecular interaction networks and PubMed abstracts. *PLoS Comput. Biol.*, **2009**, *5*, e1000450.

[37] Iorio, F.; Bosotti, R.; Scacheri, E.; Belcastro, V.; Mithbaokar, P.; Ferriero, R.; Murino, L.; Tagliaferri, R.; Brunetti-Pierri, N.; Isacchi, A.; di Bernardo, D. Discovery of drug mode of action and drug repositioning from transcriptional responses. *Proc. Natl. Acad. Sci. USA*, **2010**, *107*, 14621-14626.

[38] Jin, G.; Fu, C.; Zhao, H.; Cui, K.; Chang, J.; Wong, S. T. A novel method of transcriptional response analysis to facilitate drug repositioning for cancer therapy. *Cancer Res.*, **2012**, *72*, 33-44.

[39] DesJarlais, R. L.; Seibel, G. L.; Kuntz, I. D.; Furth, P. S.; Alvarez, J. C.; Ortiz de Montellano, P. R.; DeCamp, D. L.; Babe, L. M.; Craik, C. S. Structure-based design of nonpeptide inhibitors specific for the human immunodeficiency virus 1 protease. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 6644-6648.

[40] Bohari, M. H.; Sastry, G. N. FDA approved drugs complexed to their targets: evaluating pose prediction accuracy of docking protocols. *J. Mol. Model*, **2012**, *18*, 4263-4274.

[41] Bissantz, C.; Folkers, G.; Rognan, D. Protein based virtual screening of chemical databases. 1. Evaluation of different docking/scoring combinations. *J. Med. Chem.*, **2000**, *43*, 4759-4767.

[42] Schulz-Gasch, T.; Stahl, M. Binding site characteristics in structure based virtual screening: Evaluation of current docking tools. *J. Mol. Model*, **2003**, *9*, 47-57.

[43] Ferrara, P; Gohlke, H.; Price, D.J.; Klebe, G.; Brooks, C.L. Assessing scoring functions for protein-ligand interactions. *J. Med. Chem.*, **2004**, *47*, 3032-3047.

[44] Kellenberger, E.; Rodrigo, J.; Muller, P.; Rognan, D. Comparative evaluation of eight docking tools for docking and virtual screening accuracy. *Proteins Struct. Funct. Bioinf.*, **2004**, *57*, 225-242.

[45] Englebienne, P.; Fiaux, H.; Kuntz, D.A.; Corbeil, C.R.; Gerber-Lemaire, S.; Rose, D.R.; Moitessier, N. Evaluation of docking programs for predicting binding of golgimannosidase II inhibitors: a comparison with crystallography. *Proteins Struct. Funct. Bioinf.*, **2007**, *69*, 60-176.

[46] Cross, J.B.; Thompson, D.C.; Rai, B.K. Comparison of several molecular docking programs: Pose prediction and virtual screening accuracy. *J. Chem. Inf. Model.*, **2009**, *49*, 1455-1474.

[47] Haupt, V. J.; Schroeder, M. Old friends in new guise: repositioning of known drugs with structural bioinformatics. *Brief Bioinform.*, **2011**, *12*, 312-326.

[48] Dakshanamurthy, S.; Issa, N. T.; Assefnia, S.; Seshasayee, A.; Peters, O. J.; Madhavan, S.; Uren, A.; Brown, M. L.; Byers, S. W. Predicting new indications for approved drugs using a proteochemometric method. *J. Med. Chem.*, **2012**, *55*, 6832-6848.

[49] Kinnings, S. L.; Liu, N.; Buchmeier, N.; Tonge, P. J.; Xie, L.; Bourne, P. E. Drug discovery using chemical systems biology: repositioning the safe medicine Comtan to treat multi-drug and extensively drug resistant tuberculosis. *PLoS Comput. Biol.*, **2009**, *5*, e1000423.

[50] Defranchi, E.; Schalon, C.; Messa, M.; Onofri, F.; Benfenati, F.; Rognan, D. Binding of protein kinase inhibitors to synapsin I inferred from pair-wise binding site similarity measurements. *PLoS One*, **2010**, *5*, e12214.

[51] Pepin, J.; Milord, F.; Guern, C.; Schechter, P.J. Difluoromethylornithine for arseno-resistant Trypanosoma brucei gambiense sleeping sickness. *Lancet*, **1987**, *2*, 1431-1433.

