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REVIEW

Drug repositioning: current approaches and their implications in the precision medicine era

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ABSTRACT

Introduction: Drug repositioning implies finding new medical uses for existing drugs. It represents a cost-efficient approach, since the new indications are built on the basis of available information on pharmacokinetics, safety and manufacturing. Whereas most of the pioneering drug repurposing stories arose from serendipitous observations and clever exploitation of side effects, the drug discovery community has lately addressed repurposing initiatives in a more systematic manner. Today, in the middle of the omics era, we have the tools to explore drug repurposing opportunities in a tailored, personalized manner.

Areas covered: After a brief discussion on modern approaches to drug repurposing, the author connects the philosophies of drug repurposing and personalized medicine through the well-known and extended practice of off-label prescription. The author also discusses which, among current systematic repurposing approaches, are more appropriate to be integrated with the field of precision medicine.

Expert commentary: Personalized drug repurposing is not a new concept at all: for years, it has been known as off-label prescription, a practice widely accepted especially in some branches of medicine. Whereas in the past such approach was in many cases supported by empiric knowledge, today omics technologies allow us to face novel personalized drug repurposing options in a systematic manner.

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Drug repurposing; drug repositioning; personalized medicine; precision medicine; genome-wide association studies; GWAS; omics; off-label prescription; therapeutic indication expansion; therapeutic indication shift

1. Introduction

Generally speaking, drug repurposing involves finding novel therapeutic indications for approved, discontinued, and archived drugs, as well as drugs currently undergoing clinical trials; finding second medical uses for abandoned or discontinued drugs is more specifically referred as drug rescue [1]. In any case, the approach focuses on late-stage chemical matter (that is, drugs that are or have been approved, and drugs that are undergoing or have undergone clinical trials) [2]. Rediscovering a drug in a new therapeutic area represents a cost-efficient strategy, since the new indication is built on already available pharmacokinetic, safety, and manufacturing knowledge [3,4], resulting in major savings in time and resources. In many cases, though, dosing and formulation modifications could be required [5].

Drugs that have not achieved approval for a given indication due to safety issues might be rescued if the cost–benefit analysis justifies their administration in a new therapeutic area, or if the adverse reactions found when studying the originally pursued indication are not relevant in a different drug administration schedule or setting.

The major challenges faced by drug repurposing projects are probably related to commercial, regulatory, or intellectual property reasons [5–8]. That probably explains why it has been embraced in the field of neglected and rare conditions, where investment revenue is not the main driving force of drug discovery projects [9–11].

According to the Precision Medicine Initiative, precision medicine is 'an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person' [12]. It is closely related to personalized and stratified medicine, terms that are often used interchangeably, though subtle distinctions between them have been realized [12,13]. In any case, it is clear today that: (a) some diseases with common traits that in the past were approached as a single condition comprise in fact a spectrum of diseases and; (b) more effective and/or safe medications might be found if tailored to variations in a person's genome, transcriptome, proteome, and metabolome, or to specific types of a general condition. Besides its contribution to treatment choice, it is also becoming clear that complex dynamic changes occur both in health and disease, and thus regular monitoring of health and disease states can serve to estimate disease risk and prognosis [14]. The possibilities of personalized medicine have been greatly expanded by genotyping technologies (in particular, microarray/biochips and next-generation sequencing) and state-of-the-art computing.

Is there any bridge between drug repurposing and precision medicine? Is it possible to systematically repurpose drugs based on individual data? If so, which of the systematic approaches currently used to address drug repurposing is more adequate for such enterprise?

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2. Systematic drug repurposing

Many of the first successful repurposing stories were based on serendipitous/empirical/retrospective observations. Minoxidil was originally investigated to treat ulcers; while conducting trials in dogs, it was observed that the compound elicited a prolonged reduction in blood pressure. Later, while undergoing clinical trials to prove its efficacy as antihypertensive medication, the drug showed an unexpected positive effect on hair loss [15]. It gained US FDA approval as antihypertensive medication in 1979 and in 1988 it became the first FDA-approved drug for the treatment of androgenic alopecia. Taking into account the first pursued therapeutic indication (antiulcer therapy), we may affirm that minoxidil was repurposed not once but twice. Sildenafil was initially studied for its potential use in hypertension and ischemic heart disease; during clinical trials, scientists at Pfizer observed an unexpected effect inducing penile erection [16]. As no treatment of male erectile dysfunction had so far been approved, Pfizer shifted the focus of their research and in 1998 the drug received approval as the first oral treatment for such condition. In 2005, it also received approval as a treatment of pulmonary hypertension. The well-known antiplatelet effects of the centennial aspirin itself have been exploited to repurpose the drug, in low doses, for secondary prevention of heart attack and stroke (following a serious cardiovascular event) [17]. Moreover, aspirin has recently completed clinical trials as potential adjunctive treatment for bipolar disorder (Clinical Trial.gov identifier NCT01797575) and Phase III clinical trials to assess its effects on cancer recurrence and survival are currently under development [18].

Demonaco et al. analyzed the introduction of new clinical uses between 1999 and 2003 for compounds approved in 1998, finding that 57% of 143 drug therapy innovations were discovered by practicing clinicians through what they called 'field discovery' (an unorganized discovery process without involvement of the drug manufacturer or other laboratory, performed by the clinician during patient care) [19]. When surveyed using a standardized questionnaire to gain deeper understanding on the field discovery process, 59% of the field discoverers who answered the questionnaire reported that they had made their discovery by applying their understanding of the pharmacology of the drug to the clinical problem at hand, which indicates that not all the field discoveries are serendipitous in nature. Demonaco et al. also underlined that user-developed products (in this case, new therapeutic uses) tend to differ from manufacturer-developed products in an important way, since they tend to be 'functionally novel'; in other words, users frequently identify 'new applications of existing products not originally envisioned by the product manufacturer'.

On the basis of the impressive examples of successful drug repurposing stories based on empirical and retrospective observations, the drug discovery community is now endeavoring to systematize the (organized) identification of prospective drug repurposing opportunities [20–22], a complementary approach to field discovery.

