Keynote Review

**IN SILICO REPOSITIONING OF APPROVED DRUGS FOR RARE AND
NEGLECTED DISEASES**

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Teaser Sentence:

High-throughput screening has found many new uses for FDA approved drugs and research into neglected or rare / orphan diseases can be further accelerated using *in silico* drug repositioning approaches.

Abstract

Neglected and rare diseases traditionally have not been the focus of large pharmaceutical company research as biotech and academia have primarily been involved in drug discovery efforts for such diseases. This area certainly represents a new opportunity as the pharmaceutical industry investigates new markets. One approach to speed up drug discovery is to examine new uses for existing approved drugs; this is termed drug repositioning or drug repurposing and has become increasingly popular in recent years. Analysis of the literature reveals that using high-throughput screening there have been many examples of FDA approved drugs found to be active against additional targets that can be used to therapeutic advantage for repositioning for other diseases. To date there are far fewer such examples where *in silico* approaches have allowed for the derivation of new uses. It is suggested that with current technologies and databases of chemical compounds (drugs) and related data, as well as close integration with *in vitro* screening data, improved opportunities for drug repurposing will emerge. In this publication a review of the literature will highlight several proof of principle examples from areas such as finding new inhibitors for drug transporters with 3D pharmacophores and uncovering molecules active against *Mycobacterium tuberculosis* (Mtb) using Bayesian models of compound libraries. Research into neglected or rare/orphan diseases can likely benefit from *in silico* drug repositioning approaches and accelerate drug discovery for these diseases.

Key words

Repositioning, repurposing, *in silico*, neglected disease, rare disease, infectious disease, machine learning, pharmacophore, similarity searching.

Introduction

Neglected diseases are tropical infections common in Africa, Asia and the Americas. Infections with *Mycobacterium tuberculosis* (Mtb) or malaria are often included as neglected diseases and are estimated to kill over two million people annually [1]. Recent studies also suggest that over 2 billion individuals are infected with Mtb alone [2] and this represents approximately one-third of the global population. These statistics highlight the enormous economic and healthcare challenges for the countries and governments affected. There are also thousands of diseases that occur in small patient populations, are not addressed by any treatments [3] and are classed as rare or orphan diseases. Neglected and rare diseases traditionally have not been the focus of big pharmaceutical company research as these diseases have small patient populations in industrialized countries that make it difficult to market drugs that recoup the cost of research and development and then are profitable over the long-term. Consequently, drug discovery for neglected and rare diseases has occurred primarily at biotech companies and in academia in recent years. Traditionally, rare diseases have small patient populations though there is no global agreement on what this size is. In the US it is described as a disease that affects less than 200,000 people. Some estimates suggest this represents over 7000 rare diseases affecting 25-30 million people [4] or 5000 patients per orphan disease with approximately 4000 orphan diseases needing treatment [5]. Clearly such a 'small' market size would make drugs for these diseases less marketable compared with cancers, cardiovascular disease, diabetes, etc. which number in the millions treated annually. However, some have suggested that profits can be made on smaller and smaller patient populations in a personalized medicine strategy and have called for more

academia-pharma collaborations focused on rare diseases [5]. There are considerable challenges with regards to clinical research applied to rare diseases. Even though over 300 orphan drugs have been approved since the passage of the Orphan Drugs Act in 1983, there is still a long way to go until most diseases have a treatment [4,5].

This situation is changing primarily because pharmaceutical companies may see these rare or neglected diseases as a way to bring in more revenue as well as improve public relations. Developing treatments for rare or orphan diseases may necessitate a smaller investment upfront as, for example, in-licensing deals for an advanced therapeutic candidate targeting this area are usually less costly than the typical \$100s of millions for licensing drugs for other diseases [6]. Recently we have seen GlaxoSmithKline (GSK) make some relatively small investments in rare diseases [7], and Pfizer [8] and several other large pharma companies, as well as the World Health Organization have been working together and investing \$150M on neglected diseases [9]. These efforts may be only the tip of the iceberg, and more substantial investments will follow in the near future to solidify the trend. These investments by pharma for rare diseases are in addition to their significant investments in neglected or tropical diseases represented by the GSK Tres Cantos facility (<http://www.gsk.com/collaborations/tres-cantos.htm>), the Novartis Institute for Tropical Diseases in Singapore (<http://www.novartis.com/research/nitd/index.shtml>), the Lilly MDR-TB Partnership (<http://www.lillymdr-tb.com/>), the Lilly TB drug discovery initiative (<http://www.tbdrugdiscovery.org/>) and The Critical Path to TB Drug Regimens (<http://www.tballiance.org/cptr/>).

One approach to speeding up drug discovery is to find new uses for existing approved drugs. This is termed **drug repositioning or drug repurposing**, and traditionally has occurred by serendipity [10]. Another strategy is to look at combinations of approved drugs in the hope of finding synergy [11,12], an approach that has found some success in cancer chemotherapy. In the neglected and rare disease space academic and industry researchers look at repositioning compounds that are already approved for other indications (see references in Table 1 and Table 2). Drug repositioning has been reviewed extensively in the context of finding uses for drugs applied to major diseases such as obesity and Parkinson's disease [10]. Well known examples include thalidomide, sildenafil, bupropion and fluoxetine which found new uses beyond their initially approved therapeutic uses [10]. The example of thalidomide specifically suggests that drugs that were originally withdrawn by manufacturers or removed by the FDA, or other regulatory organizations, may be resurrected. Thalidomide was notorious for causing birth defects if taken during the first trimester of pregnancy. However, this adverse effect is not a major issue in the novel use of thalidomide in treating multiple myeloma, a disease which is not common in women of child-bearing age.

