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Do predictive technologies in drug repurposing provide validation in addition to proposing testable hypotheses, and how can these technologies be combined to reduce attrition in repurposed developments?

Predictive methods in drug repurposing: gold mine or just a bigger haystack?

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There are nearly 2000 patent and peer-reviewed literature-based examples of drug repurposing to be found at http://www.drugrepurposing.info/; yet there has recently been a spate of experimental techniques used to predict new drug repurposing opportunities. This review questions whether these new methods – from computerised modelling of drug–target interactions to retrospective analysis of clinical experience – merely add testable hypotheses without addressing their inherent validity, or whether they also partially validate the new uses so that the predictions are more likely to be successfully developed. In addition, ontological methods take existing information and link two known facts to create an unknown association. These can both enhance other methods of repurposing and provide patented, commercial products, including several case historical examples.

Introduction

Drug repurposing, which is the identification of secondary uses for existing and developmental drugs, offers significant theoretical benefits in pharmaceutical R&D. These include reduced risk of toxicology, reduced time to validate the new indication in the clinic and opportunities for patent protection of the new use [1]. However, drug repurposed developments do not necessarily have an increased chance of therapeutic success or, put another way, a lower risk of failure owing to the lack of efficacy. In fact, by decreasing the preclinical time and cost, the developmental attrition probability could increase rather than the reverse, because the preparation taken for a clinical study in a repurposing programme is likely to be less than for a conventional NCE programme. At the very least, in addition to increasing the rate of progression of compounds into clinical-stage development, companies will also face an increase in numbers of clinical-stage development failures. The risk of failure depends on how well the prediction translates into a positive clinical finding.

There has recently been a significant number of different methodological approaches to the identification of new repurposing opportunities for existing drugs. As shown in Fig. 1, the process is sequentially ordered into analysis, hypothesis generation and validation. The analytical component can be divided into two classes: experimental and ontological. According to

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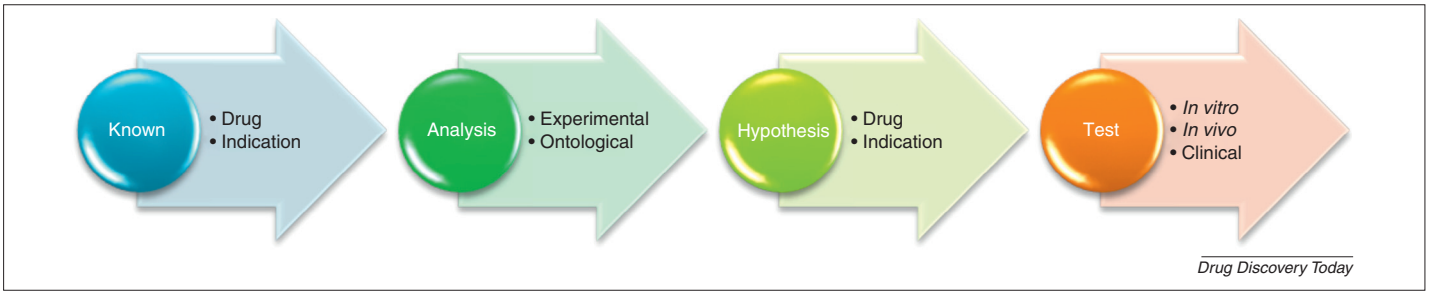

FIGURE 1

Process of drug repurposing, from existing drug in a known indication through to hypothesis generation and testing of a drug in new indication.

dictionary definitions, 'experiment' is a scientific procedure undertaken to make a discovery and 'ontology' deals with the nature of being. Thus, for the purposes of this review, experimental approaches essentially involve the generation of new knowledge, and include: *in silico* methods such as molecular modelling and gene profiling; 'wet' laboratory experiments such as *in vitro* and *in vivo* screening; and methods based on analysis of human exposure, either from clinical trial information or from prescription data. Ontological methods link two known facts to postulate a third, unknown, relationship and include semantic analysis of literature databases as well as far simpler methods that involve the intermediacy of a protein target between a drug agent and its therapeutic use. Experimental and ontological approaches do not necessarily work separately from one another, indeed they can benefit from combination.

Experimental technologies

Experimental methodologies are of interest because they can provide new associations that are presently not known. One of the main theoretical advantages of these techniques is that they can provide more intuitively obscure hypotheses, with a robust potential for patentability. In addition, as referred to below, some of the earlier computerised modelling approaches offer new leads for discovery programmes, rather than developmental opportunities.

Molecular modelling

Keiser *et al.* used a similarity ensemble approach (SEA) to relate receptor protein pharmacology to ligand chemistry, and from this went on to identify secondary target pharmacology of existing drugs [2]. SEA compares ligand topology using BLAST algorithms and naive Bayesian classifiers. Applying this technique to a total of 3665 FDA-approved and investigational drugs, the authors predicted several new drug–target associations, 23 of which were confirmed by subsequent binding studies, and five of these were potent ($K_i < 100$ nM). Previously, these drugs had been thought of as single-target agents. The paper [2] gives no information on the sensitivity of their computational approach. In other words, we do not know how many secondary target interactions there are that are not predicted by SEA; such a value would require a large-scale screening programme, looking at all the test drugs in all the receptor-binding assays. However, the paper does give information on the specificity of the approach, in other words the proportion of predicted associations that turn out to be experimentally validated. The authors tested 30 from 180 predictions obtained from their analysis that were experimentally accessible, and found 23 (77%) of these yielded K_i values of less than 15 μM.