Systematic approximations to drug repurposing may present different degrees of rationality. For example, we may resort to exhaustive, wet screening of libraries of approved drugs, either using phenotypic- or target-based screens, and low- or high-throughput assays [23–25]. High-throughput screening approximations do have a rational side since they are based on miniaturization and automation, hence displaying cost- and time-efficiency despite the high cost of technological platforms required and the high operating costs. They are nevertheless, in essence, 'brute force' approaches (systematically enumerating all possible candidates for the solution and checking whether each candidate satisfies the problem's statement). This less rational side of (wet) high throughput screening can be alleviated using focused libraries, i.e. relatively small libraries of molecules that are likely to have a pursued activity based on knowledge of the target protein and literature precedents for the chemical classes likely to have activity against a given therapeutic target [26,27]. In a very interesting recent study, for instance, Klaeger et al. used kinobeads (immobilized broad-spectrum kinase inhibitors that enable the purification of endogenous kinases from cells or tissues) and quantitative mass spectrometry to investigate the target space, selectivity, and dose-response characteristics of 243 clinical kinase inhibitors [28]. To illustrate the value of their results to detect drug repurposing opportunities, the authors demonstrated (*in vitro* and *in vivo*) the efficacy of cabozantinib for the treatment of FLT3-positive acute myeloid leukemia.

The finest examples of systematic drug repurposing methods are, possibly, computer-assisted approaches (including those methods that use computers to integrate and analyze large-scale information drawn from literature, clinical trials, or disease and treatment omics data). Using an initial broad classification, we may categorize such methods as bioinformatic-, chemoinformatic-, network-based, signature-based, and literature-mining approximations, plus some hybrid approaches [20,29]. Most of them imply, fundamentally, identifying (hidden) connections between approved drugs and diseases or pursued drug targets. This is closely related to Swamson's ABC model of disjoint but complementary structures in biomedical literature [30–32]. Consider three elements, concepts, or arguments A, B, and C. If connections between A–B and B–C have already been proven, a direct connection might probably exist between A–C even if it has not been revealed yet. We might even say that the more the indirect bridges between A and C, the best the chances of confirming a direct connection between them [33]. For instance, an association has been established between depression and chronic inflammatory response [34,35], justifying the exploration of anti-inflammatory drugs as possible new treatments for depressive disorders [36,37].

Underlying the previous discussion lies the idea that scientific data and scientific knowledge are today produced at such a meteoric rate that an increasing amount of latent knowledge awaits being exposed. Bridging data, i.e. connectivity, seems to be the key. Computer-aided drug discovery can help automatizing (or at least semi-automatizing) this kind of discovery. The link between the ABC model and computer-aided drug repurposing is illustrated in Figure 1.

2.1. Cheminformatics and drug repurposing

In silico screening constitutes the most common cheminformatic approach to drug repurposing. Of course, the chemical

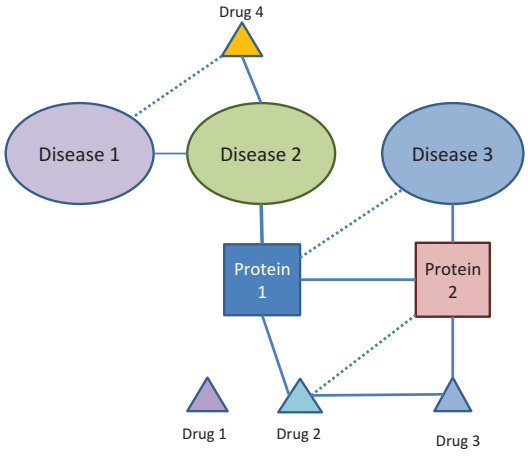

Figure 1. Swanson's ABC model can be applied to reveal hidden connections between drugs and molecular targets or diseases. In the diagram, established (direct) connections are shown in continuous lines, whereas dashed lines represent hidden (indirect, inferred) connections. In the example, the connection between Drug 2 and Protein 2 can be inferred from the established connection between Drug 2 and Drug 3 (e.g. using cheminformatics tools such as molecular similarity methods). The same hidden connection may be uncovered by connecting Protein 1 and Protein 2 through bioinformatics. A patophysiological common basis to Disease 1 and Disease 2 might also provide arguments to repurpose Drug 4 for Disease 1.

libraries submitted to an *in silico* screen should be focused on the chemical matter of drug repurposing: approved, withdrawn, shelved, and investigational drugs. Fortunately, publicly available resources such as DrugBank and Sweetlead have been developed, compiling drugs that have received approved or investigational status by the FDA and other regulatory agencies [38,39]. In addition to providing access to molecular structures of approved and investigational drugs, the latest versions of DrugBank have been expanded to incorporate, among other data, pharmacogenomic information of considerable value in the fields of stratified and personalized medicine [38,40]. Such information includes tens of thousands of coding and noncoding single nucleotide polymorphisms derived from known drug targets and drug metabolizing enzymes. DrugBank provides summary tables on these polymorphisms that include: allele variants; validation status; chromosome location; functional class (untranslated, intron, exon, etc.); mRNA and protein accession links; reading frame; and allele frequency in African, European, and Asian populations, among other fields. Furthermore, this database also informs on known drug targets of existing drugs, a remarkable feature to support the construction of drug-target networks (see Section 2.4).

A different but conceptually interesting approach has been presented by Wu and coworkers [41], closely similar to preceding work by Keiser and collaborators [42,43]. The general idea behind their work is that different therapeutic indications could be related if each of them includes chemically similar drugs. These studies speak of a pattern of repurposing opportunities between pairs of therapeutic classes, which encourages the systematic cross-screening of drugs from one therapeutic class for the other.

