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DRUG REPURPOSING: A REVIEW

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Abstract:

Drug repurposing (DR) also known as drug repositioning. It is a process of identifying new therapeutic use for old/existing/available drugs. Drug repositioning utilises the combined efforts of activity- based or experimental and in silico-based or computational approaches to develop/identify the new uses of drug molecules on a rational basis. This systemic analysis can help in computational methods implemented on biological resources. It is necessary to discover new strategies for reducing the drug discovery time frame strategies to discover new indications for FDA approved drug are are discussed in this article.Drug discovery involves de- novo identification and of new molecular entities(NME). The drug discovery by this approach is certainly advantageous. for example sildenafil (Viagra),a phosphodiesterase-5(PDE5)inhibitory initially developed for coronary artery disease(angina)by Pfizer(1985) has been repurposed for the treatment of erectile dysfunction.

Keywords*: Drug repurposing, drug discovery, insilico repositioning, activity- based repositioning, target-based screening, therapeutic indication.*

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INTRODUCTION:

Drug repurposing (DR) is also known as drug repositioning, drug re-tasking, drug reprofiling, drug rescuing, drug recycling, drug redirection, and therapeutic switching. It can be defined as a process of identification of new pharmacological indications from old / existing / failed / investigational / already marketed / FDA approved drugs /pro-drugs, and the application of the newly developed drugs to the treatment of diseases other than the drug's original / intended therapeutic use. In recent years, the drug repositioning strategy has gained considerable momentum with about one-third of the new drug approvals correspond to repurposed drugs which currently generate around 25% of the annual revenue for the pharmaceutical industry. It has been accounted that approximately 30% of the US Food and Drug Administration (FDA) approved drugs and biologics (vaccines) are repositioned drugs. According to recent estimates, pharmaceutical industries have significantly placed the market for repurposed drugs at $24.4 billion in 2015 with projected growth up to $31.3 billion in 2020. The first example of drug repositioning was an accidental discovery/serendipitous observations in the 1920s[1]. After about a century of development, more approaches were developed for accelerating the process of drug repositioning. Some most successful and best-known drugs that have been emerged out of the DR approach are sildenafil, monoxide, aspirin, valproic acid, methotrexate etc. For example, sildenafil originally developed for the treatment of hypertension and angina pectoris has currently been used to treat erectile dysfunction. many pharmaceutical companies are currently adopting drug repurposing to redevelop some of their FDA-approved and previously unsuccessful pipeline molecules as novel therapies for diverse disease conditions. The present review gives an overview of approaches currently being used for repurposing and discusses case studies illustrating the power and utility of drug repurposing observed by the substantial decrease in time needed to develop a drug because of availability of all relevant clinical and toxicological data.

Approaches of drug repurposing

Drug repositioning has two alternative and complementary approaches, one is experiment - based approach and the other is in silico- based approach. The experiment- based approach is also known as activity-based repositioning which refers to the screening of original drugs for new pharmacological indications based on experimental assays. It involves protein target-based and cell/ organism-based screens in in vitro and/or in vivo disease models without requiring any structural information of target proteins. Several approaches of experimental repositioning are targeting screening approach, cell assay approach, animal model approach and clinical approach. In contrast, in silico repositioning carries out virtual screening of public databases of huge drug/chemical libraries using computational biology and bioinformatics/cheminformatics tools. In this approach, the identification of potential bioactive molecules is achieved based upon the molecular interaction between drug molecule and protein target.The differences between activity- and in silico-based approaches of drug repositioning are summarized. Over the past few decades, the in-silico approach has gained wide popularity with significant success in drug discovery program. Many pharmaceutical companies and drug discovery research laboratories have already successfully incorporated the in-silico tools and techniques for the drug discovery from structurally diverse chemical spaces since a large amount of information on the chemical structure bioactive compounds, structure of proteins and pharmacophore models are available in the public domain[2]. Moreover, in silico repositioning has some advantages over the experimental-based approach, which includes reduced time and cost of development and low risk of failure. The limitation of this method is that it requires. new therapeutic indications for existing drugs, called mixed approach. In the mixed approach, the result of computational methods is validated by pre-clinical biological experiments (in vitro and in vivo tests) and clinical studies. The simultaneous application of computational and experimental methodologies in a systematic manner offer a robust and logical approach to the discovery of new indications, demonstrating a greater efficiency than the discovery based on serendipity. Further, mixed approach The methodologies adopted in DR can be divided into three broad groups depending on the quantity and quality of the pharmacological, toxicological, and biological activity information available.These are mainly drug-oriented, target-oriented, and disease/therapy-orienteoffers opportunities for developing repositioned drugs more effectively and rapidly[3].