Xie *et al.* [3] used a chemical systems biology approach to identify the secondary target pharmacology responsible for the anticancer effect of nelfinavir, originally a drug for HIV and AIDS. Rather than predicting new uses for this drug, the method here was to identify the interactions that are thought to be responsible for the observed new effect of nelfinavir. The technology is therefore used to deconvolute the mechanism of action for a known use, rather than predict unknown uses.

Cheng *et al.* [4] used a network-based inference technique involving the calculation of two-dimensional structural similarity between two drugs, genomic sequence similarity between two targets and topology network similarity between drug and target; from these methods, they predicted estrogenic effects of simvastatin and ketoconazole, which were confirmed in binding studies and in antiproliferative tests on the human MDA-MB-231 breast cancer cell line, with potencies in the low micromolar range. However, two other compounds (itraconazole and diclofenac) were found to be reasonably potent binding inhibitors at the estrogen receptor (ER)β but not functionally effective in terms of antiproliferative effects on the MDA-MB-231 cancer cell line. Montelukast, the leukotriene D4 (LTD4) antagonist, was also found to inhibit the dipeptidyl peptidase IV (DPP-IV) enzyme at an IC_{50} of around 10 μM. The lack of correlation of ERβ estrogen binding affinity and antiproliferative effects among the compounds identified from the screen casts doubt on the assumed causal relationship of ERβ estrogen inhibition as the prime mechanism for the antiproliferative effect. Moreover, because the predictive method was directed toward the identification of compounds able to interdict the interaction of estrogen with its receptor, it remains possible that the observed functional effect is partly driven by other factors. These observations should also be put in the context that ketoconazole is already widely used as a treatment for androgen-dependent prostate cancer and that itraconazole is known to inhibit angiogenesis and tumour growth in non-small-cell lung cancer [5]. It is plausible that additional mechanisms are associated with the antiproliferative effects observed in the MDA-MB-231 cancer cell line that were not predicted by the network inference technique. Similarly, simvastatin, like other 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors, has well-known anticancer properties derived from experimental work as well as retrospective prescription database analysis [6], making the influence of its ERβ estrogenic activity moot.

Gene profiling

Genetic expression profiling has been used by several groups in the context of drug repurposing. Sirota *et al.* compared the mRNA expression data from patients with various diseases with similar data on mRNA expressed *in vitro* by a set of 164 drugs [7]. They analysed the expression data in terms of signatures and looked for drugs that had the opposite signature to the disease. The method is appropriate for the direct relationship between drugs and indications; the new relationship might or might not involve the primary mechanism of the drug. Using this method, they predicted that the histamine H2 antagonist cimetidine would be effective in lung cancer, and the antiepileptic topiramate would be effective in inflammatory bowel disease. Both these predictions were validated in preclinical models. The functional *in vivo* benefit of topiramate against experimentally induced colitis in rats was followed up in a subsequent paper [8]. However, although the authors operated the analysis in a predictive fashion and claim their predictions are novel, in fact the associations of cimetidine with cancer and of topiramate with inflammatory bowel disease (IBD) are known in the literature. In the case of cimetidine the anticancer association was first proposed in 1979 [9]. Although not specifically for lung cancer, cimetidine has been investigated for melanoma, colorectal, gastric, renal, bladder and liver cancer, purportedly through a non-antihistaminergic effect on the immune system or cell adhesion. Topiramate has been observed to reduce inflammation of a lipoma in a dog and specifically claimed for use in IBD [10]. The prospective value of this technique to identify novel uses of existing drugs is therefore still uncertain.

Iorio *et al.* [11] used expression profiling to group 1309 drugs into communities based on similar signatures, and then cross-compared these groups with their therapeutic and chemical classification from the WHO, and targeted genes from DrugBank and ChemBank. They showed that drugs with similar gene expression signatures were also associated with similar targets and mechanisms of action. They then used this network map as a template to deconvolute the mechanism of action of test drugs based on experimentally determined gene expression signatures. One of the conclusions from this work is that fasudil is an enhancer of cellular autophagy and could be used for the treatment of neurodegeneration. However, similarly to Ref. [7], this prediction is foreshadowed in the prior art. Fasudil, a rho kinase (ROCK) inhibitor, has previously been shown to promote macroautophagy through its metabolite Y27632, with consequent effects in Huntington's disease [12]. Iorio *et al.* [11] also make other associations between cyclin-dependent kinase inhibitors and topoisomerase, but the validation of this association makes no predictions for novel uses outside the area of oncology, for which topoisomerase inhibitors are well known and widely used.

Whereas, for both these studies, the prior knowledge is to some extent a validation of the approach, it also has a negative impact on the ability of these methods to predict novel associations, particularly in so far as a great benefit of such a prediction would be the ability to penetrate new areas of patent space. These publications would benefit from a complete analysis of the sensitivity and selectivity of the technology, so that we could assess how many novel predictions are validated in binding, cell-based or functional studies, and how many non-predicted associations derive from other methods.