The existing methods to implement virtual screening to identify *in silico* repurposing opportunities include ligand-based and structure-based approximations or their hybrid combinations. Possibly, structure-based approximations are the most likely to produce results in the field of precision medicine in the short- or mid-term, since most ligand-based approximations require a minimum of training examples (including activity data) which are rarely available for drug targets variants. The general idea would be to use structural information on the target to guide the selection of new drugs active on polymorphic or mutant variants. Such molecular target could potentially be a human gene product or a structural variant responsible for modified drug sensitivity in a microorganism strain or subtype. There have been many structure-based studies applying either docking or molecular dynamics simulations to explain differences in ligand affinities to target variants [44,45], exploit such differences to design new drugs [46,47], or even conduct *in silico* screening campaigns [48–51]. However, to our best knowledge no *in silico* screening addressing variants of drug targets with a focus on drug repurposing has so far been performed.

On the other hand, it has recently been discussed that the importance of bioactivation might have been miscalculated in the context of drug repurposing campaigns based on *in silico* screening [52,53]. Since about 10% of existing drugs have been characterized as (intended or unintended) prodrugs [54], and many others produce pharmacologically active metabolites, it could be important to take into consideration possible active metabolites of the potentially repurposed compound (the major metabolites, in particular) during the screening protocol. A drug could be repurposed not only by its intrinsic therapeutic potential but because of the therapeutic potential of its metabolites; the exposure to such metabolites will vary significantly from individual to individual. This idea is in good agreement with the well-established role of polymorphic metabolic enzymes in personalized therapy [55,56]. Have in mind that drugs may be pharmacologically activated or inactivated after biotransformation, and the extent and rate of such activation/inactivation will largely depend on the genotype and expression levels of drug metabolizing enzymes.

2.2. Bioinformatics and drug repurposing

One of the general principles that support computer-aided drug repositioning is that health disorders linked to similar dysfunctional proteins may be treated with the same drugs (disease-centric approach). Bioinformatic tools, from sequence alignment to domain similarity identification tools, may be useful to reveal unknown protein–protein similarities. While experts in a given disease are naturally familiarized with target proteins associated with their specific subject of study, they might well ignore which other diseases are linked to the same or closely related targets, hence missing valuable repurposing chances.

The questions become more challenging when dealing with remote similarities between proteins which do not seem to have any evolutionary relationship or even do not share a similar fold or function. For that purpose, a lot of attention has been paid to the identification of binding site similarity as a basis for detecting repurposing prospects [57–59]: similar binding sites can be found in proteins with low or no overall similarity; several case studies reveal that the

binding of similar ligands cannot be deduced from fold but from local similarities [58]. Correlations between ligand binding promiscuity and binding site and global structure similarities have been established by Haupt and coworkers [60]. For that purpose, 164 ligands co-crystallized with three or more nonredundant targets were extracted from the Protein Data Bank. Such ligands were found in 712 nonredundant protein targets. All pairs of binding sites for all promiscuous drugs were then aligned. Direct correlations were found between the overall structure similarity and the degree of promiscuity, and between the square root of the number of similar binding sites and the degree of promiscuity. Such results suggest that the global structure similarity and the binding site similarity can be used as criteria to guide drug repositioning initiatives.

In their comprehensive review on different kinds of binding site similarity comparisons and their recent successful applications [59], Ehrt et al. have concluded that to the moment no best-performing algorithm to compare binding sites can be chosen since adequate benchmarking studies have this far not been developed. Furthermore, they highlight that poor results are often observed when using individual approaches but performance is greatly enhanced when applying different *in silico* and experimental methods in a workflow. Alike other areas of drug discovery where algorithms are routinely applied, consensus seems to be the key.

It should also be remembered that whereas similar binding sites frequently bind the same ligands, the converse does not hold: a ligand may bind to very different binding sites [59]. Consequently, binding site comparison can only cover a part of the possible drug repurposing cases.

2.3. High-throughput literature analysis and drug repurposing

The rapid expansion in the volume of the biomedical literature spawns a combinatorial explosion in the number of implicit meaningful connections between biomedical concepts; the probability that such connections remain unnoticed is substantially enhanced by the increasingly disjointed nature of knowledge as a result of specialization [61]; even for a specialist it is becoming no longer possible to keep up to date with all the relevant literature on a delimited topic [62]. The challenge increases under the perspective of drug repurposing, which intrinsically demands the researcher to reach out to other areas of knowledge related to the original or new indication of the repurposed drug. Accordingly, automated literature mining methods are highly relevant to screen large volumes of scientific literature and information to find hidden connections.

Co-occurrence methods are the simplest approaches to link biomedical terms of interest. Implicit connections between terms that do not co-occur are discovered by finding a third linking term that occurs directly with each of them. This scheme allows for two modes of discovery, termed open and closed [61]. Open discovery starts with a disease C and a set of intermediate B concepts related to this disease are identified in the literature. These B concepts are then explored to seek out A concepts (potential treatments). In closed discovery, the starting point is a hypothesis or observation of a therapeutic relationship between treatment A and disease C, and an explanation for this hypothesis or observation is sought by exploring for concepts related to both A and C. The seminal idea by Swanson's was of course later expanded and refined. For instance, Predication-based Semantic Indexing is utilized to identify sequences of relationships termed 'discovery patterns,' e.g. 'drug x INHIBITS substance y, substance y CAUSES disease z' [61]. Predications are extracted from the biomedical literature by the application of natural language processing technology.

A good example of text mining applications in the field of drug repurposing has recently been reported by Su and Sanger [63]. These authors mined ClinicalTrials.gov (which contains biomedical data from more than 220,000 clinical trials). Their idea is simple but appealing: they look for drugs where the treatment arm has fewer predefined serious adverse events than the control arm, indicating that potentially the drug is reducing the level of such adverse event. Hypotheses can then be generated for a new use of the drugs based on the predefined serious adverse event that is indicative of disease.