For pharmaceutical companies, repositioning has significant commercial value as it extends the markets for a compound and finds new uses for shelved compounds at lower financial risk and in a shorter time [13]. There has also been much discussion on how different approaches to repositioning could work but these have not focused specifically on neglected diseases [10,14]. Others have proposed that repurposing could be an invaluable tool for neglected diseases [15]. The benefits of repositioning include: working on known druggable targets, the availability of materials and data (such as on

long term toxicology) that can be used and presented to regulatory authorities and, as a result, the potential for a significantly more time- and cost-effective research and development effort than typically seen with bringing a new drug to market.

In both the major-market and neglected infectious disease realms the rapid emergence of multidrug resistant strains of pathogenic microorganisms would indicate that we need to quickly identify new scaffolds for antibiotics. This likely requires the exploration of chemical space beyond known active antimicrobial compounds. Pharma urgently needs new hits to initiate compound optimization studies. However, productivity of novel antibiotic classes over the last 30-40 years has been extremely low and this is exacerbated by the relatively low hit rates from high-throughput screening (HTS) and secondary screens [16]. There are several new scaffold search efforts that were recently reviewed [17]. For example, Pfizer has shown that pyridopyrimidine compounds derived from a eukaryotic protein kinase inhibitor pharmacophore were effective against gram negative pathogens following whole cell screening [18]. The approach is an example of **screening library repurposing** (counterbalancing the pessimism derived from recently reported antibacterial-targeted screening efforts [16]) and illustrates the pursuit of bacterial targets with high sequence or structural similarity to eukaryotic targets, in this case the bacterial and eukaryotic kinomes. The Pfizer researchers proposed that targets with high sequence and structural homology to known human drug targets are more likely to find inhibitors in the compound libraries. Others have suggested that the libraries of inhibitors for ion channel and prenyltransferases would be a good starting point for such library repurposing [19] and finding chemotypes for novel antimicrobials.

It is unclear how extensively approved drugs are screened against multidrug resistant strains of bacteria and it may be possible to find new acceptable treatments amongst them. Clearly, much more could be done to reposition existing approved drugs. The following surveys efforts to find new activities for FDA approved drugs. To date these efforts have traditionally focused on *in vitro* screening alone but it is proposed that a combined *in silico-in vitro* approach leveraging databases of molecular structures and their related information from the literature (such as ADME/Tox [20], targets, clinical trials etc.) could be a viable strategy to accelerate research in rare, neglected *and* major diseases.

Searching FDA approved drugs for new activities

High-throughput Screening

It is suggested that there are over 10,000 drugs that have been tested in clinical medicine. This may be reduced to approximately 9,000, given that many represent combinations of other drugs, different salt forms of the same molecule, or biologics (large proteins or antibodies) [21]. However, a physical library of this size does not exist for known drugs that could be screened and a virtual library of these compounds is also lacking. It should be noted that such a virtual library could likely be assembled using some of the public domain databases. Some companies like CEREP (<http://www.cerep.fr>) have screened 2,500 of the FDA drugs and reference compounds against 159 enzymes, receptors, ion channels and transporters and created a database called BioPrint [22] which is a commercial product with a cost likely out of reach of academic researchers. To date multiple groups have screened 1,000-2,000 drugs against different targets or cell types.

The John Hopkins Clinical Compound Library (JHCCL) consists of plated compounds available for screening at a relatively small charge and has been used by some groups [23]. For example, 17 novel inhibitors of *M. tuberculosis* were found after screening 1,514 compounds from the JHCCL [23]. Several new uses for FDA approved drugs have been identified by screening this or other commercially available libraries of drugs or off-patent molecules e.g. the Microsource US drug collection and Prestwick Chemical library (Table 2). The accumulation of large databases of published data and compounds screened against G-protein coupled receptors (GPCRs), such as the psychoactive drug screening program (PDSP) receptorome profile, represent good starting points for finding compounds which are active against receptors of interest. One example described is a potent 5-HT_{2A} ligand that could block the JC virus [24] which can cause the neurologic disease progressive multifocal leukoencephalopathy. A second example suggested side effects for known drugs mediated by the 5-HT_{2B} receptor [24]. The number of examples of groups finding new uses for approved drugs by HTS appears to be growing (Table 2) on a laboratory by laboratory basis. It is intriguing to ponder whether an organized effort to experimentally screen the set of all known drugs against all known targets validated for a given disease would be feasible. Certainly, the potential for success with one disease, let alone many human diseases, appears to be quite significant.