In vitro *screening*

Some investigators, in particular Rothstein *et al.* from Johns Hopkins [13], have strategically deployed libraries of known compounds (particularly FDA-approved compounds) in an *in vitro* screen using primary organotypic spinal cord slice cultures, and as a result have identified interesting and previously unknown effects for ceftriaxone and harmine as upregulators of the glutamate transporter GLT-1 [13,14]. GLT-1 loss selectively in glia had previously been reported to be associated with the development of amyotrophic lateral sclerosis (ALS). Ceftriaxone increases the expression of GLT-1 and, through its effects on glutamate transport, provides functional neuroprotection in animal models of ALS, delaying neuronal loss and loss of muscle strength and increasing survival. The original report, published in 2005, then rapidly led to long-term clinical trials of ceftriaxone in ALS, which began Phase III efficacy determination in 2009. Although this trial was unfortunately curtailed in 2012 because it was anticipated to be unlikely to reach the predetermined efficacy criteria, the drug repurposing strategy was pivotal in being able to advance clinical testing rapidly for this disease, for which riluzole is the only FDA-approved agent and there remains substantial unmet medical need.

Phenotypic screening

A systematic analysis of drugs approved between 1999 and 2008 recently reported that phenotypic screening exceeded target-based approaches in discovering first-in-class small-molecule drugs [15]. Melior Discovery has a proprietary discovery platform for screening known drug-like compounds using a battery of 40 *in vivo* assays spanning a broad range of therapeutic areas including inflammation, immunology, diabetes and metabolic syndrome, dermatology, cardiovascular, gastrointestinal, psychiatric, neurological and neurodegenerative disorders [16]. They have used this or similar techniques to identify and repurpose tolimidone, an old drug from Pfizer. Now known as MLR1023, the compound acts as a potential new antidiabetic agent *via* insulin sensitisation [17]. Following identification of the functional effect, the company then deconvoluted the mechanism of action of the drug, showing it to act through lyn kinase activation. Previously, Pfizer had discontinued the development of MLR1023 (tolmidone) for gastric ulcers, owing to lack of efficacy.

Side effect analysis

Side effect data, as a basis for prediction of repurposing opportunities, have the substantial advantage that they are derived from humans rather than animals or cells. Campillos *et al.* assessed the similarity of side effects for marketed drugs to identify shared protein targets, and validated the predictions using *in vitro* assessments [18]. They identified the 5-HT uptake transporter as a hitherto unknown target for the Alzheimer's disease drug donepezil, and the dopamine D3 receptor is inhibited by the antiulcer drug rabeprazole. This technology is good for the identification of secondary target pharmacology (the association of an existing drug with a new target protein), particularly because it is based on effects in humans. Unlike the molecular modelling approach, the prediction is based on levels of the drug that pertain in therapeutic situations, so that the drug–target interaction probably takes place in a prescription setting. However, most drug

repurposing opportunities derive from a new association of an existing target with a new therapeutic indication, and this technology does have limited applicability in this area.

In an extension of the previous work, Yang and Agarwal analysed the side effects and therapeutic effects of clinically used drugs to construct a 'side effectorome' [19]. This analysis places in close proximity side effects and the particular indication with which they are commonly associated. The authors then looked for drugs known to cause a particular side effect but not known to be useful in nearby indications. Examples include drugs that cause priapism and obsessive compulsive disorder (OCD). For various examples of drugs with this side effect that are not indicated for OCD (e.g. oxcarbazepine) there is a literature precedent for this activity, for example from case reports [20].

This method offers strong validation evidence for use, but the disadvantage is that the predictions tend to be limited to diseases for which there are existing treatments. This is because, for therapeutic indications for which there are no treatments such as many orphan diseases, there is no side effect profile associated with such treatment upon which to base a repurposing prediction. Furthermore, the method is less useful for predicting repurposing opportunities that work through novel biochemical pathways, because again the side effect profile for such drugs is not known. Nevertheless, side effect analysis offers significant power.

Retrospective analysis of human trials or experience

This area concerns the retrospective analysis of clinical trials or prescription data. In both cases, the advantage is that it involves human data, although with the caveat that it is not prospective in nature, and therefore could provide false-positive associations [21]. The analysis is only useful for a secondary indication that can be predicted from information available in the patient history while being treated for the primary indication. A common area where this applies is in the incidence of cancer, which is always recorded in either a trial or prescription setting because it represents a possible treatment-related serious adverse event. Thus, several drugs have been proposed to have anticancer properties as a result of analyses of this kind, although reduction in cancer incidence is not the same as effective cancer treatment.

An interesting example of this is the discovery of anticancer properties of the Na^+/K^+-ATPase inhibitors like ouabain and digitoxin. Platz *et al.* identified digoxin as a possible treatment for prostate cancer based on a high-throughput screen and a prospective cohort study [22]. Others have previously discussed the potential for cardiac glycosides as cancer therapeutics [23], but the mechanistic basis for their action has not been conclusively defined. One disadvantage of this method is the time required to mine the trial data for the required effect, and indeed knowing what to look for in the first place. It presumes that a hypothesis exists before conducting the analysis. The source of such a hypothesis could be any one of the methods described above, or pre-existing knowledge from a related area.

Ontological methods

In addition to the computerised methods for predicting new uses for existing compounds, there are ontological (knowledge-based) methods that can be used either on the predictions cited above or on pre-existing literature information. At its simplest, the ontological approaches take two known relationships (for example, a drug and an indication) and extend this (for example a link between two related indications) to create an unknown relationship (Fig. 2). This method, although simple, depends on the particular circumstances of the relationships (discussed below) for its patentability. More-complicated analyses can take multiple information sources and seek links based on semantic data mining [24].