Methodologies of drug repurposing

In the drug-oriented methodology, the structural characteristics of drug molecules, biological activities, adverse effects, and toxicities are evaluated. This strategy is meant for identifying molecules with biological effects based on cell. This type of repositioning methodology is based on traditional pharmacology and drug discovery principles, where studies are usually conducted to determine the biological efficacy of drug molecules without really

knowing about the biological targets. Significant successes in DR have been achieved with this orientation profile, through serendipity or clinical observation, such as discoveries with sildenafil. Target-based methodology comprise in silico screening or virtual high-throughput screening (vHTS) of drugs or compounds from drug libraries/compound databases such as ligand-based screening or molecular docking followed by in vitro and in vivo high-throughput and/or high-content screening (HTS/HCS) of drugs against a selective protein molecule or a biomarker of interest[4].In this method, there is a significant success rate in drug discovery as compared to drug-oriented method, because most biological targets directly represent the disease pathways/ mechanisms. The application of disease/therapy-oriented methodology in DR is relevant when there is more information on the disease model is available. In this case, DR can be guided by the disease and/or treatment based upon availability of information given by proteomics (disease specific target proteins), genomics (disease specific genetic data), metabolomics (disease specific metabolic pathways/profile) and phenotypic data (off-target mechanism, pharmacological targets, disease pathways, pathological conditions, adverse and side effects etc.) concerning the disease process. It, therefore, requires construction of specific disease networks, recognizing genetic expression, considering key targets, identifying disease causing protein molecules related to cell and metabolic pathways of interest in the disease model delineates the methodologies and steps involved in drug repositioning. Drug-based phenotypic screening and target-based methods account for more than 50% of the FDA approved small drug molecules and biologics[5]. Phenotypic drug screening methods identify drug candidates from small molecule libraries. Methodologies and steps involved in drug repositioning. Drug Repurposing by serendipitous observations. Target-based methods discover drugs based upon known target molecules.

Blinded search or screening methods:

Involve serendipitous identification from biological tests/experimental screens aimed at specific disease models and drugs. The advantage of these methods is that they possess higher flexibility for screening many drugs or diseases.

Target-based methods:

Carry out in vitro and in vivo high-throughput and/ or high-content screening (HTS/HCS) of drug molecules for a protein target or a biomarker of interest and in silico screening of compounds or drugs from large compound libraries, such as ligand-based screening or molecular docking. In these methods, there is a higher possibility of finding useful drugs/drug leads as compared to blinded search methods. It also requires less time for the entire screening process to complete.

Knowledge-based methods:

Utilize bioinformatics or cheminformatics approaches to gather the available information of drug profile, chemical structures of targets and drugs, drug-target networks, clinical trial information including adverse effects, signalling or metabolic pathways. This information content of knowledge-based methods is rich enough as compared to blinded or target-based methods. The known information can be used to predict therefore, be used to predict the unknown new mechanisms, such as unknown targets for drugs, unknown drug–drug similarities, new biomarkers for diseases etc.

Signature-based methods:

Use gene signatures derived from disease omics data (genomics data) with or without treatments to discover unknown off-targets or unknown disease mechanisms. Genomics data are publicly available as databases. The advantage of these methods is that they are useful to explore unknown mechanisms of action of drugs. In comparison to knowledge-based methods, signature-based methods investigate drug mechanisms at more molecular-level, such as changes in expression of genes by using computational approaches

Pathway- or network-based methods

Make use of disease omics data, available signalling or metabolic pathways, and protein interaction networks to reconstruct disease-specific path- ways that provide the key targets for repositioned drugs. The advantage of these methods is that they can narrow down general signalling networks from many proteins to a specific network with a few proteins (or target molecules).

Targeted mechanism-based methods:

Integrate treatment omics data, available signalling pathway information and protein interaction networks to describe the unknown mechanisms of action of drugs. The advantage of these methods is that they are not only used to discover the mechanisms related todiseases or drugs, but also to identify those directly related to treatments of drugs to specific diseases[6].