2.4. Network-based approaches and drug repurposing

Integrating large amounts of data is the key concept underlying network analysis. Networks deal with complexity by simplifying complex systems: entities are represented as nodes while relationships between nodes are depicted as edges [64]. Such degree of simplification allows visualizing and analyzing the system with a holistic perspective, unraveling informative associations from the topological architecture of the network (e.g. modularity, 'date' and 'party' hubs, nodes neighboring hubs, etc.). Networks may incorporate different levels of information (for instance, different types of nodes representing categories of elements may be allowed, e.g. drugs, proteins, genes, diseases). Moreover, the edges could be established using experimental data (e.g. the value of an experimentally measured affinity constant might be used to link a given drug to a given protein) and/or predicted/theoretical data (e.g. an association between two drugs might result from a similarity measure or from proximity in the chemical space). Edges could be weighted to reflect different strengths or reliability of associations; occasionally, they might even encode some sort of dynamic link between two linked elements (e.g. semantic edges) [65,66]. Note that all the approaches to drug repurposing described in previous sections (cheminformatics, bioinformatics, literature-mining), jointly with experimental data, can be used to build networks.

There are currently a vast number of public resources that provide valuable data to develop protein–protein, drug–protein, drug–disease, and drug–protein–disease networks. Just to mention a few examples, DisGeNET [67] offers hundreds of thousands of associations between human genes and diseases, and (interestingly) disease-variants associations; the Therapeutic Target Database (TTD) [68] is a drug database that provides information about known and explored protein and nucleic acid drug targets, the targeted disease, pathway information, and the corresponding drugs directed at each of these targets (including investigational drugs); BindingDB [69] is a database of experimental protein–small molecule interaction data that today collects over a million data entries

extracted from scientific articles and patents; STRING is a public repository that provides protein–protein interaction data on the basis of both experimental (e.g. co-expression patterns) and *in silico* predictions (for instance, co-occurrence in biomedical literature) [70]; STITCH integrates experimental and predicted information about interactions between small molecules and targets [71]: it currently holds information on more than 430,000 chemicals retrieved from different sources of verified protein–chemical interactions, which is complemented by automated text-mining and structure-based predictions and displayed as an interaction network.

In a very interesting application of network analysis to drug repurposing, Vitali et al. implemented a network-based study to identify drug repurposing opportunities against triple negative breast cancer, a subtype of breast cancer whose biology is still poorly understood [72]. First, they selected a list of genes and proteins known to have a role in the disease. The list was generated from a previous study that enumerated genome aberrations from 104 cases of primary triple negative breast cancer. The most significantly mutated and differentially expressed genes were extracted and high-confidence protein–protein associations with the correspondent disease proteins were extracted from STRING to produce a protein–protein network, with the edge weights being proportional to the confidence level of the association. The topology of the resulting network was used to identify potential therapeutic target proteins; for that purpose, three criteria were used: (a) hubs were discarded (impairing their function results in rapid deterioration of network information transfer, which may translate into considerable side effects); (b) bridging nodes were selected; and (c) the selected targets should be druggable (Figure 2). Next, envisioning the possibility of selecting multi-target drugs, the authors resorted to the previously reported Topological Score of Drug Synergy [73] to select target triplets; such score also takes into account the network topology: it considers the topological distance between the targets and the network edge weights, favoring shortest paths with higher edge weights. DrugBank and the Comparative Toxicogenomics Database were then explored to extract a list of approved drugs known to interact with the target proteins; this search was complemented by the application of a data fusion approach based on matrix tri-factorization. Finally, 18 pathways involved in the progression of triple negative breast cancer were extracted from the KEGG repository. Each pathway was modeled as a Boolean Network and gene expression microarray data were later integrated to the model, to identify up- and downregulated genes. The effects of imatinib (one of the drugs selected for repositioning) alone or in combination with other drugs was estimated; it was predicted that imatinib alone or in combination with vemurafenib or vemurafenib and flucytosine would have positive effects against triple negative breast cancer, which was validated *in vitro*. In the light of the scope of this article, we believe that the study by Vitali and collaborators is an excellent example on how to integrate gene expression data to network analysis to repurpose drugs for specific subtypes of a given condition.

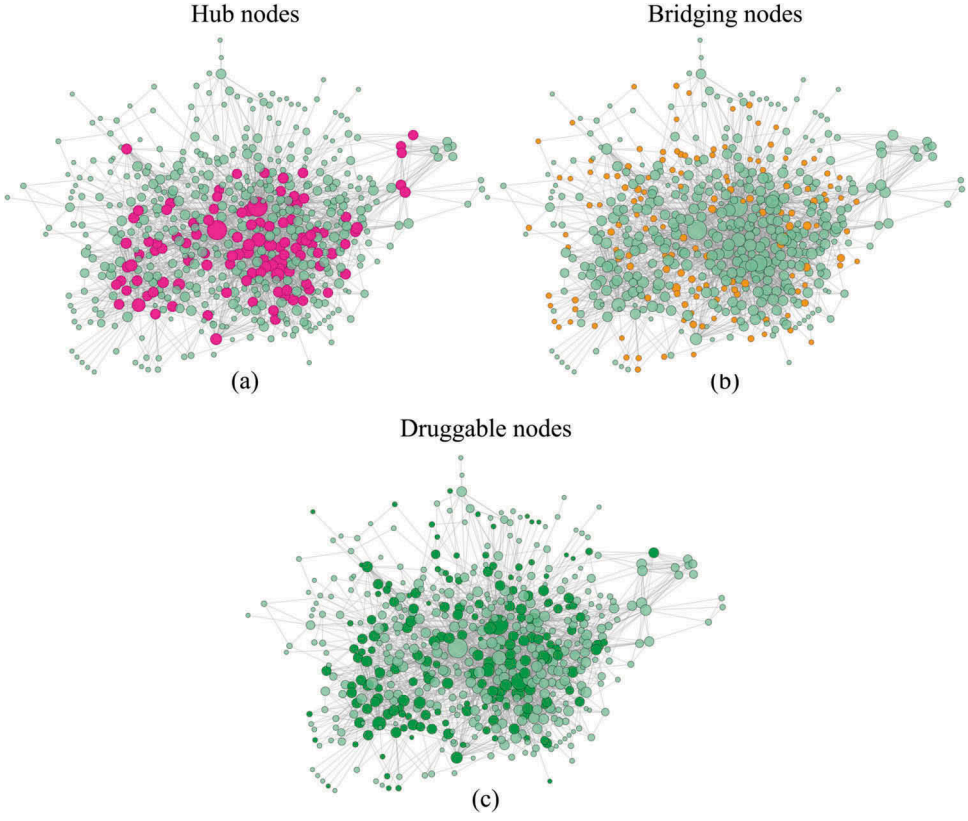

Figure 2. Network constrains were applied to the protein-protein network build by Vitali et al. Hubs (a) were excluded from the list of potential targets to reduce the probabilities of significant adverse reactions. Bridging nodes (b) that were also druggable (c) were selected instead. Reproduced under Creative Commons Attribution license from reference [72] © 2016 Vitali et al.