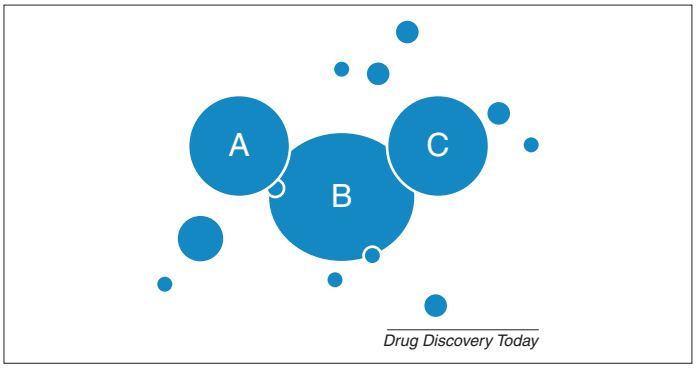

FIGURE 2

The basic principle of ontological drug repurposing. Here, B is known to be related to A and C but the relationship between A and C is unknown.

Ontological repurposing is also necessary for finding opportunities in virgin drug discovery space. For example, side effect analysis is strictly limited to indications where there are existing drugs. However, virgin therapeutic indications are accessible through 'neighbouring' areas. An example of a neighbouring area would be two indications that are often, but not always, associated such as rheumatoid arthritis (RA) and psoriasis; or Paget's disease and osteoporosis.

A third way in which this type of analysis can be useful is in enhancing, or even optimising, the relationship between drug and indication. This is a complex area but, to put it simply, there can be particular attributes that favour a related indication or related compound to the ones originally identified. At one extreme, the original association provides merely a foundation for a new discovery programme, perhaps identifying a new structural class of compound that interdicts a known receptor or enzyme; by contrast, the association between compound and indication is stratified among related (known) compounds, or close analogues, and among a range of related indications, to choose a particularly appropriate pairing of the two. It is highly advantageous to conduct this type of analysis before embarking on a drug repurposing development, and very important to have selected the best pairing. Because method of use patents do not (by definition) cover all possible avenues for the particular therapeutic, it is possible that two competitor groups of investigators could coincide in choice of therapeutic agent, but for different indications and probably in different formulations. This situation can result in confusion and complication later in development and commercialisation.

Using mechanism of action as a bridge between indications

Mechanism can play an important, intermediary role in the drawing together of two relationships. Thus, the knowledge that a mechanism can be related to multiple indications offers *a priori* opportunities for compounds known to work *via* such a mechanism. Despite the ostensible issues of obviousness behind such an approach,

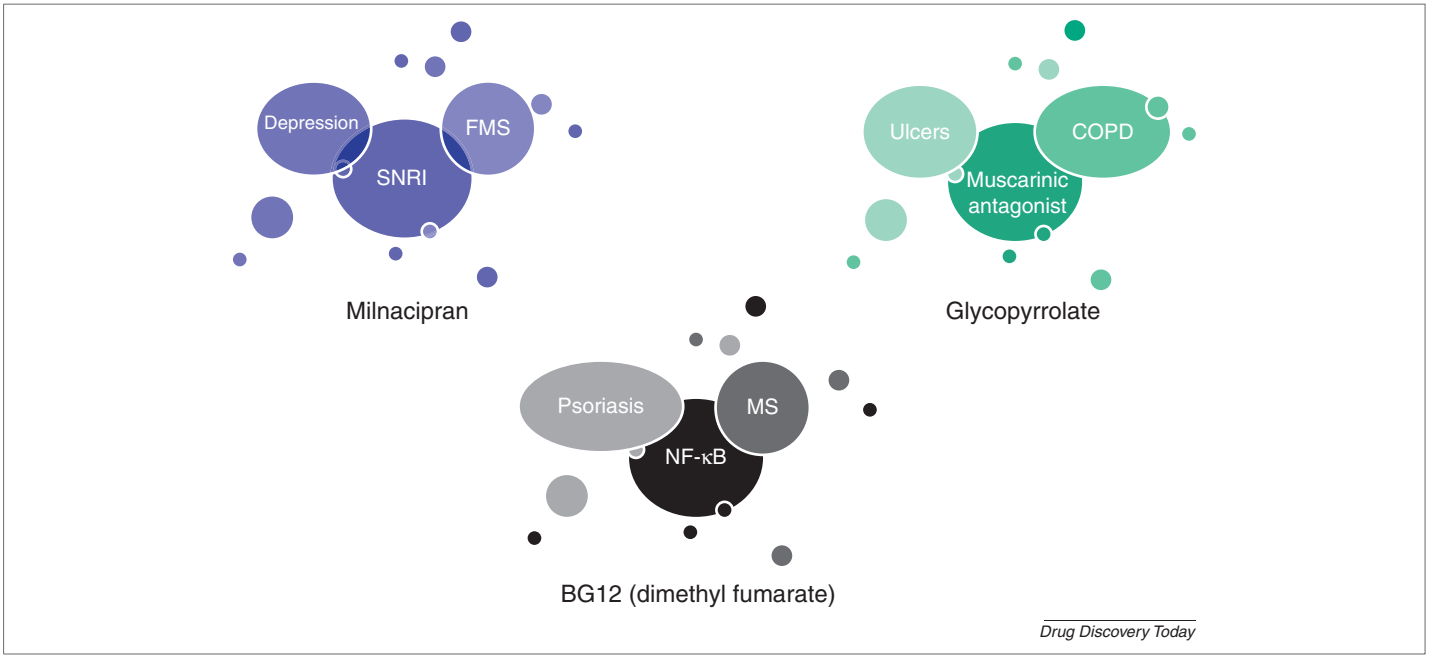

FIGURE 3

Three examples of drug repurposing based on common mechanisms of action. All examples here are patented and approved for a major market. *Abbreviations*: COPD, chronic obstructive pulmonary disease; FMS, fibromyalgia syndrome; MS, multiple sclerosis; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SNRI, serotonin-noradrenaline reuptake inhibitor.