Repositioned drugs

Drug repositioning is an alternative approach to traditional drug discovery. With increasing market demand many pharmaceutical companies are developing new drugs or new therapeutic uses from existing/old/available drugs by drug repositioning approaches in less time, yet at low cost. In drug discovery program, the repositioning is usually essentially carried out in two stages as described follows. In the first stage, the in-silico screening of approved drugs against a particular disease target is carried out, which is followed by the second step, in which the selected identified molecules are further experimentally investigated both in vitro and in- vivo in specific disease models of interest. After successful preclinical studies in the second stage of repositioning, identified drug candidates enter the clinical trials in human subjects. delineates several potential strategies (with suitable examples) of drug repositioning. depicts examples of some repositioned drugs already developed or currently under development from various approved (FDA) or marketed drugs and investigational new drugs (IND). Some repositioned drugs currently under clinical trials in COVID-19 are also included in the list. Colchicine, a well-known anti-inflammatory drug used in the treatment of gout and pericarditis, is currently under clinical trial for treating COVID-19 patients. This drug has been proved to be effective in preventing massive cytokine storm induced pneumonia caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). The antiviral effect of an older antimalarial drug, chloroquine (used as phosphate salt) against SARS-CoV-2 infection has also been investigated worldwide. Studies suggest that chloroquine may be beneficial in preventing coronavirus induced pneumonia in COVID-19. As per recent reports from NIH (National Institutes of Health, US), the clinical trial of a combination of hydroxychloroquine/azithromycin for the treatment of COVID-19 patients has already been started. In this combination, both the drugs are FDA approved, here hydroxychloroquine is an antimalarial drug and azithromycin is an anti-bacterial antibiotic. An anti-viral drug, favipiravir intended for the treatment of influenza is currently under phase-2/phase-3 clinical trials on COVID-19 patients around the world (China, Japan, US, India). Glenmark has initiated phase-3 trial on favipiravir for the treatment of COVID-19 patients in India. An investigational anti-retroviral drug called remdesivir (originally developed by Gilead Sciences Inc. for the treatment of Ebola but failed in clinical trial) is also under clinical trial for treating COVOD-19 patients in several countries like China, US, UK and India. In India, clinical trials on favipiravir, remdesivir and colchicine are currently underway by CSIR (Council of Scientific & Industrial Research) laboratories. A fixed dose drug combination called lopinavir/ritonavir earlier approved. The concept of drug repositioning thus excludes any structural modification of the drug. Instead, repositioning makes use in a new indication of either the biological properties for which the drug has already been approved or the side properties of a drug that are responsible for its adverse effects. Drug repositioning relies that on two main scientific bases:(1)the discovery, through the human genome elucidation, that some diseases share sometimes common biological targets and(2) the concept of pleiotropic drugs.The description of the elements associated to the complex interplay between diseases, drugs and targets with in silico approaches is one of the key for drug repurposing[7].It is now possible to describe diseases by their molecular profile and to use computational methods, especially data mining, to determine the degree of similarity between diseases that share a number of these molecular feature to treat HIV/AIDS under the brand name Kalita is currently being studied to treat COVID-19 patients in several countries. This drug combination was investigated along with the flu drug, oseltamivir (Tamiflu) to cure infection caused by SARS-CoV-2 in Thailand. The clinical trial of an anti-parasitic drug called ivermectin (used traditionally as an approved treatment in worm infestations) for the treatment of COVID-19 is being undertaken in several parts of the world after a successful in vitro effectiveness against SARS-CoV-2 infection at Monash University in Melbourne, Australia. The clinical trial of tocilizumab, an IL-6 receptor antagonist (marketed under the brand name Actemra) used for the treatment of inflammatory illness such as rheumatoid arthritis is also being conducted for the treatment of patients with COVID-19.

Significance of drug repurposing

For a new drug to enter the market, it needs to abide by stringent regulations. To identify a drug and develop it further requires significant investment, primarily because of diverse physicochemical properties of the chemical entities and complexity of scaling up the production This limitation further empowers pharmaceutical companies or academic centres to utilize already-approved medications quickly and efficiently for a new indication, not yet available to the patients with that disease[8]. Investigational molecules that fail to show efficacy for a predetermined indication typically provide a good start for their revival by repurposing. They can be further rediscovered for a new indication(s), ultimately being developed as viable therapies, particularly useful in cases of rare diseases, which present

significant challenges in diagnosis, treatment, and lack of resources. For instance, some autoimmune disorders, bacterial infections, and rare cancers are not inherited, thus making it more difficult to treat because they are idiopathic in nature. Drug repurposing, being a less expensive and shorter approach, brings effective therapies to patients compared with cumbersome traditional discovery and development processes. Moreover, this approach helps overcome the inflating costs for drug development, thus lowering out of pocket cost for patients, and ultimately reducing the actual cost of therapy. For a new investigational molecule, safety and efficacy data are not yet available, resulting in higher attrition during the drug discovery process leading to the most failures regarding safety or efficacy. By contrast, all safety, preclinical and efficacy data are readily available for a repurposed molecule, thus enabling the investigator to make an informed decision at each stage of drug development. Availability of prior knowledge regarding safety, efficacy and the appropriate administration route significantly reduces the development costs and cuts down the development time resulting in less effort required for successfully bringing a repositioned drug to market. For example, sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor represents one of the successful repurposing efforts. Sildenafil was originally developed for hypertension treatment but was later identified to have significant benefits in erectile dysfunction and was approved by the FDA for the same. It was later repurposed for treatment of a rare disorder: pulmonary hypertension. With the advent of new technologies as well as availability of computational tools, drug discovery is a much more affordable approach when starting with an already-approved drug. Drug repositioning offers a great deal of potential for out-licensing as well because these drugs possess characteristics attractive to potential buyers. Although attractive, it is also important that identification of a new disease target should not cripple the marketing potential of the drug for its original indication. A major area of interest for drug repurposing could be rare disorders, representing a significantly unmet medical need owing to no availability of standard therapies and worsening clinical outcomes. summarizes key differences and benefits of drug repurposing approaches compared with traditional drug discovery approaches.