[52] Hopkins, A. L.; Groom, C. R. The druggable genome. *Nat. Rev. Drug Discov.*, **2002**, *1*, 727-730.

[53] Austin, C. P.; Battey, J. F.; Bradley, A.; Bucan, M.; Capecchi, M.; Collins, F. S.; Dove, W. F.; Duyk, G.; Dymecki, S.; Eppig, J. T.; Grieder, F. B.; Heintz, N.; Hicks, G.; Insel, T. R.; Joyner, A.; Koller, B. H.; Lloyd, K. C.; Magnuson, T.; Moore, M. W.; Nagy, A.; Pollock, J. D.; Roses, A. D.; Sands, A. T.; Seed, B.; Skarnes, W. C.; Snoddy, J.; Soriano, P.; Stewart, D. J.; Stewart, F.; Stillman, B.; Varmus, H.; Varticovski, L.; Verma, I. M.; Vogt, T. F.; von Melchner, H.; Witkowski, J.; Woychik, R. P.; Wurst, W.; Yancopoulos, G. D.; Young, S. G.; Zambrowicz, B. The knockout mouse project. *Nat. Genet.*, **2004**, *36*, 921-924.

[54] Zambrowicz, B. P.; Sands, A. T. Knockouts model the 100 best-selling drugs--will they model the next 100? *Nat. Rev. Drug Discov.*, **2003**, *2*, 38-51.

[55] Zambrowicz, B. P.; Turner, C. A.; Sands, A. T. Predicting drug efficacy: knockouts model pipeline drugs of the pharmaceutical industry. *Curr. Opin. Pharmacol.*, **2003**, *3*, 563-570.

[56] Barabasi, A. L.; Oltvai, Z. N. Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.*, **2004**, *5*, 101-113.

[57] Albert, R.; Jeong, H.; Barabasi, A. L. Error and attack tolerance of complex networks. *Nature.*, **2000**, *406*, 378-382.

[58] Chen, Y.; Zhu, J.; Lum, P. Y.; Yang, X.; Pinto, S.; MacNeil, D. J.; Zhang, C.; Lamb, J.; Edwards, S.; Sieberts, S. K.; Leonardson, A.; Castellini, L. W.; Wang, S.; Champy, M. F.; Zhang, B.; Emilsson, V.; Doss, S.; Ghazalpour, A.; Horvath, S.; Drake, T. A.; Lusis, A. J.; Schadt, E. E. Variations in DNA elucidate molecular networks that cause disease. *Nature*, **2008**, *452*, 429-435.

[59] Csermely, P.; Agoston, V.; Pongor, S. The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol. Sci.*, **2005**, *26*, 178-182.

[60] Korcsmaros, T.; Szalay, M.S.; Bode, C.; Kovacs, I.; Csermely, P. How to design multi-target drugs: target search options in cellular networks. *Expert Opin. Drug Discov.*, **2007**, *2*, 1-10.

[61] Liang, D.; Han, G.; Feng, X.; Sun, J.; Duan, Y.; Lei, H. Concerted perturbation observed in a hub network in Alzheimer's disease. *PLoS One.*, **2012**, *7*, e40498.

[62] Elgoyhen, A. B.; Langguth, B.; Vanneste, S.; De Ridder, D. Tinnitus: network pathophysiology-network pharmacology. *Front. Syst. Neurosci.*, **2012**, *6*, 1.

[63] Chu, L.H.; Chen, B.S. Construction of a cancer-perturbed protein-protein interaction network for discovery of apoptosis drug targets. *BMC Syst. Biol.*, **2008**, *2*, 56.

[64] Cheng, F.; Liu, C.; Jiang, J.; Lu, W.; Li, W.; Liu, G.; Zhou, W.; Huang, J.; Tang, Y. Prediction of drug-target interactions and drug repositioning via network-based inference. *PLoS Comput. Biol.*, **2012**, *8*, e1002503.

[65] Campillos, M.; Kuhn, M.; Gavin, A. C.; Jensen, L. J.; Bork, P. Drug target identification using side-effect similarity. *Science.*, **2008**, *321*, 263-266.

[66] Zhang, A.; Sun, H.; Yang, B.; Wang, X. Predicting new molecular targets for rhein using network pharmacology. *BMC Syst. Biol.*, **2012**, *6*, 20.