history tells us that drug repurposing based on this strategy has been successfully patented and commercialised (Fig. 3).

i. Milnacipran was an antidepressant marketed in France by Laboratoires Pierre Fabre with a mechanism of action based on serotonin noradrenaline reuptake inhibition (SNRI). Cypress Biosciences recognised the opportunity in fibromyalgia syndrome and pain, and obtained method of use patents in these areas [25–27]. These patents were granted despite the known use of certain antidepressants, including ones with serotonin and noradrenaline reuptake blocking effects (such as amitriptyline), in the treatment of fibromyalgia and pain. The patents were licensed to Forest, and it undertook the late development and registration studies, obtaining FDA approval in 2009 under the tradename Savella™.

ii. Glycopyrrolate was an oral drug from the 1950s for the treatment of peptic ulcers with a mechanism of action based on muscarinic antagonism. Arakis (later taken over/merged to form Sosei) recognised the opportunity in COPD, and obtained patents that were based eventually on a dry powder inhaled formulation of the drug [28]. Again, these patents were granted despite the known use of other antimuscarinic compounds such as tiotropium for respiratory disorders. The patents were licensed by Novartis who funded later development and registration trials of glycopyrrolate itself and a combination product with their proprietary β2-adrenoceptor agonist indacaterol. The former has been approved in Europe as a once-daily inhaled maintenance bronchodilator treatment to relieve symptoms in adult patients with COPD.

iii. Finally, dimethyl fumarate was marketed for the treatment of psoriasis by Fumagen in Germany; the mechanism of action, although complex, was known to involve the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. Fumagen explored another autoimmune disease involving the NF-κB pathway, multiple sclerosis, and obtained experimental data supporting this new use and patents [29,30] that were licensed to Biogen-Idec. Biogen codenamed it BG12 and then undertook the later stages of development and obtained fast-track designation from the FDA for its review of the application to market it in relapsing-remitting multiple sclerosis (RRMS). They also looked in more detail at the mechanism of action and proposed that BG-12 worked through covalent modification of the Kelch-like ECH-associated protein 1 (KEAP1) leading to activation of the Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway.

Despite these successful examples, a common mediator does not always translate into efficacy of a single agent, or group of agents, in multiple indications. A good counter example is in the area of anti-inflammatory therapy, where the tumour necrosis factor (TNF) blockers such as etanercept and infliximab are currently approved for treatment of the diseases shown in Table 1. Most of these are based around arthritis and gastrointestinal inflammation, in which there is good, although not complete, similarity between the indications for which etanercept (a TNF receptor antagonist) and infliximab (a TNF antibody) are

TABLE 1

Approved indications for TNF blockers

Disease	Etanercept	Infliximab
Rheumatoid arthritis	Yes	Yes
Psoriatic arthritis	Yes	Yes
Ankylosing arthritis	Yes	Yes
Juvenile rheumatoid arthritis	Yes	Under investigation
Crohn's disease	No	Yes
Ulcerative colitis	No	Yes

TABLE 2

Indications where TNF has been implicated in the pathophysiology but where TNF blockers are not clinically effective

Indication	Drug	Pubmed ID
Systemic inflammatory response syndrome	Infliximab and/or etanercept	15585509
Multiple sclerosis	Lenercept	10449104
Systemic vasculitis	Infliximab	17360788
Sjogren's syndrome	Infliximab	15077311
Wegener's granulomatosis	Etanercept	15673801
Autoimmune inner ear disease	Etanercept	16151336
COPD	Infliximab	17290043
Giant cell arteritis	Infliximab	17470830
Polymyalgia rheumatica	Infliximab	17470831
Chronic heart failure	Infliximab	12796126
Pulmonary sarcoidosis	Etanercept	12853521

approved. In addition, there are investigations underway that are studying the effect of these agents on uveitis and vasculitis.

TNF has been found to mediate a range of other inflammatory diseases, as shown in Table 2. In many of these conditions, either the blockade of TNF has been shown to be beneficial in preclinical models of the disease or TNF has been found to be over-produced in the human condition. For instance, in COPD the support for a benefit of TNF blockade is based on increased production in sputum of COPD sufferers, natively [31] and during exacerbations [32]; TNF-receptor knockout mice having substantially less cigarette-smoke-induced emphysema [33] and infliximab protecting against pulmonary emphysema in rats passively exposed to cigarette smoke [34]. Despite this evidence base, in a multicentre, randomised, double-blind, placebo-controlled, parallel-group, dose-finding clinical trial in 232 subjects with chronic obstructive pulmonary disease there was no treatment benefit in the primary or secondary endpoints [35]. In fact, in all of the diseases in Table 2 a lack of effect has been shown clinically by one or other of the marketed TNF blockers. It is interesting to speculate what might have happened to this class of therapeutics if the initial pivotal clinical studies had taken place in multiple sclerosis rather than arthritis, because TNF-blocking drugs can promote onset or exacerbation of MS, a risk that is correlated with genotype [36].

Thus, although the disease pathway is an important factor in *de novo* drug discovery and has important implications for drug repurposing, understanding the differences as well as the similarities between indications is a key skill for drug repurposing. The variable efficacy demonstrated in certain indications relative to others despite a common pathophysiological role and similar evidence base in both diseases is a problem for many kinds of predictive drug repurposing technology, but also offers an argument for non-obviousness that is used and, on a case-by-case basis, might be accepted in patent prosecution.