Strategies of drug repurposing

There are two main strategies of DR, viz., on-target and off-target . In on-target DR, the known pharmacological mechanism of a drug molecule is applied to a new therapeutic indication. In this strategy, the biological target of the drug molecule is same, but the disease is different. For example, in the repositioning of minoxidil (Rogaine), an on-target profile is observed, since the drug acts on the same target and produces two different therapeutic effects. Minoxidil was transformed from an antihypertensive vasodilator anti hair loss drug. As an antihypertensive vasodilator, minoxidil has the property of widening blood vessels and opening potassium channels, which allows more oxygen, blood, and nutrients to the hair follicles and this pharmacological action helps its use in the treatment of male pattern baldness (androgenic alopecia[9]. Strategies in Drug Repurposing for COVID-19 For rapidly establishing drugs for arising and re-emerging viruses, drug repurposing is one of the most substantial preferences nowadays. Given the SARS-CoV-2 pandemic, compared to de novo drug development, drug repositioning appears as a favourable perspective to develop efficient therapeutics. Traditional drug repurposing techniques establish drug effects and their mode of action. This process employs screening techniques of current pharmacopoeia to reveal novel drug indications of already established drugs..Drug repurposing for COVID-19 undergoes three steps before getting considered for development and marketing: candidate drug identification; mechanistic evaluation of the drug effect in preclinical models; and evaluation of candidate drugs' efficacy in phase II clinical trials. Among these three steps, the first step—the screening and identification of drugs with a high potential for repurposing—is the most crucial. Fig.No-4:on target and off-target strategies of drug repurposing. As such, drug repositioning has two alternative and complementary approaches: an experiment-based approach and a theoretical or in silico-based approach. COVID 2022, 2 153 ered for development and marketing: candidate drug identification; mechanistic evaluation of the drug effect in preclinical models; and evaluation of candidate drugs' efficacy in phase II clinical trials. Among these three steps, the first step—the screening and identification of drugs with a high potential for repurposing—is the most crucial. As such, drug repositioning has two alternative and complementary approaches an experiment-based approach and a theoretical or in silico-based approach, Two alternative and complementary drug repurposing approaches: one is an experiment-based approach, and the other is a theoretical or in silico-based approach.The experiment-based approach, also known as activity-based repositioning, refers to screening original drugs for new pharmacological applications based on experimental assays. It involves protein-target-based and cell-based screens in disease models without requiring any structural information of target proteins[10]. Approaches of experimental

