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Drug Repurposing

ABSTRACT

The discovery and development of new drugs is slow, time-consuming and costly. An increasingly attractive option is 'repurposing', which is the process of finding new uses for existing drugs. This proposition of repurposing (also called 'drug repositioning') has spurred the research community as it has a promising potential to fast track already used drugs into clinical studies for new clinical indications.

INTRODUCTION

There are several advantages of repurposing drugs. Perhaps the most important one is that there is a lower failure risk. This is because the repurposed drug has already been found to be safe in clinical trials. Another advantage is that there is less investment to repurpose a drug. In fact, it has been estimated that on average 275 million euros are needed to repurpose, while for a new drug discovery it ranges between 2 to 3 billion euros. The repurposing strategy also has a reduced time frame when compared to the conventional drug discovery. Indeed, generally, a conventional drug discovery takes from 10 to 17 years. On the other hand, it takes from 3 to 12 years for a drug to be repurposed. The shorter time frame becomes more practical in pandemics. For example, in the ongoing Covid-19 pandemic, there have been about 25 repurposing trials in clinicaltrial.gov.

DRUG REPURPOSING STRATEGIES

The task of repurposing navigates around the relationship between drugs, targets and diseases. Traditionally, drug targets were selected from approximately 20,000 genes in the human genome. So only about 1-2% of the human genome was currently a potential drug target. This is surely to increase in the near future, given the fact that the non-coding regions of the genome are also being deciphered and targeted. When it comes to the disease aspect, one finds difficulty to give an estimate because diseases are really a spectrum, and one cannot draw boundaries. But one can say that there are about 14,400 diseases in total, of which about 7,000 are rare diseases. The rare diseases were traditionally neglected by pharmaceutical companies but this trend is also changing.

Drug repurposing can be approached through three main methods, specifically 'target-centric', 'drug-centric', and 'disease-centric' repositioning. The target-centric strategy tries to find a new indication for a target that is already deciphered. In drug-centric repurposing, the aim is to find a new target for a known drug. In the disease-centric repurposing, focus is on the pathophysiological pathways of diseases, whereby any revealed similarity is investigated and exploited. Through a retrospective analysis, Parisi et al.[1] found that several cases of repurposing were based on drug-target interaction approaches.

This essay will not be dealing with serendipitous discoveries, even though such discoveries can be a source of untapped opportunity. Suffice to say that serendipity here means the accidental discovery of a new clinical drug indication. Examples include Viagra® (researchers were conducting research o n drug moieties for antihypertensive properties and during the process they found that sildenafil could be used for erectile dysfunction) and Regaine® (initially minoxidil was used as an antihypertensive but later found a niche market for hair growth).

A. DRUG-CENTRIC APPROACHES

Table 1 lists some of the drug-centric approaches. Often these approaches are coupled together.

Technologies
1. Computational Ligand-based approach
2. Computational Structure-based approach
3. Protein expression profiling (Chemical Proteomics)
4. Off-target screening

1. *Computational ligand-based approaches* are virtual screening approaches. They aim to reveal pharmacophores, which are functional groups within a prospective compound that are responsible for a biological response.[2] Generally, pharmacophore mapping is used when no information about the target structure is available.

Daoud et al.[3] used this approach, with other tools, to create a pharmacophore model to repurpose antiviral drugs against SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), which is responsible for the current Coronavirus Disease 2019 (COVID-19) pandemic. Specifically, they found five antiviral drugs which successfully docked into the binding site of COVID-19 main protease (lopinavir, remdesivir, ritonavir, saquinavir and raltegravir) which are already approved by the FDA.

Pérez-Sánchez et al.[4] used pharmacophore mapping and 3D shape similarity (see paragraph below) on a comprehensive drug database called Drugbank and identified 108 hits from 11,353 compounds. From these hits

they found eight molecules that were structurally similar to pyridostigmine. The latter is an acetylcholinesterase inhibitor (AChEI) which is used in the management of Alzheimer's disease.

2. *Computational structure-based approach* is also a virtual screening approach. It uses the 3D structure of the biological target to identify how an active drug binds to the 'active site' of the target. Knowledge of this 'molecular docking' between the target and drug is then used to discover other drugs.[5] Such a virtual molecular docking method is also used in 'target fishing' or 'inverse docking', where drugs are investigated to bind to other diverse types of targets from databases of known clinical targets.[6]

Choudary et al.[7] used the structure-based virtual screening approach and investigated a library of drugs called LOPAC. Their aim was to find prospective drugs against the ACE2 receptor of human cells and against the receptor binding domain of spike protein (S-RBD) of SARS-CoV-2. They found some promising molecules and propose that these can be used against SARS-CoV-2.