In line with the emerging paradigm of using multi-target therapies to improve efficacy in the treatment of complex disorders and reduce drug resistance rates [74,75], this type of network-based approximations are being increasingly applied in oncology to select drug combinations that may cope with robustness conferred by abundant regulatory loops and redundant pathways in cancer [76]. Whereas initial computational tools to detect effective drug combinations such as Combinatorial Drug Assembler [77] or DrugPairSeeker [78] were simply based on calculating connectivity scores of drug pairs that maximized the reversal of disease-associated gene signatures (see next section for further details), more recent tools such as DrugComboRanker [79] or SynGeNet [80] combine transcriptomics with network-mining algorithms. For example, Regan and coworkers reported the application of SynGeNet to identify drug combinations to treat melanoma [80]. They retrieved gene expression data of patient-derived $BRAF^{V600E/K}$ mutant melanoma tumor samples and normal skin samples from the Gene Expression Omnibus dataset. Melanoma-associated genes were obtained via DisGeNET and a melanoma disease signaling network was constructed, integrating gene expression fold change information and protein–protein interactome data. The top-ranked melanoma-associated disease genes from DisGeNET were used as root nodes to uncover the dysfunctional disease signaling network. Gene expression profiles of more than 600 FDA-approved drugs tested in the BRAF-mutant A375 melanoma cell line were obtained from LINCS L1000 transcriptomics database. Individual drugs were ranked by negative connectivity scores (i.e. by its hypothesized ability to reverse disease-signature). Such rank was then used to weight each drug; the 61 top-ranked drugs were clustered into drug communities according to their targeted pathways, in order to choose possible synergistic drug combinations. Drug target interaction data was obtained from DrugBank and STITCH databases. Drug target genes were mapped on the constructed melanoma disease signaling network, and the centrality of each target gene within the overall network was calculated based on the average of betweenness, closeness, and page-rank centrality metrics. The weighted sum of the centrality parameters for each of the unique drug targets was computed to determine the synergy score of drug pairs (note that this approach does not take into account the distance between two drug targets, though such distance can be taken into consideration by assessing if the selected drugs come or do not come from the same drug community). The method was validated using results from a previously published *in vitro* combinatorial drug screening study across different melanoma cell lines, seemingly demonstrating that the approach outperformed other transcriptomics-based methods. SynGeNet was able to predict 12 validated drug combinations against melanoma, 11 of which involved drugs from different communities.

2.5. Signature-based drug repurposing

Signature-based drug repurposing uses gene expression data to identify new drug repositioning opportunities. By comparing the expression profile of a cell before and after exposure to a drug the changes induced by active compounds on the transcriptome can be quantitatively assessed [81]. This drug-induced gene expression profile can then be compared with a disease-associated signature. It is hypothesized that inverse similarities between drug- and disease-associated signatures will speak of a possible beneficial effect of the drug against the disease of interest. Although it can be argued that this method does not consider mechanistic aspects in a direct manner, and that the effect of a drug on the transcriptome of a reference cell type may not be exactly the same that its effect on an unhealthy cell, the approach has shown promising results in several difficult-to-treat complex disorders, such as cancer [82], Alzheimer's disease [83], and inflammatory bowel disease [84].

Public resources specifically oriented to signature-based drug repurposing have been developed [85], adding to the pioneering signature repository from the Broad Institute, the Connectivity Map (cMap) (https://portals.broadinstitute.org/cmap/) [86], the first large public database of genome-wide gene expression profiles from a diversity of human cell lines treated with more than a thousand bioactive small molecules. To query the cMap, the authors devised a pattern-matching tool based on Gene Set Enrichment Analysis (GSEA) [81], through which connections can be statistically evaluated. Essentially, GSEA assesses expression data at the level of gene groups which are defined on the basis of prior biological knowledge (e.g. belonging to the same biochemical pathways, coexpression, proximally located in chromosomes); GSEA determines if the members of a gene group tend to occur toward the top (or the bottom) of a ranked list of differentially expressed genes between two cell types, in which case the gene set is correlated with the phenotypic class distinction [87]. The resultant 'connectivity score' is normalized using random permutation, assuming values from −1 to 1 to reflect the closeness or connection between the expression profiles. Identifying drugs with similar mode of action constitutes another possibility of cMap application to drug repurposing. In this case, it is assumed that if two drugs elicit similar transcriptional responses they could display a common mode of action and thus they might be applied to treat the same pathological condition. The cMap query tool allows sscMap extension and the mode of action by network analysis (MANTRA) tool [81]. sscMAP is a free-to-download java implementation of the cMap algorithm bundled with the reference dataset, enabling integration of user-defined data [88]. The approach uses a connectivity score computation with permutation tests at treatment instance and treatment set levels, offering a statistical mean to control false connections between the gene signature and the reference profiles [89]. First, sscMAP introduces a new ranking score by making the following considerations: treatment and control instances are treated similarly; the genes that are highly differentially expressed are given more weight and; the up- and downregulated genes are handled equally. The new scoring scheme for representing a query gene signature either with ordered or unordered gene list is computed as follows. The connections strength is calculated between the reference profile R and the gene signature s:

$$C(R,s) = \sum_{i=1}^{m} R(g_i)s(g_i) \quad (1)$$

where g_i represents the ith gene in the signature, $R(g_i)$ denotes the ith gene rank in the reference profile, and $s(g_i)$ denotes the ith gene rank in the signature. m is the number of genes in the

gene signature. For ordered gene signatures, the maximum connection between the reference profile and the gene signature is achieved by matching the m genes and their regulation status in the reference profile and the gene signature in the correct order:

$$C^0_{max}(N,m) = \sum_{i=1}^{m}(N-i+1)(m-i+1) \quad (2)$$

where N is the total number of genes. For unordered gene signatures, all the genes in the list would have equal weight. The overall connectivity score is calculated by dividing the connection strength with the maximum connection strength of a given gene signature and reference profile; it ranges from −1 to 1, where 1 indicates a maximum connection of s with R and −1 indicates a negative connection.