repositioning include the target screening approach, cell assay approach, animal model approach, and clinical approach. In contrast, in silico repositioning involves virtual screening of public databases of huge drug/chemical libraries using computational biology and bioinformatics/cheminformatics tools. In this approach, the identification of potentially bioactive molecules is achieved based upon the molecular interaction between drug molecules and protein targets. Although experimental explorations are functional for drug efficacy determination, they may be time-consuming with small-scale results. Therefore, computational methodologies have enhanced this approach by digging deeper into drug executions, being capable of evaluating the interaction of ligand(s) with respective target proteins, predicting the novel signalling pathways, and making rapid development in lesser time with reduced costs, which is significant for the current pandemic (COVID-19) situation. 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Although experimental explorations are functional for drug efficacy determination, they may be time-consuming with small-scale results[11]. Therefore, computational methodologies have enhanced this approach by digging deeper into drug executions, being capable of evaluating the interaction of ligand(s) with respective target proteins, predicting the novel signalling pathways, and making rapid development in lesser time with reduced costs, which is significant for the current pandemic (COVID-19) situation . Binding Assays to Identify Target Interactions Today, analyses of the targets and off-targets of drugs and drug repurposing complement each other. Proteomic techniques such as affinity chromatography and mass spectrometry have been used as approaches to identify binding partners for many drugs Experimental assays of pre-approved drugs are being adopted in laboratories to bridge the gap between instant diagnosis and long-term research required for the availability of treatment for COVID-19 . The cellular thermostability assay (CETSA) is one such technique introduced to map target engagement in cells using biophysical principles that predict thermal stabilization of target proteins by drug-like ligands that possess the appropriate cellular affinity . Examples of execution of this technique include the confirmation of cellular targets for the tyrosine kinase inhibitor (TKI) crizotinib and the detection of quinone reductase 2 (NQO2) as a cellular offtarget of acetaminophen (paracetamol) .In a study COVID 2022, 2 154 by Friman et al., a panel of drugs was screened using the CETSA MS format on HepG2 cells to identify host proteins. The experiment was designed to study off-target effects of remdesivir and chloroquine as repurposed drugs for targeting SARS-CoV-2. This leads to a hopeful venture to further improve or develop fortuitous therapies for SARS-CoV-2 infection .The promiscuity of protein kinase inhibitors has increased efforts to develop better compounds for preclinical research that can help clinical drug development and repurposing through evidence-based pharmacological assays. It is also noteworthy that fallacies made in various kinase drug discovery approaches have led to seeking out beneficial pathways of cellular effects through early-stage affinity approaches. Numerous kinases have been considered essential mediators of various viral infections, particularly SARS-CoV and MERS-CoV.These same proteins are also predicted to be involved in mediating infection by SARS-CoV-2 as well. Many kinase inhibitors with pharmacologic effects that may be beneficial in mitigating the severe and potentially life-threatening symptoms of COVID-19 are already approved. Kinase inhibitors can be tested in combination with antiviral agents or other targeted therapies that work against COVID-19 to achieve greater efficacy. Tyrosine-protein kinase (ABL) inhibitors have been demonstrated to inhibit replication of several unrelated viruses like dengue and Ebola in in-vitro cell-based studies. ABL inhibitors, imatinib and dasatinib, were identified as inhibitors of both SARS-CoV and MERS-CoV replication. Nilotinib was identified as an inhibitor of SARS-CoV. Investigation of the mechanism for imatinib against both SARS-CoV and MERS-CoV revealed inhibition of the early stages of the virus life cycle and inhibition of viral replication through blocking the fusion of the coronavirus virion with the endosomal membrane[12]. Importantly, authors show that targeted knockdown of ABL2, but not ABL1, significantly inhibited SARS-CoV and MERS-CoV replication/entry in vit JAK inhibitors belong to a

family of DMARDs or disease-modifying antirheumatic drugs, which have been repurposed to treat COVID-19.Chen et al. have summarized the observational studies of the clinical uses of Janus Kinase (JAK) inhibitors, including rollatini, baricitinib, and tofacitinib for COVID-19 patients. They compared the clinical efficacy of JAK inhibitors of different meta-analyses and concluded that they are safe agents and can lead to a better clinical outcome for COVID-19 patients. Diving into chemical genetics, one can also better understand the relationship between binding and efficacy in the cellular context. Non-kinase targets of small molecules designed initially to inhibit protein kinases are increasingly recognized. Their validation has led to repurposing opportunities in cancer, as Zika virus modulators and potential agents to treat cancer antibiotic-resistant microorganisms. Small-molecule–kinase binding is analysed in a kinome-wide fashion using various in vitro and increasingly organism- based assays to generate heat maps of biologically essential interactions. Thus, findings can be rapidly translated into new clinical developments to address drug-resistance outcomes in cancer. Many of these studies stem from industry-driven high-throughput direct binding or catalytic assays Karaman and colleagues used an in vitro competition binding assay to evaluate 38 kinase inhibitors against a panel of 317 distinct human protein kinases in one such study[13]. Their analysis identified a total of 3175 binding interactions. Interestingly, some kinase inhibitors such as sorafenib and dasatinib showed higher affinity to secondary kinase targets than their known primary target . Such studies show that drug repurposing may indeed be a quick and effective combat strategy against the current pandemic. 3.1.2. Phenotypic Screening Phenotypic screening puts forward drug candidates to proteins in more biologically relevant contexts than screens involving purified proteins. These screens determine cellular function without the requirement of prior knowledge of the relevant targets and signalling pathways, and they offer the possibility of discovering new therapeutic targets . Phenotypic screening can identify compounds that show disease-relevant effects in model systems without prior knowledge of the target(s). In the context of drug repurposing, COVID 2022, 2 155 if the compounds screened are approved or investigational drugs, this may fast-track repurposing opportunities that can readily be pursued. Typically, in vitro phenotypic screens use a wide range of cell-based assays in a 96-well format.For example, Chen et al., in 2021, tested a total of 8810 approved and investigational compounds, out of which 319 compounds were found to have anti-SARS-CoV-2 activities . These included 91 approved drugs and 49 investigational drugs. The antiSARS-CoV-2 activities of 230 of these confirmed drugs had not been previously reported, including 38 approved drugs from the same. The three most potent FDA-approved drugs with anti-SARS- CoV-2 activities were chlorprothixene, methotrimeprazine, and piperacetazine . The compounds that were tested were either mechanism-based bioactive compounds or natural products. They were selected from compound libraries like NCATS Pharmaceutical Collection (NPC), NCATS Mechanism Interrogation Plate (MIPE), NCATS Pharmacologically Active Chemical Toolbox (NPACT), Epigenomic library, Autophagy library, and anti-infective library. A SARS-CoV-2 CPE assay was conducted to check for anti-SARS-CoV-2 activities[14] . The SARS-CoV-2 CPE is a 72 h assay that measures the phenotypic consequence of viral infection and cell replication . SARS-CoV-2 induces cell death after 48 to 72 h of infection, and thus cell viability is an indirect measure of viral replication in vitro. Due to its dependence on the host response and its indirect measurement of SARS-CoV-2 infection and replication, the CPE assay may have certain limitations. The phenotypic outcome can also vary depending on culture conditions, the viral multiplicity of infection (MOI), and the number of virions added per cell during infection.