Wang et al.[8] also used a structure-based virtual screening to interrogate an FDA approved drug database for prospective Cytochrome P450 1B1 (CYP1B1) inhibitors. Cytochrome P450 1B1 is a target in cancer prevention and therapy. They identified six compounds, amongst which were carvedilol and indacaterol.

3. *Chemical Proteomics* is a main branch of proteomics. Its aim in repurposing is to identify the numerous protein targets of active small molecules.[9,10] Le et al.[11] screened commercially available kinase inhibitors for any antibacterial properties that could kill methicillin-resistant *Staphylococcus aureus* (MRSA). They found sorafenib, an anticancer drug, to be a potential candidate. Chemical proteomics, in their investigation, also revealed the probable mode of action of sorafenib that was responsible for the killing of MRSA.

Chemical proteomics were pivotal in the study of Lum et al.[12] Specifically, they investigated the global cellular interactions between lipids and proteins and the effects of active small molecules that included natural products but also repurposed drugs. They showed that the antifungal miconazole inhibits sphingosine-1-phosphate (S1P) lyase. This inhibition decreases the severity of multiple sclerosis in experimental models.

4. *Off-target-screening* is based on the fact that drugs cause side-effects because they bind with other targets that are somewhat similar to the original target. Thus, the study of these other homologous targets in other diseases offers another platform for drug repurposing.

Sprinolactone is an antagonist of the mineralocorticoid receptor and as such is used in the treatment of heart failure, hypertension and cirrhosis. However, it also is an antagonist of the androgen receptor and has been repurposed for the treatment of acne, polycystic ovary syndrome and hirsutism in women.

Another example is the repurposing of doxepin. Doxepin is an antidepressant of the tricyclic class. However, its off-target effects, mediated by histamine H1 and muscarinic receptors, are responsible for its repurposing in pruritus, chronic urticaria and psychodermatology.

Dapsone and thalidomide are another two drugs that were repurposed based on their off-target screening. Dapsone, which is used in leprosy, has been repurposed for its anti-inflammatory effects in several skin conditions like pemphigoid, IgA pemphigus, prurigo pigmentosa and neutrophilic dermatosis, amongst others.[13] Thalidomide, on the other hand was used for morning sickness in pregnancy, but was withdrawn because of its teratogenic effect. Specifically it caused phocomelia (a congenital deformity where the limbs are underdeveloped or absent). This off-target effect was methodically studied and it was found that thalidomide inhibits vascular endothelial growth factor (VEGF) and tumour necrosis factor alpha (TNF-α). Because of its inhibition of VEGF it was repurposed for the treatment of multiple myeloma. Because of its inhibition on TNF-α, it was repurposed to manage erythema nodosumleprosum.[14,15]

B. DISEASE-CENTRIC APPROACHES

Here the homology of the pathophysiological pathways of diseases is the basis of repurposing. Table 2 lists some of the main methods used in disease-centric repositioning.

Technologies
1. Gene expression profiling
2. Phenotypic screening
3. Data Mining (Neural networks, Graph Theory Algorithms)

1. *Gene Expression Profiling*

Bioinformatics and gene expression profiling-has led to the ability to link genetic profiles and drug response. This approach is also being used to systematically repurpose drugs by expanding their spectrum for treatment.

For example, Lee et al.[16] built a strong bioinformatics platform called DeSigN, which can be used for repurposing drugs. By using gene expression profiling, it predicts the candidate drug/s against the cancer cell lines of interest. Another platform called DeepCodex was designed by Donner et al.[17] It links functional similarity of compounds based on data of gene expression.

Another resource for drug repurposing that uses gene expression changes or signatures is the Connectivity Map (CMap). Donertas et al.[18] used it to repurpose drugs to counteract aging in the human brain. They found 24 drugs and propose that some of these drugs can be used as anti-aging drugs.

Qu et al.[19] also used gene expression profiling to find repurposed drugs for the treatment of psoriasis. Their analysis revealed several candidates, amongst which were monobenzone, tiabendazole, resveratrol, doxycycline, parthenolide, and methotrexate.