Computational Methods

Computational methods, including target- and ligand-based strategies, are an excellent complement to experimental techniques, and are used widely in industry and academia [25,26]. There have been many studies establishing relationships between

ligand molecular structures and broad biological activities, both on and off-target [27-30]. Several examples using pharmacophore-based studies and searching databases of FDA drugs [31] to find new transporter inhibitors *in vitro* represent attempts at understanding off-target effects. For example, in our previous work we have used pharmacophores for various transporters such as the human peptide transporter 1 (HPEPT1) [32], P-glycoprotein (P-gp) [33], the human organic cation/carnitine transporter (hOCTN2) [34,35] (Figure 1) and the human apical sodium-dependent bile acid transporter (ASBT) [36] to search a subset of FDA approved drugs compiled from a small physician's handbook (SCUT) [31] and to identify previously unknown inhibitors based on *in vitro* testing (Table 3). What is interesting about this approach is that for each transporter we found inhibitors belonging to different therapeutic classes and obviously these represent molecules with overlapping pharmacophores. What has not been examined to date is whether the distinct therapeutic class hits for a single transporter are also shared by other common biological activities. These transporters were selected because of the inhibition of hPEPT1 or P-gp involved in drug-drug interactions [33], the putative role of hOCTN2 inhibition in rhabdomyolysis [34,35] and the potential for drugs inhibiting ASBT to promote several adverse drug reactions (ADRs) including colon cancer [36]. The transporters also represent a class of proteins for which *in vitro* models may be limited in throughput and where *in vivo* study is even more complicated due to the presence of multiple transporters with overlapping substrate specificities. Therefore, the *in silico* – *in vitro* approach has value in targeting testing of compounds with a high probability of activity.

Pharmacophores and 3D database searching could, in theory, be readily used for repositioning. 2D approaches may, however, be more readily available for both similarity and substructure searching and have been used with success in studies to predict cross-reactivity of drugs and drug metabolites with immunoassays utilized in clinical medicine. Common applications of immunoassays include drug of abuse (DOA) screening, endocrinology testing, and therapeutic drug monitoring (TDM). Immunoassays may be limited by the occurrence of false positives (or ‘cross-reactive’ compounds). For example, drugs with structural similarity to amphetamine and methamphetamine such as ephedrine and pseudoephedrine can cross-react with drug of abuse screening assays designed to detect the presence of amphetamine or methamphetamine. Diagnostic companies manufacturing clinically used immunoassays often test a limited number of compounds for cross-reactivity against their immunoassay while there is a potentially large array of compounds (metabolites, herbals, environmental chemicals) that could possibly interact. Consequently, cross-reacting compounds are discovered on a case-by case basis.

Computational 2D similarity (using the MDL public keys fingerprint descriptors) of test compounds to that of the antigen used in immunoassays, has been used to predict cross reactivity. The same SCUT database of frequently used FDA-approved drugs was used for similarity searching and was supplemented with some metabolites of drugs (see Supplemental data). This relatively simple computational approach showed a statistically significant separation between cross reactive and non cross reactive compounds for TDM immunoassays [37,38] and DOA/toxicology immunoassays [39,40], and was further used

to identify novel inhibitors of DOA/toxicology immunoassays [40]. These examples show how modeling can build on existing data and focus *in vitro* testing.

The examples above also illustrate how 2D similarity alone may be useful for finding compounds that may possess similar pharmacophore features to other drugs. This raises the question whether such similar molecules may share overlapping biological activities. Similarity searching could likely be a component of a compound repositioning strategy, using computational methods to predict likely cross reactive compounds by similarity and then quickly confirming with immunoassays that are commercially available. Similarly other computational approaches to searching by similarity, such as those involving LASSO descriptors [41], can make use of large publicly available databases like ChemSpider [42] to compare existing drugs with virtual libraries. Similarly, methods such as PASS (prediction of activity spectrum for substances) could also be used to predict potential new bioactivities for existing drugs [43]. Methods that account for molecular shape may be generally useful for searching for compounds with common bioactivity [44]. Molecular docking is one example that has been successful in finding molecules with complementary shape and electrostatic interactions to known protein active sites. For example, docking approaches have been used to dock 1055 known drugs (from DrugBank) into 78 unique human drug targets and the authors found 52 interactions of interest (although no verification was reported) [45].

Networks and Systems Biology

In the last decade our understanding of biological mechanisms has been significantly enhanced by the curation of vast ligand and protein-protein interaction

databases and the use of top-down and bottom-up network modeling leading to a systems biology approach [46-50]. In the past five years alone, 2D-ligand-based approaches have been increasingly used along with sophisticated algorithms and networks. Fliri *et al.* used biological spectra for a cross section of the proteome [51]. They implemented hierarchical clustering of the biological activity spectra similarity and created a relationship between structure and bioactivity before extending this to identify receptor agonist and antagonist profiles [52]. The same group from Pfizer took this concept further and applied a probabilistic approach to link adverse effects for drugs with biological spectra (similar molecules had overlapping profiles, in the same way that they had similar biological spectra), thus linking preclinical with clinical effects [53]. Such an approach could be useful for repositioning compounds as well as for differentiating compounds that might have some desirable off-target effects, thereby mitigating the undesirable off-target effects.