On the other hand, MANTRA makes use of a post-processed version of the cMap dataset, where compounds are grouped into a drug similarity network. In this network, two drugs are connected if they induce similar transcriptional effects in human cell lines [81]. MANTRA users can either integrate a drug under investigation into the network and deduct its mode of action by analyzing the surrounding subnetwork, or identify repurposing opportunities by searching the neighborhood of a query compound with a desired mode of action for other compounds non-previously linked to that mode of action.

Fortney et al. have recently adapted a parallel CMap approach across multiple gene signatures of a disease, and named their method CMapBatch [89,90]. CMapBatch has interesting perspectives in the field of stratified medicine: instead of applying CMap to one individual gene signature, it applies it to an ensemble of signatures for the same disease and then combines the resulting outcomes. CMapBatch thus resembles meta-analysis [89].

Recently, a new version of cMap was reported as part of the NIH LINCS Consortium [91] using the L1000 assay (a new, low-cost, high-throughput reduced representation expression profiling method that measures the expression of only 978 landmark genes, whereas the expression values for the remaining genes are computationally estimated, allowing a 1000-fold scale-up of the cMap) [92]. It was shown that L1000 is highly reproducible, comparable to RNA sequencing, and suitable for computational inference of the expression levels of 81% of non-measured transcripts. This next-generation cMap can be used to discover mechanisms of action of small molecules, functionally annotate genetic variants of disease genes, and inform clinical trials.

It is also worth mentioning that valuable specific resources compiling genomic data in support of precision medicine in the field of cancer exists, such as the National Cancer Institute's Genomic Data Commons (which centralizes, standardizes, and makes accessible data from previous large-scale NCI programs such as The Cancer Genome Atlas and Therapeutically Applicable Research to Generate Effective Treatments, granting public access and exchange of cancer genomic data) [93] or the Cancer Cell Line Encyclopedia (providing public access analysis and visualization of DNA copy number, mRNA expression, mutation data and more, for 1000 cancer cell lines) [94]. CIVIC [95] constitutes another formidable initiative that intends to provide a centralized, open-access, open source, open license knowledge base for expert crowdsourcing of clinical interpretation of gene variants in cancer. It accepts public contributions but requires experts to review these submissions. Clinical interpretations are captured and displayed as evidence records consisting of an 'evidence statement' and several structured attributes. Each evidence record is associated with a specific gene, variant, disease, and clinical action. Evidence records belong to one of four evidence types indicating whether a variant is predictive of response to therapy, prognostic, diagnostic, and/or predisposing for cancer. They are assigned to an evidence level ranging from established clinical utility to inferential evidence, and the quality of the underlying evidence is rated from one to five stars.

3. Is individual or stratified drug repurposing new? And if not, what is new to it?

Off-label use of drugs (unlicensed prescribing) constitutes a possible precedent to drug repurposing in the field of personalized medicines. Off-label use comprises the use of a drug in a different dose or frequency, by a different route or in populations groups different from the ones included in the product identification (e.g. children) [96–98]. Medicines may also be used for different indications to those contained within the product license [96,97], which is clearly in direct relation with the drug repurposing approach, whereas many unlicensed prescriptions can be related to the personalized medicine paradigm. It is a frequent practice to treat patients who have proven resistant to a range of treatment approaches [98]. Various reasons can justify off-label uses [97]: the existence of therapeutic gaps, the strong research conducted in certain areas, and the lack of commercial interest by some companies about extending their treatment indications. Absence of license not always equals absence of evidence. For example, the mood-stabilizing effects in bipolar disorder of valproic acid, carbamazepine, and lamotrigine were well-established through controlled trials way before receiving FDA approval [99,100] and they were extensively prescribed off-label to treat that condition. An off-label use may have undergone a proper therapeutic trial but excluded on clinical grounds such as contraindications or risk of interactions [98]. Off-label use is a particularly common practice in certain areas, such as oncology, psychiatry, and pediatrics [98,101–103].

It should however be mentioned that off-label use is often based on empirical observations instead of evidence from clinical trials [97]; there is evidence that shows that unlicensed use can be associated to increase risk of adverse reactions [104] and unlicensed prescriptions increase professional liability [98]. It has also been noted that undisclosed unlicensed used is contrary to the philosophy of patient-focused care [104].

The idea of using a drug outside label specifications to provide better treatment for specific patients is thus not new: it has been with us for decades. What is the novelty to it? The answer is obvious: we now have the tools to do it on a more solid scientific basis. We will be able, in the immediate

future, to universally characterize an individual's disease at the molecular level in a time frame compatible with clinical decisions.