[67] Keiser, M. J.; Setola, V.; Irwin, J. J.; Laggner, C.; Abbas, A. I.; Hufeisen, S. J.; Jensen, N. H.; Kuijer, M. B.; Matos, R. C.; Tran, T. B.; Whaley, R.; Glennon, R. A.; Hert, J.; Thomas, K. L.; Edwards, D. D.; Shoichet, B. K.; Roth, B. L. Predicting new molecular targets for known drugs. *Nature*, **2009**, *462*, 175-181.

[68] Xie, L.; Evangelidis, T.; Xie, L.; Bourne, P. E. Drug discovery using chemical systems biology: weak inhibition of multiple kinases may contribute to the anti-cancer effect of nelfinavir. *PLoS Comput. Biol.*, **2011**, *7*, e1002037.

[69] von Eichborn, J.; Murgueitio, M. S.; Dunkel, M.; Koerner, S.; Bourne, P. E.; Preissner, R. PROMISCUOUS: a database for network-based drug-repositioning. *Nucleic Acids Res.*, **2011**, *39*, D1060-6.

[70] Chow, W. A.; Jiang, C.; Guan, M. Anti-HIV drugs for cancer therapeutics: back to the future? *Lancet Oncol.*, **2009**, *10*, 61-71.

[71] Gills, J. J.; Lopiccolo, J.; Tsurutani, J.; Shoemaker, R. H.; Best, C. J.; Abu-Asab, M. S.; Borojerdi, J.; Warfel, N. A.; Gardner, E. R.; Danish, M.; Hollander, M. C.; Kawabata, S.; Tsokos, M.; Figg, W. D.; Steeg, P. S.; Dennis, P. A. Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis *in vitro* and *in vivo*. *Clin. Cancer Res.*, **2007**, *13*, 5183-5194.

[72] Parsons, D. W.; Jones, S.; Zhang, X.; Lin, J. C.; Leary, R. J.; Angenendt, P.; Mankoo, P.; Carter, H.; Siu, I. M.; Gallia, G. L.; Olivi, A.; McLendon, R.; Rasheed, B. A.; Keir, S.; Nikolskaya, T.; Nikolsky, Y.; Busam, D. A.; Tekleab, H.; Diaz, L. A.,Jr; Hartigan, J.; Smith, D. R.; Strausberg, R. L.; Marie, S. K.; Shinjo, S. M.; Yan, H.; Riggins, G. J.; Bigner, D. D.; Karchin, R.; Papadopoulos, N.; Parmigiani, G.; Vogelstein, B.; Velculescu, V. E.; Kinzler, K. W.

An integrated genomic analysis of human glioblastoma multiforme. *Science*, **2008**, *321*, 1807-1812.

[73] Jones, S.; Zhang, X.; Parsons, D. W.; Lin, J. C.; Leary, R. J.; Angenendt, P.; Mankoo, P.; Carter, H.; Kamiyama, H.; Jimeno, A.; Hong, S. M.; Fu, B.; Lin, M. T.; Calhoun, E. S.; Kamiyama, M.; Walter, K.; Nikolskaya, T.; Nikolsky, Y.; Hartigan, J.; Smith, D. R.; Hidalgo, M.; Leach, S. D.; Klein, A. P.; Jaffee, E. M.; Goggins, M.; Maitra, A.; Iacobuzio-Donahue, C.; Eshleman, J. R.; Kern, S. E.; Hruban, R. H.; Karchin, R.; Papadopoulos, N.; Parmigiani, G.; Vogelstein, B.; Velculescu, V. E.; Kinzler, K. W. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*, **2008**, *321*, 1801-1806.

[74] Shim, J. S.; Rao, R.; Beebe, K.; Neckers, L.; Han, I.; Nahta, R.; Liu, J. O. Selective Inhibition of HER2-Positive Breast Cancer Cells by the HIV Protease Inhibitor Nelfinavir. *J. Natl. Cancer Inst.*, **2012**, *104*, 1576-1590.

[75] Gottlieb, A.; Stein, G. Y.; Ruppin, E.; Sharan, R. PREDICT: a method for inferring novel drug indications with application to personalized medicine. *Mol. Syst. Biol.*, **2011**, *7*, 496.

Received: January 10, 2013 **Revised: January 29, 2013** **Accepted: January 29, 2013**