Strategies in Drug Repurposing for COVID-19

For rapidly establishing drugs for arising and re-emerging viruses, drug repurposing is one of the most substantial preferences nowadays. Given the SARS-CoV-2 pandemic, compared to de novo drug development, drug repositioning appears as a favourable perspective to develop efficient therapeutics. Traditional drug repurposing techniques establish drug effects and their mode of action. This process employs screening techniques of current pharmacopoeia to reveal novel drug indications of already established drugs Drug repurposing for COVID-19 undergoes three steps before getting considered for development and marketing: candidate drug identification; mechanistic evaluation of the drug effect in preclinical models; and evaluation of candidate drugs' efficacy in phase II clinical trials. Among these three steps, the first step—the screening and identification of drugs with a high potential for repurposing—is the most crucial. As such, drug repositioning has two alternative and complementary approaches: an experiment-based approach and a theoretical or in silico-based approach, COVID 2022, 2 153 ered for development and marketing: candidate drug identification; mechanistic evaluation of the drug effect in preclinical models; and evaluation of candidate drugs' efficacy in phase II clinical trials.

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Drugs Repurposed against SARS CoV-2 Drug Targets :

Drug repositioning is not only a current scientific trend but spans across several decades. Drugs with a specific clinical indication have been further tested to discover alternative clinical indications for different diseases. The most common drug repurposing examples are NSAIDs (anti-inflammatory drugs) being used as anticancer agents. Chloroquine, an antimalarial drug, and azithromycin, an antibacterial antibiotic are under development as antiviral drugs against COVID-19 [50]. Drugs currently being tested for repositioning in COVID-19 can be distinguished as drugs potentially able to inhibit one or more steps of the coronavirus lifecycle and those that can counteract the effects of SARS-CoV-2 infection, such as the amplified immune response and the massive cytokine release, both of which lead to severe complications such as coagulopathy and acute respiratory distress syndrome (ARDS). The first is remdesivir, first developed in 2009 to treat hepatitis C, then repurposed to treat Ebola. Although ineffective in treating both diseases, later animal studies found that it effectively managed other coronaviruses such as SARS and MERS[16]. It has proven effective in shortening recovery time from COVID-19 in some patients if administered early. However, it is to be used with only the most severely affected patients in critical care units. Another group of drugs that have previously been widely used among critically ill patients with SARS and MERS are glucocorticoids, powerful antiinflammatory drugs that inhibit the production and survival of T-cells and macrophages. Although controversial, glucocorticoids have been used to treat patients critically ill with COVID-19. A comprehensive list of repurposed drugs used against drug targets of SARS-CoV2.

Nafcillin

Nafcillin is a semi-synthetic, narrow-spectrum antibiotic, a beta lactamase-resistant penicillin. The bactericidal action of penicillin inhibits cell wall synthesis due to the presence of the beta-lactam ring. However, certain bacteria develop resistance against the beta-lactam ring by synthesizing beta-lactam inhibitors (i.e., beta-lactamase or penicillinase). Penicillinase resistance drugs were introduced to combat this resistance . Currently, nafcillin is being used to treat penicillinase-producing staphylococcal species, particularly methicillin-sensitive Staphylococcus aureus (MSSA). Nafcillin is also being used to treat non-specific lower respiratory tract infections and community-acquired pneumonia (CAP) [26]. Nafcillin is not known to cause life-threatening adverse side effects. An analysis by Das et al. shows the highest binding affinity with the TMD domain of monomeric E-protein [17]. Thus, nafcillin can be considered for redirecting its purpose for COVID 2022, 2 161 the treatment of SARS-CoV-2 infection as it could also combat bacterial co-infection in a COVID patient, which produces the same symptoms as seen in SARS- CoV-2 infection.