There have been many efforts to look at compound or protein promiscuity or polypharmacology. Specifically, there has been considerable discussion of predicting undesirable drug interactions with promiscuous proteins *in silico*. This is a particular issue for hydrophobic compounds that may bind to cytochrome P450 (CYP) 3A4, the pregnane X receptor (PXR), P-gp or the human ether-a-go-go-related gene (hERG) [54]. Quantitative structure activity relationship (QSAR) models for these proteins have been used to predict potential molecule-protein interactions and then visualize this as a node on a network, simultaneously showing other endogenous and exogenous ligand-protein interactions [49,50,55] as well as allowing overlay of any gene expression or other high content data [56-58]. Such an approach could be useful for ensuring that repurposed

compounds do not have negative effects on biological networks through binding other off-targets. A global mapping of pharmacological space focusing on a polypharmacology network of 200,000 molecules with activity against 698 proteins has been produced. [59] A further published study created a drug-target network of approved drug data for 890 molecules from DrugBank [60] and OMIM (<http://www.ncbi.nlm.nih.gov/omim>) with over half of these molecules forming the largest connected network with multiple target proteins (also illustrating polypharmacology or promiscuity) [61]. Such networks might help understand likely nodes involved in toxicity and add to the similarity maps for enzymes and receptors [62] and human polypharmacology networks [59] that have also been developed to date. A recent study from Abbott introduced a sequence-independent kinome inhibitor interaction map [63] while another study established links between over 44,000 small molecules and 160 cardiovascular targets, with kinases having on average 7 connections to cardiovascular targets [64]. An example from Berg *et al.*, has merged chemical target and pathway toxicity mechanisms that can be defined from profiling in primary human cell systems covering many readouts and enabling known reference compounds to be mapped by functional similarity [65].

A complimentary approach taken by a group at Novartis uses chemical substructures relevant for toxicology related adverse effects [66] for approximately 4000 molecules with side-effect information from the World Drug Index. The same group related promiscuity of compounds to their safety [67] as compounds hitting several targets cause more undesired effects. More recently the group has related over 4000 MedDRA [68] terms for ADRs for over 1800 drugs using the ECFP_4 descriptors and Bayesian models [69,70]. This resulted in a map of ADRs in chemical space and an

approach that could be used to predict, *in silico*, the ADR likelihood for new molecules based on substructures. Interestingly, the recent similarity ensemble analysis described by Keiser *et al.* also used the ECFP_4 descriptors and Bayesian models to predict off-target effects of 3665 FDA approved drugs and investigational compounds [71]. This study clearly showed the promiscuity of many compounds. Their *in vitro* validation of the computational predictions focused on compounds with predicted GPCR activity other than the known targets. The approach may be particularly useful for understanding the potential targets for compounds where these have been previously unknown.

Machine learning models have also been applied with various types of literature data. Decision tree induction has been used to predict the adverse drug reactions for 507 drugs from the Swiss drugs registry, and resulted in models that looked internally predictive [72]. A machine learning method has also been used with a set of 390 drugs to demonstrate that anatomical therapeutic chemical classification, a system used for drug repurposing, can be predicted by using a binary feature vector derived from extraction of drug property data from text alone [73]. Chiang and Butte have compiled a drug-disease knowledge base (DrDKB) to capture the 3,517 FDA approved drug indications and 8,130 off-label uses of 2,022 distinct drugs used to treat each of 726 diseases [74]. They were able to make 57,542 unique novel drug use suggestions and, leaving out 10 to 20% of the data as a test set, resulted in over 85% recovery of the drug uses [74]. Others have generated a database called PROMISCOUS (<http://bioinformatics.charite.de/promiscuous/index.php?site=drugdev>) representing a set of 25,000 withdrawn or experimental drugs annotated with 21,500 drug-protein and 104,000 protein-protein relationships, using public resources (e.g. DrugBank,

SuperTarget, etc.) and text or data mining [75]. These data can be searched using a network visualization tool and several anecdotal examples were provided of molecule or side effect similarity, although no prospective testing was described [75]. Another tool suggested to be useful for drug repositioning is IDMap which integrates the Elsevier MDDR database, Asinex compounds, PASS and molecular descriptors from Cerius² [76]. Text mining was used to compare PASS and MDDR bioactivity and provide a co-occurrence frequency although again, no prospective testing was shown [76]

By connecting data on drugs, proteins and diseases these various databases, network and computational methods may not only be useful for understanding and identifying promiscuity, polypharmacology and toxicity mechanisms, but also potentially for repurposing molecules for new uses that could focus and accelerate *in vitro* screening efforts [21,24,77-79] as we have previously described with transporters. [32-36]. For some, finding molecules with manageable ADRs may be useful and lead to new indications. Many of these examples illustrate how molecules can be put into a biological context in networks. The integration of approaches and data may lead to a more complete understanding than using a single approach in isolation and could enable network-based drug discovery described elsewhere [80,81]. Others have also suggested that data integration platforms for systems biology (whether using ligand [62,71] or binding site similarity [82]) could support repositioning and drug discovery, although no solid examples of bringing new treatments into the clinic have been provided as yet [83].

Examples specific to infectious diseases

As a proof of principle that computational methods could accelerate neglected disease research, a machine learning method has been used and validated with multiple datasets. Laplacian-corrected Bayesian classifier models are computationally fast and have been used widely for several drug discovery applications in recent years, including with Mtb [84]. Bayesian classification methods with molecular function class fingerprints of maximum diameter 6 (FCFP_6) [85] have been previously used for CYP, transporter and toxicity models [36,86-89] as well as to identify substructures that are important in recent tuberculosis screening datasets [90]. We have validated the Mtb Bayesian models (training sets from 2000 to > 200,000 molecules) with external compounds using the published NIAID, GVKbio datasets (which include known drugs and other experimental compounds) and a set of 102,000 compounds (TAACF-NIAID CB2) containing 1702 molecules with $\geq 90\%$ inhibition at 10 μM (representing a hit rate of 1.66 %) [91]. We were able to show 10 fold enrichments in finding active compounds in the top ranked 600 molecules for the TAACF-NIAID CB2 [91] which came from the same source [90,92] as the training sets used in the original models and represents an ideal scenario from modeling to limit any experimental variability. The 3 test sets ranged from 2880 to over 102,000 compounds. The largest test set also contained a more realistic percentage of hits representative of HTS screens.