Current and emerging tools to assess the genetic profile of a patient or group of patients seem to provide a scientific framework for safer, evidence-based off-label use (e.g. adapting the licensed dose of a medication to the genetic makeup of a patient; choosing the best therapeutic alternative). The still meager literature exploring or discussing the link between precision medicine and drug repurposing underlines the central role of last-generation sequencing and gene expression profiling tools to bridge both concepts [105–108]. The fundamental notion involves recognizing disease and patient heterogeneity and characterize such heterogeneity with genetic diagnostic tools, so to choose drug targets and therefore drugs or combination of drugs in a more informed and specific manner. For instance, the web-based platform DeSigN for predicting drug efficacy against cancer cell lines using gene expression patterns has recently been released [105], which will help choosing anticancer treatment for specific cancer types or subtypes. In their excellent article, Li and Jones discuss and exemplify the horizons of personalized drug repurposing to treat cancer and rare diseases [106]. A number of DNA sequencing-based tests are today used in cancer medicine, from single gene tests for mutations with recognized prognostic and predictive significance to panels that may include hundreds of defined cancer genes. Back in 2010, those authors were the first to publish an example of clinical treatment decision-making based on whole-genome analysis [109]. They described the treatment choice for a patient presenting a rare tongue adenocarcinoma with no standard treatment options: the oncologists used sequencing tools to reveal amplification and upregulation of the rearranged during transfection (RET) proto-oncogene and the treatment with a (repurposed) RET inhibiting kinase drug conferred 8-month disease stabilization. The same team later expanded the application of such approach through the Personalized OncoGenomics (POG) program [110]. Between 2012 and 2014, 100 adult patients and 6 children with incurable cancers (representing 30 different cancer types) agreed to take part in the study. Fresh tumor and blood samples were obtained and used for RNA sequencing and whole-genome analysis. Sequencing and analysis was completed for 85 patients, among them 78 adults. In 83% of these cases, the clinician who was making treatment decisions indicated that the data information had been informative. A total of 71% of patients received a POG-informed treatment, 62% of which achieved some disease control. A recent case report by this same group on a patient with metastatic colorectal cancer, treatment-related toxicity, and resistance to chemotherapy and radiation [111] seems particularly relevant in relation to drug repurposing. The patient underwent immunohistochemical analysis for expression of the MMR proteins MSH2, MSH6, PMS2, and MLH1 and the V600E mutant BRAF protein. Whole-genome sequencing was carried out on the pretreatment tumor and blood, and whole-genome sequencing and whole transcriptome sequencing on the metastatic tumor. Somatic point mutations, small insertions or deletions, and copy-number alterations were identified. Whole-genome sequencing identified a large number (more than 2000) genomic alterations, consistently with defective MMR arising from the loss of MLH1 observed using immunohistochemistry. Among the most differentially expressed genes identified through transcriptome analysis, there were the founding members of two proto-oncogene families, FOS and JUN (their expression levels were 10- and 4-fold greater than for normal colon tissue, and 3.7- and 4-fold when compared with the mean expression of the malignant colon adenocarcinoma, respectively). c-FOS and c-JUN comprise the AP-1 transcriptional complex, which is known to be a key regulator of disease initiation and progression in many cancer types, including colorectal cancer. These data supported that blocking the renin–angiotensin system could provide therapeutic benefit, which led to the repurposing of the antihypertensive angiotensin II receptor antagonist irbesartan as an anticancer therapy, resulting in the patient experiencing a radical and persistent response. The previous example is particularly interesting since the treatment of choice has not traditionally been used as cancer chemotherapy, representing a case of 'hard' (nonapparent) drug repurposing.

Similarly, but at a more basic level of research, Jahchan et al. used a systematic drug-repositioning bioinformatics approach querying a large compendium of gene expression profiles to identify candidate FDA-approved drugs as potential treatments of small cell lung cancer [112]. It was predicted that tricyclic antidepressants (imipramine, clomipramine) and first-generation histamine H1 receptor antagonists (promethazine), among others, could act as novel treatments for small cell lung cancer. Such predictions were validated in both chemonaïve and chemoresistant small cell lung cancer cells in culture, in mouse and human small cell lung cancer tumors transplanted into immunocompromised mice, and in endogenous tumors from a mouse model for small cell lung cancer, which led to the implementation of a Phase II clinical trial. Remarkably, the best performing candidates displayed a complex pharmacological profile typical of classic central nervous system agents.

The application of genome analysis to drug repurposing has slowly begun to spread to other fields of medicine besides oncology, though they have not reached clinical applications in those areas so far. In a very recent work, So et al. used data from genome-wide association studies to screen for potential drug repurposing opportunities to treat seven psychiatric conditions [108]. Interestingly, they discriminated their results not only by disease but also by brain region. Their methods provided abundant cross-repurposing opportunities between psychiatric disorders (e.g. antidepressants for anxiety, antipsychotics for major depression, and so on). They also validated the general approach by 'rediscovering' classical treatments for some of the conditions. Siavelis et al. resorted to a comprehensive approach using five Alzheimer-related microarray data sets with three different methods of evaluating differential gene expression and four drug repurposing tools [83]. A list of 27 potential anti-Alzheimer agents was found which was further refined with molecular similarity tools, ontology enrichment, and network analysis. Zhang et al. compared Alzheimer-associated genes with approved

drug targets and found 23 approved drugs that may be efficacious against the disease [113]; *in vitro* and *in vivo* evidence suggests that four angiotensin-converting enzyme inhibitors have potential to treat Alzheimer's disease. Similarly, Zhang et al. generated a list of known and putative anti-Alzheimer drug targets by analyzing available genomic, epigenomic, proteomic, and metabolomic data [114]. Drug-target data was then extracted from DrugBank and the TTD and a list of 75 existing drugs that may be repurposed as medications for Alzheimer was obtained. The same group has applied a very similar protocol to repurpose drugs against diabetes [115]. Data from genome-wide association studies, proteomics, and metabolomics studies revealed a total of 992 proteins as potential anti-diabetic targets. Information on the drugs that target these proteins was retrieved from the TTD, finding that 108 of those proteins are drug targets with drug projects information. After excluding research and preclinical drug targets, 35 of the 108 proteins were selected as druggable proteins. A total of 58 drugs were found to have a new indication for treating diabetes. The cMap was used to compare the gene expression patterns of cells treated by those 58 drugs and that of cells treated by existing antidiabetic drugs and diabetes-risk-causing compounds. A total of 9 drugs were found to have the potential to treat diabetes, among them 4 drugs targeting COX2 and 2 drugs targeting the alpha-2A adrenergic receptor.