Nabumetone

Nabumetone is an FDA-approved non-selective anti-inflammatory drug (NSAID) that is currently being used for its anti-inflammatory and antipyretic effects. It is a prodrug that goes through biotransformation within the liver to produce the active component, 6-methoxy-2- naphthyl acetic acid (6MNA), that inhibits the synthesis of prostaglandins by acting on cyclooxygenase (COX) I and II. Prostaglandins are

responsible for initiating fever by signaling the hypothalamus to increase body temperature. Prostaglandin acts as an inflammatory mediator acting on blood vessels to promote an inflammatory response. NSAIDs mediate antiinflammatory effects by preventing vasodilation, reducing capillary permeability and cytokine release from endothelial cells. Altogethectacosanol is the main component of plant-extracted natural wax and is a lowmolecular-weight primary aliphaticalcohol. Its role is mainly investigated for the treatment of Parkinson's disease. It is approved as a nutraceutical by the FDA and is marketed as the main component of policosanol (PC), a genericr, these effects impede the migration of immunocompetent cells to the injury site, thereby preventing uncontrolled immune system activation and inflammation.

Octacosanol

O term for a natural mixture of primary alcohols isolated originally from sugarcane wax.

Cinametic Acid

Cinametic acid is an FDA-approved food additive, mainly obtained from oil of cinnamon and other plant sources. Among the many therapeutic functions of cinnamic acid, one of its roles has also been linked to inhibiting angiotensin-converting enzyme (ACE). ACE converts angiotensin (Ang) I to Ang II. Ang II is responsible for constricting blood vessels and increasing blood pressure or hypertension, one of the risk factors for COVID-19, via binding to angiotensin1 receptor (AT1R) and activating a cascade of signaling pathways. The role of cinametic acid in inhibiting ACE will hamper conversion of Ang I to Ang II, which can reduce hypertension. Further, Ang II gets converted to Ang-(I-VII) by ACE2 in the absence of ACE.

Ascorbyl Palmitate

Ascorbyl palmitate is an FDA-approved small molecule. Mainly, it is a fat-soluble form of vitamin C formed by the ester of ascorbic acid and palmitic acid. Being an amphipathic molecule, it has the advantage of being more stable and easily enters into cell membranes.

Guaifenesin

Guaifenesin is an FDA-approved over-the-counter (OTC) or non-prescription expectorant for treatment of cough and the common cold. It aids in the clearance of mucous and other respiratory tract secretion by increasing the volume of trachea and bronchi and reducing mucus viscosity, otherwise leading to congestion, chronic bronchitis, and COPD, commonly seen in ARDS[18] As a result of this, the action of guaifenesin results in a more productive cough, thus combating the condition of ARDS. This is also expected to happen if administered to COVID-19 patients as it can potentially disrupt the formation of the pentameric structure of E-protein, which causes ARD.

Remdesivir

Remdesivir, a nucleoside analogue prodrug, was developed for use against the Ebola virus, is currently under trial at many medical institutions, and is known to be effective against MERS- CoV. It has demonstrated a better safety profile than other drugs in treating acute Ebola viral infections. It gets activated into triphosphate, inhibits viral COVID 2022, 2 162 RNA polymerase, and has manifested in vitro and in vivo activity against MERS-CoV and SARS-CoV-2. It effectively treated a severe patient with severe pneumonia who needed mechanical ventilation but not inotropic agents for support of circulation. Findings have been mixed in studies being conducted regarding the efficacy of remdesivir for COVID-19 treatment. A multinational cohort study supported by Gilead Sciences showed clinical improvement for 68% of severe COVID-19 patients treated with compassionate use of remdesivir.

Molnupiravir

Molnupiravir is an isopropyl ester prodrug, which initially emerged as a possible treatment of influenza viruses, and encephalitic alphaviruses such as Venezuelan, Eastern, and Western equine encephalitic viruses. It is derived from the ribonucleoside analog β-D-N4- hydroxycytidine (NHC) triphosphate that converts to its active form molnupiravir (MTP) in the cell. This drug appears to work by the mechanism of "error catastrophe"; this is essentially the concept that by increasing the rate of mutation in the viral genome beyond a biologically tolerable threshold, the virus will no longer be able to exist. It is a broad-spectrum antiviral drug with a two-step mutagenesis mechanism[19]. It targets the virally encoded RdRp of the SARS- CoV-2 and competitively inhibits the cytidine and uridine triphosphates and incorporates MTP. The RdRp utilizes the NHC triphosphate to incorporate either A or G in the active centers. This, in turn, helps in escaping proofreading of a mutated RNA. The resulting mutagenesis is lethal to the virus. This drug appears to work by the mechanism of "error catastrophe"; this is essentially the concept that by increasing the rate of mutation in the viral genome beyond a biologically tolerable threshold, the virus

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Ganciclovir;

Ganciclovir, originally used to treat cytomegalovirus (CMV) infection, has shown effectiveness in a study for COVID-19 treatment at 0.25 g intravenously every 12 h. Several other studies have also found such antiviral drugs to reduce viral load and avert possible respiratory impediments.