More recently we have used a dataset from Novartis of Mtb aerobic assay hits as a test set for the previously described Bayesian models to provide further validation of the approach. Over 35% of the total hits (minimum inhibitory concentration, MIC, < 10 μM) are found with a Bayesian model in the top 8% of molecules (enrichment > 4 fold) [93]. In addition the JHCCL set of 1514 known drugs were screened experimentally against

Mtb and the MIC values determined using the Alamar blue susceptibility assay (published by others [23]). 21 of the actives identified were used as a test set in a larger set of 2108 FDA approved molecules downloaded from the Collaborative Drug Discovery database (CDD) database. After removal of compounds that were also in the Bayesian models, it was shown that the Bayesian models initially had ~10 fold enrichments. One model identified >60% of the drug hits in the top 14% of compounds [93] (Figure 2). The Bayesian models were also used to suggest molecules with high probability of predicted Mtb activity that could be tested *in vitro* in future including: sertaconazole (antifungal), clofarabine (antineoplastic), tioconazole (antifungal) and amodiaquine (antimalarial), quinaldine blue (antineoplastic), atorvastatin (anti-hyperlipidemic) and montelukast (antiasthmatic) [93].

Resources For *In silico* Repositioning Of Molecules

If we are going to try to accelerate rare/orphan and neglected disease research *in silico* what resources are currently available and what are still needed? One accessible tool is the CDD database [94] with a focus on neglected diseases which has been recently described in detail [91,95,96]. Dr. Chris Lipinski (Melior Discovery) provided a database of 1055 FDA approved drugs with designated orphan indications, sponsor name and chemical structures. In addition, Dr. David Sullivan (Johns Hopkins University) collated and provided a database of 2815 FDA approved drugs. Dr. Bryan Roth (University of North Carolina) provided the PDSP database, which currently consists of nearly 1500 molecules structures that have been screened against an array of GPCRs [24,62,78,79].

These data, in addition to the > 20 screening datasets for malaria and tuberculosis (Table 4), have enabled recent analysis of the physicochemical properties of active compounds [91,97,98] and filtering with readily available substructure alerts or “filters” [91,97,98]. All these datasets allow for free access of substructure, similarity or Boolean searches upon registration (e.g., see: <http://www.collaborativedrug.com/register>). The data has also been used for similarity searching and pharmacophore approaches to find mimics of essential metabolites for Mtb [99].

In addition, a license to CDD can enable download of datasets that are not freely available. This may be advantageous if they need to be searched with third party cheminformatics software, e.g. pharmacophore models or QSAR methods etc (Figure 3). This suggests an additional approach for repurposing using *in silico* models to find compounds of interest in the FDA approved drugs set. For instance, models generated with data from one or more public datasets (or the users own private data) could be used to search other datasets and find new molecules for screening (Figure 3). This strategy could be readily taken with other databases and software tools taking advantage of freely available content and tools in databases like ChemSpider [42], PubChem [100], DrugBank [60] and ChEMBL [101] or others [20,102,103]. This overall approach is analogous to the pharmacophore approach we have taken with transporters searching the SCUT database of commonly used drugs (Table 3), similarity searching for drugs cross-reactive with DOA and TDM immunoassays [37-40] and with Mtb Bayesian models [91,95] for searching the FDA approved drugs. Recent efforts to validate the Bayesian models with data from other laboratories (MRC and Novartis) would indicate that the *in silico* approach certainly has some merit for neglected diseases.

What is still needed is a single comprehensive resource that has validated chemical structures (and properties) of both FDA and international approved drugs, as well as those no longer used or removed from the market. A database containing information on studies in which these compounds show activity (e.g. enzyme, receptor, whole cell data, etc.) as well as clinical data would be invaluable. Such a database could then be linked with other mining tools that enable 1D-3D similarity searching. Once created it could be used as the authoritative virtual screening database for repurposing prior to testing physical compounds in whole cells or target assays.

SUMMARY

Analysis of the literature suggests that using HTS, there have been many examples of FDA approved drugs that are found to be active against additional targets that can be used to therapeutic advantage for repositioning. For example, there are several examples for neglected disease including compounds with antimalarial, anti-tuberculosis, trypanosomal and Chagas disease activity (Table 2). To date there are far fewer such examples where *in silico* approaches have derived new uses for approved drugs (Table 2) [82,104]. However, with current technologies and databases, as well as a close integration with *in vitro* screening, this will change. Although computational approaches such as ligand- and structure-based methods have been widely used for searching libraries of commercial compounds for neglected diseases [105], few have tried to use already existing drugs with computational methods [82]. A recently described apparent disconnect has been noted in the Mtb community between the generation and utilization

of computational models for drug discovery [106]. These *in silico* models are not well disseminated and certainly not used for repositioning FDA approved drugs. This situation needs to be rectified. Another important consideration should be the quality of the structures in the databases used whether of FDA drugs or other molecules, as these will impact the *in silico* results [102]. If neglected diseases can benefit from *in silico* methods so can rare or orphan diseases, and of course repositioning approved drugs brings with it other benefits such as 7 years market exclusivity [106]. In our opinion, some or all of the aforementioned *in silico* approaches should be used alongside other methods to drug repurposing, if for no other reason but to speed up the process of drug discovery at little additional cost.