Another ontological method of predicting secondary uses for a drug is to look for a mechanism and/or indication combination and transfer it to another mechanism upstream of the first mechanism on the same pathway. For instance, the establishment of efficacy by an angiotensin AT1 antagonist such as candesartan in hepatic fibrosis [37] might give reasonable grounds for considering the efficacy of an ACE inhibitor like enalapril, particularly because ACE inhibitors are effective in an animal model of radiation-induced lung fibrosis [38]. However, the relative merits and demerits of each drug need to be considered, and one class could have a superior profile relative to the other. In the renin–angiotensin pathway, ACE inhibitors also have an effect on bradykinin production, because the angiotensin-converting enzyme also governs the breakdown of this inflammatory mediator. There is evidence that activation of the bradykinin B2 receptor also reduces renal fibrosis [39]. This evidence might suggest that ACE inhibition could invoke an additional benefit in fibrotic situations.

Using linked indications to identify secondary uses

The similarities between indications can also provide a useful tool for progressive enlargement of treatment groups. Some companies have made good commercial use of this type of drug repurposing, particularly when the initial trials are in tightly focused, rare indications. An example of this strategy is to be found in the development of canakinumab, a recombinant monoclonal antibody for interleukin (IL)-1β marketed by Novartis. This product was originally unsuccessful for RA, but the company persisted in clinical testing in Muckle-Wells syndrome, a very rare condition in which patients are genetically predisposed to high levels of IL-1β. In this population, canakinumab produced rapid and sustained improvement in almost all clinically tested patients, and the FDA granted orphan regulatory status and approval for this drug. Subsequently, further approval was obtained for other rare conditions. Novartis is now conducting trials to extend the drug to other inflammatory indications such as COPD, gout, RA, osteoarthritis (OA) and vasculitis in stratified groups of patients whose disease is highly dependent on IL-1β overproduction. This example supports the central importance of choice of indication in determining clinical efficacy, regulatory approval and ultimately commercial success.

Patents

Most, although not all, drug repurposing projects seek some form of patent protection to provide commercial exclusivity in return for the investment made in getting these projects to market. There are some exceptions to the general rule that patents are a necessary component for successful drug repurposing. One is where a

non-profit organisation (e.g. a charity or government research body) intends to carry through a project to registration (http://numedicus.co.uk/blog/?p=213). A singular example in the commercial arena is that of Aspreva, this company partially developed mycophenolate mofetil for the treatment of certain orphan diseases including systemic lupus erythematosus (SLE) without patent cover but with financial rewards from off-label use derived from an agreement with Roche (http://strategy.sauder.ubc.ca/hellmann/pdfs/AppendixB.pdf). Given the interest in pursuing drug repurposing approaches for the identification of new products for orphan diseases [40,41], it is appropriate to mention that in most developed territories the protection afforded by orphan regulatory protection offers a non-patent route to commercial protection. However, in the vast majority of cases patents are the primary articles of exchange by which early discovery companies raise finance and commercialise their efforts through transfer of intellectual property rights to a larger company that will undertake the later developmental and registration activities. Without a patent, and in reference to this article's title, there is unlikely to be much gold mined. The normal form of such protection is a 'method of use' patent.

One of the perceived advantages of the more exotic associations that can be derived from the experimental predictive methods is that they are more likely to be patent protectable. However, as the examples in Fig. 3 show, patentability can ensue perfectly well from ontological approaches. This is because the three components shown in Fig. 2 are not mutually associated. The circle B can be linked to A and C without implying that A and C are linked. In patent law, this comes into the area of combining elements of prior art, or mosaicing [42]. Ontological analysis can also be helpful in qualifying the output from experimental methods to assess patentability (novelty and obviousness) and to assess freedom to operate.

A good example of the way in which overlaying patents of increasing focus can be used for the protection of drug repurposing projects is that of Arcion's development of a topical clonidine gel for the treatment of diabetic neuropathic pain. The original development was protected by method of use patent WO/1992/014453, entitled 'Compositions and methods of treatment of sympathetically mediated pain', which was filed in 1992 [43]. A successor filing was based on WO/2007/056460, entitled 'Treatment of length dependent neuropathy' [44], followed by a patent protecting a formulation of clonidine, WO/2009/026178, entitled 'High concentration local anesthetic formulations' [45]. Finally, and most recently, a diagnostic procedure to identify the patients most likely to benefit from the treatment was encapsulated in WO/2012/012333, entitled 'Topical treatment of neuropathic pain and methods of diagnosis' [46]. If the latest of these filings is granted it will offer patent protection until at least 2032 for a technology where the patent life began in 1992.