2. *Phenotypic screening*

This is a method to identify molecules that can alter the phenotype of a cell. Two main methods are used, 'in vivo assays' and 'cell-based (in vitro) assays'. In the in vivo method, screening of compounds is done on preclinical disease models. In the second method, as the name implies, screening of compounds is done on cultivated cells 'in vitro'; the cell culture systems are validated disease models. This phenotypic screening approach is seeing a revival and is being used to overcome the bottleneck of cancer therapeutics, but not only.[20,21]

Using this method, Iljin et al.[22] found that disulfiram can act as an anti-neoplastic drug in prostate cancer. However, a clinical trial (Identifier: NCT02963051) found that disulfiram is ineffective in metastatic, castration resistant prostate cancer because it is metabolically changed into an inactive metabolite. Nevertheless, the authors propose further research to find a stable formulation of disulfiram. Corsello et al.[23] also used this approach using the method 'PRISM' (profiling relative inhibition simultaneously in mixtures) and found 49 non-oncology drugs to have high anti-neoplastic activity.

Phenotypic screening on whole organisms, besides identifying potential cancer drugs, can also reveal their pharmacokinetics and organ-toxicity. Using a transgenic model of zebra fish, Ridges et al.[24] screened the effectiveness of 26,000 small molecules against leukaemia. They fished a molecule called lenaldekar that had potential against various blood malignancies. Although lenaldekar is currently not being investigated in clinical trials, its discovery triggered further research on quinoline derivatives, as prospective candidates to treat leukaemia.[25]

3. *Data Mining – Graph Theory Algorithms; Neural Networks*

Data mining also has great potential in drug discovery and drug repurposing. In recent years, research into cellular functions and processes has generated great knowledge. But this great knowledge has generated difficulties in how to integrate the data into a rational meaningful way. This led to an increase of computational techniques to process, analyse and store the data.

One such tool is that of graph theory, where biological structures and relationships are represented graphically. Gramatica et al.[26] used this mathematical technique to enable drug repurposing by discovering connectivity between drugs and disease.

Another pipeline is that based on 'artificial neural networks', simply called 'neural networks'. Again, these are a series of algorithms that try to decipher relationships in a set of data. These computing systems mimic processes of how biological neural networks work. When such artificial neural networks are made in several layers, the platform is called a 'deep learning' approach. These neural networks offer another strong approach for developing 'in silico' drug repositioning.

Zhou et al.[27] used such a network to rapidly identify potential repurposable drugs against SARS-CoV-2. They identified melatonin, mercaptopurine, and sirolimus as repurposable drugs. Melatonin (N-acetyl-5-methoxytryptamine) is in clinical trials (ClinicalTrials.gov Identifier: NCT04474483, NCT04470297) for its anti-oxidative and anti-inflammatory roles, offering possible protection against organ injuries.

Similarly, Hsieh et al.[28] used a neural network approach together with other tools to repurpose drugs for Covid-19. They fished 22 potential candidate drugs amongst which are Atorvastatin, Azithromycin, Aspirin, Acetaminophen, and Albuterol. Atorvastatin is in clinical trials (ClinicalTrials.gov Identifier: NCT04952350, NCT04380402). As is Azithromycin (ClinicalTrials.gov Identifier: NCT04381962).

Wei Zhang et al.[29] used both network-based and graph theory based algorithms in oncology, to reposition drugs and offer personalized treatment. Sidders et al.[30] coupled a network approach with a phenotypic screening approach to study and identify small molecules that could be potential drug candidates in complex chronic pain diseases.

C. TARGET-CENTRIC

In target-centered drug discovery, researchers use analytical tools (already mentioned) to detail and define a molecular target like a gene or its product or a molecular mechanism. Knowing the molecular target, they then try to find a drug to hit it. In theory, this allows more specificity and less side-effects.

A good example of this approach is the use of antisense oligonucleotides targeting a specific nucleotide sequence in messenger RNA (mRNA). Once there is binding, the mRNA cannot be translated into the protein. Fomivirsen provided the first proof-of-concept for the use of such

antisense oligonucleotides. Fomivirsen was approved by FDA (Food and Drug Administration) in 1998 (and later also approved by EMA (European Medicines Agency)) for treating cytomegalovirus (CMV) retinitis. Other antisense-based drugs were approved like mipomersen which is an antisense oligonucleotide that inhibits apolipoprotien B. It was FDA approved in 2013 for treating homozygous familial hypercholesterolemia. Other examples beside antisense oligonucleotides, are those previously discussed by Grech et al.[31] and include locked nucleic acid anti-miRs, miRNA sponges, miRNA mimics, siRNAs and ribozymes.

CONCLUSION

Recycling old drugs is definitely an attractive form of drug discovery. This has yielded new methods, some of which have been discussed, to identify new clinical uses for drugs that are either already in use or even those that have been shelved. Still, new computational pipelines are being developed to make sense of the big data which originates from various studies along the years. This will surely continue to augment the successes of drug repurposing.

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