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Conflicts of Interest

SE consults for Collaborative Drug Discovery, Inc on a Bill and Melinda Gates Foundation Grant#49852 “Collaborative drug discovery for TB through a novel database of SAR data optimized to promote data archiving and sharing”.

Supporting Information Available: Supplemental material is available online. The updated SCUT database is provided as an sd file as used in recent similarity studies and pharmacophore searches [35-37,39,40].

ABBREVIATIONS

ASBT, apical sodium-dependent bile acid transporter; CCR5, Chemokine receptor 5; DHFR, Dihydrofolate reductase; DOA, Drugs of abuse, FDA, Food and Drug Administration; GLT1, Glutamate transporter 1; HSP-90, Heat shock protein 90; hERG, human ether-a-go-go-related gene; hPEPT1, human peptide transporter 1; NK-1, neurokinin- 1 receptor; OCTN2, Organic Cation/Carnitine Transporter; P-gp, P-glycoprotein; PXR, pregnane X receptor; QSAR, quantitative structure activity relationship.

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Table 1. Approved drug molecules identified with new uses against other diseases other than originally intended using low throughput methods.

Molecule	Original use	New use	How discovered	Ref
Aprepitant	Nausea – NK-1 receptor antagonist	Drug resistant HIV-1 Infection downregulates CCR5 in macrophages.	Initial hypothesis tested with another NK-1 receptor antagonist <i>in vitro</i> .	[107, 108]
Aprepitant	Nausea – NK-1 receptor antagonist	Cryptosporidiosis in immunosuppressed hosts.	Tested <i>in vivo</i> in immunosuppressed mice infected with <i>Cryptosporidium parvum</i> – decreased substance P levels.	[109]
Amiodarone	Class III anti-arrhythmic	Chagas Disease blocks ergosterol biosynthesis.	Literature search.	[110]
Glybenclamide	Antidiabetic	Antithrombotic activity in mouse models IC ₅₀ 9.6μM.	Common pharmacophore with an	[111]

			experimental TP receptor antagonist SQ29,548.	
Tamoxifen	Antiestrogen	Anti-protozoal – <i>Leishmania amazonensis</i> IC ₅₀ 11.1- 16.4 µM.	Focused screening to test hypothesis and <i>in vivo</i> mice studies.	[112, 113]
Trimetrexate	Anti folate used in <i>Pneumocystis carinii</i> infection in AIDS patients	Inhibitor of <i>T. cruzi</i> Dihydrofolate reductase (DHFR) IC ₅₀ 6.6 nM.	Enzyme activity and antiparasite activity assays for one compound.	[114]
Riluzole	Amyotrophic Lateral Sclerosis - inhibits glutamate release and reuptake	Riluzole is in clinical trials for melanoma but may have activity against other cancers.	Riluzole treatment of GRM1-positive human melanoma cells reduced levels of released glutamate, suppressed human melanoma cell growth and also suppressed tumor growth in xenograft model.	[115]

			induced cell cycle arrest leading to apoptosis.	
Sertraline	Antidepressant (selective serotonin reuptake inhibitor)	Neuroprotective, prolongs survival, improves motor performance and ameliorates brain atrophy in the R6/2 HD model.	Previously shown that another SSRI was neuroprotective.	[116]

Table 2. Approved drug molecules identified with new uses against other diseases other than originally intended using HTS or *in silico* methods.

Molecule	Original use	New use	How discovered	Ref
Itraconazole	Antifungal – lanosterol 14 α -demethylase inhibitor	Inhibition of angiogenesis by inhibiting human lanosterol 14 α -demethylase IC ₅₀ 160nM.	<i>In vitro</i> HUVEC proliferation screen against FDA approved drugs (JHCCL)	[117]
Astemizole	Non-sedating antihistamine (removed from USA market by FDA in 1999)	Antimalarial IC ₅₀ 227nM against <i>P. falciparum</i> 3D7.	<i>In vitro</i> screen for <i>P. Falciparum</i> growth of 1937 FDA approved drugs (JHCCL)	[118]
Mycophenolic acid	Immunosuppressive drug inhibits guanine nucleotide biosynthesis	Inhibition of angiogenesis by targeting Type 1 inosine monophosphate dehydrogenase IC ₅₀ 99.2nM.	<i>In vitro</i> HUVEC proliferation screen 2450 FDA and foreign approved drugs (JHCCL)	[119]
Entacapone and	Parkinson's Disease	Anti-tuberculosis –	Used a chemical	[82]