Despite the opportunities offered by increasingly refined intellectual property (IP) strategies, and the ability to obtain protection despite close prior art, the caveat is that the eventual claims might only offer narrow protection, from which a commercialised product could suffer commercial competition. For example, as mentioned above, the granted claims for the patent protecting the development of glycopyrrolate as a treatment for COPD were based on its formulation as a dry powder inhaler. With this in mind, two successor biotechnology companies (Pearl Therapeutics and Elevation Pharmaceuticals) have initiated development programmes based, respectively, on metered dose aerosol inhaler and nebulised formulations of glycopyrrolate for COPD. These formulations ostensibly lie outside the scope of the dry powder formulation claims. Elevation Pharmaceuticals was rapidly able to develop

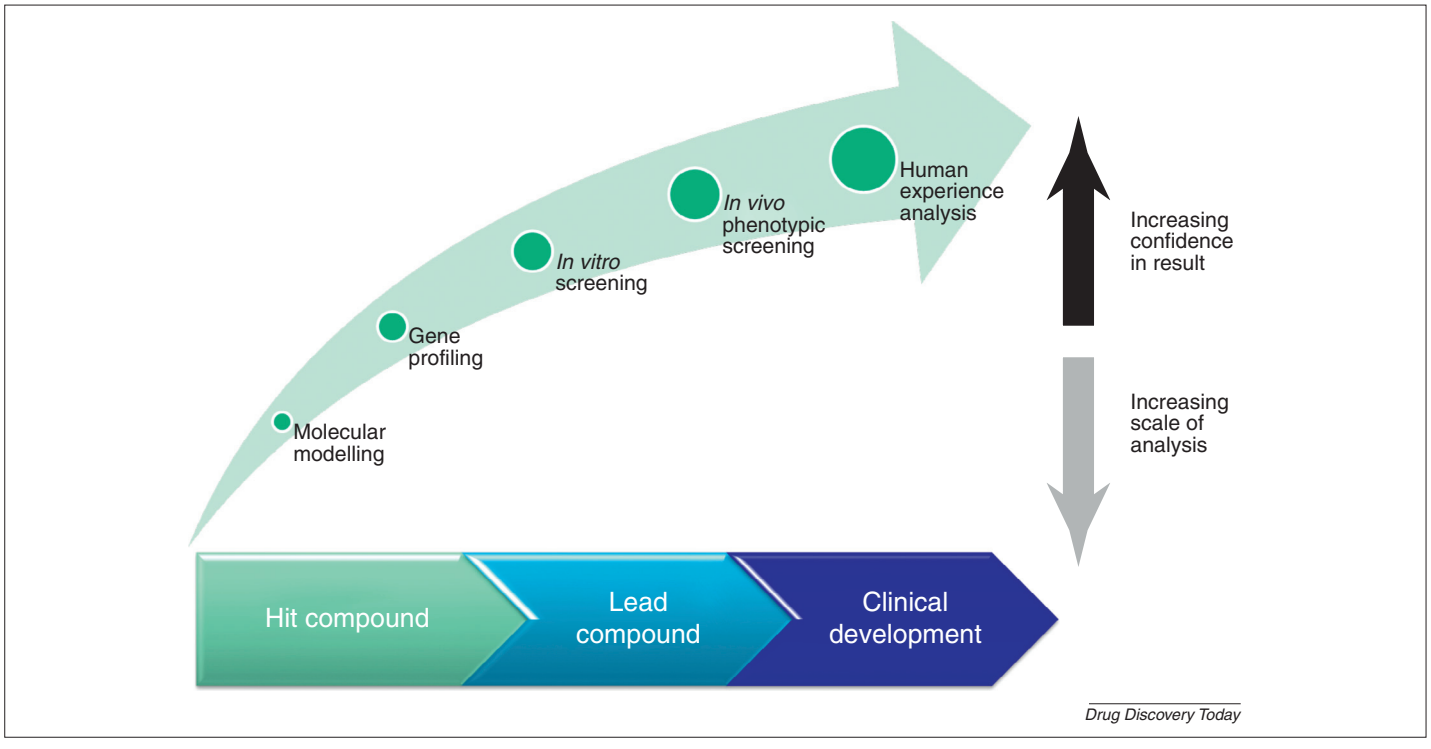

FIGURE 4

Different levels of validation, confidence, scale and stage of R&D associated with different methods of experimental drug repurposing prediction.

its product through to Phase IIb clinical trials, and was recently acquired by Sunovion Pharmaceuticals for ~US$430m, assuming all milestones are met (Sunovion Pharmaceuticals Press Release 30 August 2012, http://www.sunovion.com/news/pressReleases/20120830.pdf).

Improving the success of repurposing drug development

A central feature of drug repurposing is that it offers improved efficiency in generating clinical candidates or the discovery phase of R&D. Although existing drugs will have toxicological information that reduces their risks of unacceptable safety, or tolerability, they will not *a priori* have reduced chances of efficacy failure in the subsequent developmental phase. From that perspective, the strategy increases the size of the haystack but offers improved efficiency in finding the needle and undertaking the pivotal tests for its aptitude for the task ahead.

Each of the experimental methods has a different development course resulting from the hypothesis created. Figure 4 shows that each of the predictive methods is associated with a range of different stages of R&D. As well as providing differing levels of validation, each method is also associated with different scales of operation. Whereas the molecular modelling and gene profiling techniques can be applied to complete libraries of approved drugs in a matter of hours, retrospective clinical trial analysis can take weeks per compound.