4. Conclusion

Drug repurposing presents remarkable opportunities to accelerate and lower the costs of drug discovery. In the beginning of drug repurposing, discoveries of this type were serendipitous or based on empirical observations. Today, due to the potential of the approaches, repurposing prospects are purposely and systematically explored.

Systematic drug repurposing approaches vary in terms of rationality, from the essentially 'brute force' approximations (exhaustive screening) to computational and signature-based methods. These methods have slowly and steadily begun to be applied in the framework of the precision medicine paradigm. The first successful reports in clinical practice have already been observed in the field of cancer, whereas other therapeutics areas are beginning to show their own application examples in a more basic research level. Genetic profiling tools are probably the best suited approaches for personalized or stratified drug repurposing, though benefits may arise from their integration to other approximations.

5. Expert commentary

Whereas the successful drug repurposing cases so far are dominated by serendipitous discoveries or empirical knowledge, current repurposing campaigns deliberately pursue opportunities in an organized manner. Still, most repurposing initiatives to date have been oriented to a traditional, 'one medication for all' perspective. Off-label prescriptions may be regarded as the historical precedent to personalized drug repurposing: a considerable fraction of unlicensed prescriptions corresponds to a therapeutic indication shift and many of them are focused on specific patient subpopulations, e.g. children or refractory patients. If off-label prescribing is the bridge that connects drug repurposing and personalized medicine, the challenge today involves boosting the safety and efficacy of unlicensed prescription by integrating genomics, transcriptomics, proteomics, and metabolomics to the stage of treatment choice.

It is now accepted in most fields of medicine that broad disease categories encompass heterogeneous conditions that are now being segregated. Recent (but cumulative) reports in the cancer arena have made clear that it is possible to rethink drug repurposing under the view of personalized and precision medicine. The key to this approximation are last generation sequencing and genome-wide association studies. Accordingly, signature-based approximations are the most likely to deliver personalized drug repurposing for complex disorders in the near future. They could also provide hints on the mechanism of action of known drugs, which is sometimes only partially known. Eventually, though, signature-based approximations may be complemented by target-based drug discovery approaches with a focus on protein variants linked to disease; so far, structure-based studies on variants have mostly focused on CYP450 polymorphism and we have not been able to find reports on target-based approaches oriented to drug repositioning.

As mentioned, most of the current examples of personalized drug repurposing come from oncology, where plenty of successful cases have been reported. The strategy is, however, beginning to flourish in other therapeutic areas, such as psychiatry and neurology, still under the form of nonclinical research.

Signature- and computer-aided approaches will be soon fully integrated under the network pharmacology paradigm, where diverse levels of observed and predicted data may be comprehensively, and synthetically, presented, and topological analysis of the network allows selecting the best point or point combination for therapeutic intervention. In an era where the multi-target and network pharmacology paradigms are being actively and progressively pursued to develop new medicines or drug combinations to treat complex diseases, protein networks provide hints on the best combination of drug targets, whereas disease networks might uncover unsuspected relationships between diseases. It is possible that those networks will be constructed or adapted in the near future by integrating data from individual patients or patient subgroups, expanding the horizons of personalized medicine.

6. Five-year view

Until no much time ago, it was my opinion that the current interest on drug repositioning would eventually reach its peak and then slowly begin to decrease. My reasoning was simple: the number of candidates for drug repurposing is finite. As more and more systematic drug repositioning campaigns were implemented, the drug repurposing opportunities would be drained (or at least severely reduced). My view has completely changed after seeing how drug repurposing is being now integrated with precision medicine, finding

unexpected solutions for individual patients (e.g. an antihypertensive drug that is used to efficaciously treat a patient with cancer!). The number of approved or clinical drugs is still finite but, under the precision medicine paradigm, what is not finite is the number of health conditions to be treated. In the limit, each ill person represents a specific situation that requires diagnose and treatment. Such statement becomes particularly true in the field of complex disorders whose etiology and evolution depends on an intricate combination of genetic, epigenetic, and environmental factors. Under this new light, I believe that the interest on drug repositioning will continue to grow vigorously in the short- and mid-term, as known drugs are repurposed for particular subtypes of a given disease.

Due to the global burden of cancer and the comparatively large investment in that field, it is common that novel technologies aiming to develop new therapeutic solutions are first applied in oncology and then more or less slowly 'spill' to other areas of medicine. Accordingly, although successful case reports on the application of personalized drug repurposing based on omics technologies have so far come from the cancer arena, it can be speculated that new successful stories will be told in other fields of medicine in the following years. How fast this will be realized will depend on how fast the omics technologies are developed and universally integrated into the medical practice.

It is to be seen how the pharmaceutical industry will adapt and rethink itself in the era of precision medicine, where off-label uses of medications will surely be a much more common and well-founded practice than today. Physicians and data analysts will surely have a prominent, leading role in this scenario.

Key issues

- Drug repurposing is a time- and cost-efficient strategy to develop new medications. Whereas the initial successful drug repurposing stories emerged from serendipitous observations or exploitation of drugs side effects, the drug discovery community has shifted in favor of more systematic drug repurposing in the late years.
- So far, molecular docking has not been applied to perform virtual screening applications to detect drug repurposing opportunities on the basis of structural knowledge of polymorphic or mutant variants of a given molecular target.
- Since about 10% of the drugs in the market are prodrugs and many marketed drugs are subjected to bioactivation, drug repurposing initiatives under the philosophy of precision medicine may consider major metabolites of the studied drugs and should stratify the population or characterize the individual patient on the basis of the pharmacogenetics of CYP450 and other major biotransformation enzymes.
- There are precedents that connect drug repurposing and personalized medicine. In particular, unlicensed prescribing is an extended practice to approach certain patient populations. The omics era (genomics, transcriptomics, proteomics, metabolomics) provides new tools to guide such practices in a rational, informed manner.
- New opportunities for individual or stratified drug repurposing will possibly emerge once data from genome-wide association studies and genetic profiling are integrated to in silico-aided drug repurposing.

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•• **To the author's best knowledge, the first paper explicitly linking personalized medicine and drug repurposing. Several examples are provided.**
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