Tolcapone	– Catechol-O-methyltransferase inhibitors	Entacapone inhibits InhA IC ₅₀ 80µM.	systems biology approach	
Nitazoxanide	Infections caused by <i>Giarda</i> and <i>cryptosporidium</i>	Anti-tuberculosis – multiple potential targets.	Screens against replicating and non replicating M. tuberculosis	[120]
(±)-2-amino-3-phosphonopropionic acid	Human metabolite, mGLUR agonist	Antimalarial – Inhibits HSP90 IC ₅₀ 0.06 µM against <i>P. falciparum</i> 3D7.	HTS screening 4000 compounds	[121]
Acrisorcin	Antifungal	Antimalarial – Inhibits HSP90 IC ₅₀ 0.05 µM against <i>P. falciparum</i> 3D7.	HTS screening 4000 compounds	[121]
Harmine	Anticancer	Antimalarial – Inhibits HSP90 IC ₅₀ 0.05 µM against <i>P. falciparum</i> 3D7.	HTS screening 4000 compounds	[121]
Acetophenazine, Fluphenazine, Periciazine	Antipsychotics – D2 and 5-HT ₂ inhibitors	Human androgen receptor antagonists acetophenazine (K _i 0.8 µM),	Docking of known drugs into Androgen receptor followed by <i>in</i>	[104]

		fluphenazine(K_i 0.8 μ M) , periciazine (K_i 3.0 μ M).	<i>vitro</i> screening	
Levofloxacin, Gatifloxacin, Sarafloxacin, Moxifloxacin, Gemifloxacin	DNA gyrase	Active against ATCC17978 inactive against BAA-1605 MIC <0.03 – 0.04 (mg/L)	Screened microsource drugs library of 1040 drugs versus <i>A. baumannii</i>	[122]
Bithional, Bortezomib, Cantharidin, Chromomycin A3, Duanorubicin, Digitoxin, Ectinascidin 743, Emetine, Fluorosalen, Manidipine HCl, Narasin, Lestaurtinib, Ouabain,	Various	NF- κ B inhibitors IC ₅₀ 0.02-39.8 μ M.	Screened NCGC pharmaceutical collection of 2816 small molecules <i>in vitro</i>	[123]

Sorafenib tosylate, Sunitinib malate, Tioconazole, Tribromsalen, Triclabendazolu m, Zafirlukast				
Pyrvinium pamoate	Antihelmintic	Anti-tuberculosis – Alamar blue assay MIC 0.31 μ M.	<i>In vitro</i> screen against 1514 known drugs – many other previously unidentified hits found.	[23]
Pyrvinium pamoate	Antihelmintic	Anti-protozoal – <i>Cryptosporidium</i> <i>parvum</i> IC ₅₀ 354nM.	<i>In vitro</i> screen for <i>P. Falciparum</i> growth of 1937 FDA approved drugs hypothesized to be active due to confined to intestinal	[124]

			epithelium.	
Pyrvinium pamoate	Antihelmintic	Anti-protozoal – against <i>T Brucei</i> IC ₅₀ 3 µM.	Screened 2160 FDA approved drugs and natural products from Microsource. 15 other drugs active IC ₅₀ 0.2 – 3 µM	[125]
Riluzole	Amyotrophic Lateral Sclerosis - inhibits glutamate release and reuptake	Riluzole enhanced Wnt/β-catenin signaling in both the primary screen in HT22 neuronal cells and in adult hippocampal progenitor cells. Metabotropic glutamate receptor GRM1 regulates Wnt/β-catenin signaling.	Screened 1857 compounds (1500 unique) <i>in vitro</i> treating melanoma cells with riluzole <i>in vitro</i> enhances the ability of WNT3A to regulate gene expression.	[126]
Closantel	A veterinary	Onchocerciasis, or	Screened 1514	[127]

	anthelmintic with known proton ionophore activities	river blindness IC ₅₀ 1.6 µM competitive inhibition constant (K _i) of 468 nM.	FDA approved drugs (JHCCL) against the chitinase OvCHT1 from <i>O. volvulus</i> .	
Nitroxoline	Antibiotic used outside USA for urinary tract infections.	Antiangiogenic agent inhibits Type 2 methionine aminopeptidase (MetAP2) IC ₅₀ 54.8nM and HUVEC proliferation. Also inhibits Sirtuin 1 IC ₅₀ 20.2 µM and Sirtuin 2 IC ₅₀ 15.5 µM.	Screened 2687 FDA approved drugs (JHCCL) for inhibition of HUVEC cells. Also found the same compound in HTS of 175,000 compounds screened against MetAP2. Also active in mouse and human tumor growth models.	[128]
Glafenine	Analgesic	Inhibits ABCG2 IC ₅₀ 3.2 µM could be used with chemotherapeutic	Screened FDA approved drugs (JHCCL) with bioluminescence	[129]

		agents to counteract tumor resistance.	imaging HTS assay. Discovered 37 previously unknown ABCG2 inhibitors.	
Tiagabine	Antiepileptic (enhances gamma – aminobutyric acid activity).	Neuroprotective in N171-82Q and R6/2 mouse models of Huntington’s disease (HD).	Initial screen of NINDS Microsource database of drugs (1040 molecules) against PC12 cell model of HD found nepeptotic acid which is related to tiagabine.	[130]
Digoxin, Oubain, Proscillaridin A	Cardiac glycosides used to treat congestive heart failure and arrhythmia.	Anticancer – Inhibition of hypoxia-inducible factor 1 $IC_{50} < 400$ nM	Screened 3120 FDA approved drugs (JHCCL) screened against reporter cell line Hep3B-c1. Digoxin also tested	[131]