As shown in Fig. 4, different methods fit into pharmaceutical R&D in different ways:

i. Larger scale approaches (molecular modelling, gene profiling) provide larger numbers of opportunities, with lower levels of predictability, so these are normally validated *in vitro* first, rather like a conventional discovery programme. It is possible that large companies with interests in repurposing and computational chemistry and biology resources could conduct an exhaustive analysis of the repurposing opportunities achievable by their particular technology. However, the comprehensiveness of such work will depend on the particular method used – whether it is focused on secondary targets for existing drugs (as opposed to indications); whether it considers orphan indications in addition to the mainstream; whether it considers all existing drugs or only those entities within its own corporate library; whether it considers all possible indication areas or only those within the strategic intent of the company; and so on.

ii. *In silico* approaches to the discovery of secondary target interactions for existing drugs are likely to unveil weaker binding or functional interactions that occur at higher drug concentrations than the primary, most potent interaction upon which a drug's first life is based. A rare exception to this is the discovery that doxycycline for periodontitis through matrix metalloproteinase inhibition is effective at subantibacterial doses (http://en.wikipedia.org/wiki/Tetracycline_antibiotics). To identify selective agents (assuming they are necessary), further medicinal chemistry needs to be conducted and the output from these methods is akin to a hit or a lead in a conventional R&D programme. This does not mean, however, that the value of the repurposing experiment is entirely lost, because it might be possible to test the hypothesis in a clinical situation providing the envelope of systemic toxicological exposure is not exceeded, and this might be possible with topical testing for example, as long as bridging sensitivity studies have been conducted.

iii. Human analysis (side effect comparison or retrospective clinical trial analysis) is particularly valuable because it relates to the species of interest, following approved doses of an existing drug. The logical next step is often to undertake prospective clinical trials to see whether the retrospective analysis is replicable. However, a significant limitation of this kind of analysis is the scope of the data available. Incidence of cancer is a universally reported serious adverse event that can readily be analysed by this means, but the same cannot be said of arthritis. Side effect profile analysis is far more versatile as a means of matching compounds but, as pointed out above, has limitations with the identification of new therapeutic indications from existing targets, or identification of drugs for new indications for which there are no known therapeutics.

iv. Despite the logical 'next step' for validation of a given proposal according to Fig. 4, there are other things that can be done to build an evidence base before prospective studies are undertaken. For instance, retrospective analysis can be used to validate proposals made for individual drugs in new indications from gene profiling analysis directly linking drugs and indications. If we start from the prediction about cimetidine in lung cancer, the natural next prospective study from such a proposal could be to test *in vivo* in a model of lung cancer. However, given the ontological support from the observations of cimetidine's effect in other forms of cancer, another route could be to conduct a retrospective analysis of prescription data to see if there is a reduced incidence of lung cancer for patients treated with cimetidine. If this retrospective analysis proved sufficiently positive, one might decide to skip the *in vivo* model and initiate a prospective clinical trial to evaluate whether cimetidine was therapeutically effective in the treatment of existing lung cancer. This sequence of investigational studies is particularly attractive in therapeutic areas lacking well-validated *in vivo* models.

Concluding remarks

Given the profusion of drug repurposing hypotheses from the existing literature, the first question is: why do we need any more? One answer could be that drug repurposing offers advantages in terms of cost to enter clinical stages of development, as well as time to do so; however, the existing hypotheses are subject to attrition through lack of (sufficient) clinical efficacy in the same way as conventional drug development programmes. Therefore, all else being equal, the more compounds we enter into the R&D pipeline the more successful products will emanate from the other end.

The 'more shots on goal' argument is a rather one-dimensional reason to pursue this strategy, and arguably an insufficient one. To go beyond this situation, to improve the efficiency of drug repurposing as an overall strategy, we need to improve the attrition rate in development. Even though the toxicological information that accompanies the existing drug can improve attrition as a result of safety, to improve rates of efficacy attrition we need to do more.

Improvements in attrition efficacy can be achieved if we can use the method of identifying a repurposing opportunity to also partly

validate the new hypothesis. Information from human experience, either from side effect analysis or retrospective analysis of clinical trials or prescription data, can provide certain levels of validation, and because it is based on the animal of interest, at drug exposure levels that correlate with real experience, this information is of significant value.

At the other extreme, the repurposing that derives from molecular modelling is normally the precursor to a long discovery and development campaign that lacks the time advantages associated with repurposing-based development. Computerised modelling of drug–target interactions provides the foundation for a different kind of repurposing, associating drugs with new targets rather than indications, in which there is normally chemical modification before the identification of an optimised development candidate molecule. The computer techniques can enable scaffold-hopping, where for instance there are known deficiencies in the existing structures effective for a certain biological target – be they selectivity, metabolic or toxicological in nature.

In the middle, between these extremes, lie the 'wet'-laboratory-based screening methods. The use of existing libraries such as the 1040 FDA-approved compounds used by Rothstein's laboratory group [13] enabled the direct and rapid initiation of clinical studies in ALS, an area of huge unmet need. *In vivo*, mechanistically agnostic screening methods have identified new mechanisms, such as Melior's identification of lyn kinase as a target in diabetes, that were theoretically unpredicted by the prevailing biological theory.

Although the methods that provide little or no validation of the repurposing hypotheses leave much to be proved, the use of existing drugs offers a rapid method of testing in a human being. This is particularly valuable when the existing animal models for a disease are poorly developed, poorly translatable or do not exist at all, and where the existing drug can be tested against a disease biomarker in a human population at drug exposure levels that are within the envelope provided by the existing toxicological information. It is also valuable for testing a mechanistic hypothesis for a disease treatment.

Ontological methods can complement experimental approaches to drug repurposing, particularly in adding validity to a hypothesis. By contrast, the stronger the evidence for the utility of a repurposing hypothesis then the weaker the scope is for patent protection in the new area. The identification of robust IP is one of the main drivers for using experimental approaches as opposed to ontological ones, yet experience has taught us that, even in areas where there is close prior art, there remain patent opportunities sufficient to generate significant commercial successes. This is particularly the case where small modifications of one (or more) of the components of the product opportunity can be optimised beyond what is published [47].

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