			<i>in vivo</i> xenograft models.	
Tacrine, Carvedilol, Hexamethylenamine, Phenoxybenzamine	Acetylcholinesterase inhibitor, Beta 2adrenergic blocker, diuretic, alpa-1 adrenergic blocker	Prevention of hearing loss – lowest dose tested that shows protection is 10 μ M	Initial screen of NINDS Microsource database of drugs (1040 molecules) against neomycin induced hair cells in zebrafish. Tacrine was also active in mouse utricle	[132]
Ceftriaxone	β -lactam antibiotic	Neuroprotection – Amyotrophic lateral sclerosis (ALS) - increasing glutamate transporter (GLT1) expression EC ₅₀ 3.5 μ M. Other β -lactams also active	Screen of NINDS Microsource database of drugs (1040 molecules) against rat spinal cord cultures followed by immunoblot for GLT1 protein expression. Also	

			tested in ALS mouse model, delaying neuron loss, increased survival.	
Flufenamic acid	Non steroidal anti-inflammatory drug	Familial amyloid polyneuropathy – Inhibits transthyretin.	Screening library not described.	[133]

Table 3. FDA approved compounds found to inhibit transporters by *in silico-in vitro* approach. Additional examples of transporter pharmacophore searches can be found in a recent review [134].

Compounds	Transporter	Biological effect	Pharmacophore features	reference
Aspartame Fluvastatin, Repaglinide,	hPEPT1	Inhibit uptake of natural substrates, other drug that are substrates.	2 Hydrophobic, one hydrogen bond acceptor, one hydrogen bond donor, one negative ionizable feature	[32]
Acitretin, Cholecalciferol, Misoprostol, Nafcillin, Repaglinide, Salmeterol, Telmisartan	P-gp	Decrease clearance of drugs by inhibiting efflux into intestine of P-gp substrates.	3 Hydrophobic features and 2 hydrogen bond acceptor features	[33]
Thioridazine, Vinblastine,	OCTN2	Inhibition may cause	3 Hydrophobic features and 1	[35]

Clozapine, Amlodipine, Gefitinib, Trifluoperazine, Dibucaine, Tamoxifen, Amiodarone, Atracurium, Nefazodone, Argatroban, Nelfinavir, Prochlorperazine, Raloxifene, Metoclopramide, Desloratidine, Duloxetine, Carvedilol, Olanzapine, Amitriptyline, Imatinib, Desipramine, Quinine, Quinidine,		rhabdomyolysis.	positive ionizable feature	
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Haloperidol, Bromocriptine				
Nimodipine, Fluvastatin, Latanoprost, Lovastatin, Pentamidine, Simvastatin, Pioglitazone, Tioconazole	ASBT	ASBT inhibition can cause diarrhea, hyperglyceridemia, gallstone disease and colon cancer.	2 hydrophobic features, 2 hydrogen bond acceptors and shape restriction around mesoridazine.	[36]

Table 4. A subset of the >20 CDD publicly available antimalarial and tuberculosis (TB) data sets.

Database Name/Source	Description	Molecules
U.S. Army Survey	An extensive collection of antimalarial drug animal SAR data, including structures, bioactivity etc, published originally by the U.S. Army in 1946.	12,318
St Jude Childrens Research Hospital	Supplemental data for Nature Article [135]- structures tested in a primary screen, with additional data in eight protocols: Bland-Altman analysis, calculated ADMET properties, Phylochemogenetic screen, Sensitivity, Synergy, and Enzyme Assays, as well as a Thermal Melt Analysis.	1524
Novartis Malaria	Data from Nature paper [136] Plasmodium falciparum strains 3d7 (drug-susceptible) and W2 (chloroquine-, quinine-, pyrimethamine-, cycloguanil-, and sulfadoxine-resistant), obtained from MR4, were tested in an erythrocyte-based infection assay for susceptibility to inhibition of proliferation by selected compounds	5695
Johns Hopkins- Sullivan	Percent inhibition of approved drugs at 10 μ M	2693
MLSMR	A diverse collection tested by the Southern Research Institute against Mtb H37Rv. The most active compounds have dose response and cytotoxicity data.	214,507
TB efficacy data from	TB Efficacy Data from over 300 published literature	6,771

the literature	sources. Data includes PubMed citations, targets, cells and organisms testes, MIC, % Inhibition, EC ₅₀ , IC ₅₀ , etc.	
TAACF- NIAID-CB2	Results of screening a commercial compound library by the Southern Research Institute to inhibit the growth of M. tuberculosis strain H37Rv.	102,634
Novartis Mtb	Aerobic and Anaerobic hits	283

LEGEND TO FIGURES

Figure 1. A. Cetirizine. B. Cetirizine mapped to a Catalyst common features pharmacophore based on 3 actives and 2 inactives for OCTN2 [35]. The pharmacophore contains 2 hydrophobic features (cyan) and a positive ionizable feature (red). C. Cetirizine mapping to a Catalyst pharmacophore derived from 22 drugs with K_i data for OCTN2 (observed and predicted data described in Diao et al., [34]). The pharmacophore contains 2 hydrophobic features (cyan), a hydrogen bond acceptor (green) and a positive ionizable feature (red).

Figure 2. Receiver operator characteristic plot for the FDA approved Mtb hits ($N = 21$) used as a test set ($N = 2108$) for a previously published Bayesian model [95]. Purple = best rate of finding hits, Yellow = Bayesian model, Blue = random rate of finding hits.

Figure 3. A repositioning strategy using the CDD (see: <http://www.collaborativedrug.com/register>), ChemSpider (www.chemspider.com) or other databases (bioactivity data for target or disease of interest and FDA drug dataset) in combination with computational methods (pharmacophore, similarity assessment, machine learning etc).