Opportunities and challenges

Computational or machine learning approach has significantly improved the performance of drug repositioning. In comparison to computational approaches, using experimental approaches (such as target protein-based screening, cell-based assay, testing in animal model, and clinicaltesting) that provide direct evidence-based understanding of links between drugs and diseases are more reliable and credibel[20]. However, in recent years computa-tional approaches are usually combined with the experimental approaches to identify new indications for old drugs, called mixed approaches. In this approach, computational methods are validated by biological experiments and clinical tests. Mixed approach of repositioning offers a rational and exhaustive exploration of all possible repositioning opportunities, taking into consideration improved access to available databases and technological advances. Furthermore, the R&D investment required for drug repositioning is lower than that for traditional drug discovery. Thus, drug repositioning offers an opportunity for many pharmaceuti-cal companies to develop drugs with lower. Mixed approach of DR offers opportunities for developing repositioned drugs more effectively and rapidly. From the market perspective, a large number of diseases require new drugs to be treated with a potential market demand and economic impacts. For example, the discovery of drugs for rare/neglected diseases has a large potential market to explore. There is, therefore, an opportunity for repurposing of drugs for the treatment of rare, neglected, orphan diseases or difficult to treat diseases. There are over 6000 rare diseases that lack proper treatment. About 5% of them are being researched. Rare diseases have a large potential market to explore. Given the high attrition rates, substantial costs and slow pace of drug discovery and development, repurposing of old drugs to treat both common and rare diseases is increasingly becoming an attractive area of research because it involves the use of drug molecules with reduced risk of failure at shorter time and lower cost devel-opment .With the advent of technologies such as genomics, proteomics, transcriptomics, metabolomics, etc., and availability of huge databases resources including drug omics data, disease omics data, etc., there are a plenty of opportunities to discover drugs by drug repositioning in a collective and integrated effort of all the above methods/approaches mentioned above[21]. Researchers are currently equipped with the latest reliable tools and data to explore the novel unknown mechanism of actions/pathways based upon disease-specific target proteins/genes and/or specific bio-markers associated with the progression of the disease. Various databases and software are available publicly for genomics, proteomics, metabolomics and pathway analysis. Several computational strategies are already developed to increase the speed and ease of the repurposing process. However, opportunities come often with many challenges in drug repositioning. The identification of a new therapeutic indication for an existing drug poses a major challenge in repositioning. However, drug repositioning is a complex process involv-ing multiple factors such as technology, commercial models, patents, and invest-ment and market demands. Some multiple challenges which include choosing the right therapeutic area for the drug under investigation, issues related to clinical trials such as need to run new trials from start if the data from clinical or preclinical trials for the original drug or drug product are outdated or are not satisfactory Since the new applications are found based on the previously known data such as pharmacokinetics and mechanism of action, the time for clinical evaluations for a new application of a drug is reduced. However, certain drawbacks need to be considered during the utilization of this approach. The primary concerns associated with repurposing existing drugs is the intellectual property rights and additional national, as well as international legislations associated with the drug patents. These are the major obstacles of investigating the novel applications of previously known drugs. Further, if the drugs or their previous applications are patented, then that provides the original developers the market exclusivity. This not

only makes the data unavailable, but also makes the molecule or chemical scaffolds unavailable for further investigation. An extension of this, deviating from the chemical structure based on structure- activity relationship violates the principle of drug repurposing and makes the new chemical entity subject to the dogma of pre-clinical and clinical evaluation. These drawbacks associated with the drug repurposing should be addressed scientifically as well as legally to utilize the approach to its maximum potential and make the drugs rapidly available during global pandemics and worldwide emergencies. Furthermore, the previous application of drug/s are reported at a particular dose range, which may not necessarily be effective for its new application. Hence, it is critical to determine the effective dose range of a drug/s for its new application/s along with the determination of toxicity and off-target effects at the effective dose. Despite the associated advantages and promising results over the period, global financial support for the drug repurposing approach has been lackin[22]g. Finally, the low market price of the drugs, shorter duration of the patent with new applications, and low returns on the investments are the primary reasons why pharmaceutical companies are not extensively interested in the drug repurposing approach.

Recommendations for drug repurposing

Bearing in mind the opportunities and challenges for drug repurposing discussed above, we conclude by put- ting forward six recommendations to help realize the full potential of drug repurposing. First, there is a need for better integrative platforms for data analysis. The benefits of big data and how it can aid identification of repurposing opportunities are clear. However, data access and integration remain a bottleneck, particularly for clinical data (including clinician notes in patient case records). There is a need for advanced technological solutions that can reduce the need for manual curation and help integrate different types of omics data (BOX 5) such that subsequent ana- lyses can be more refined and analysed in user-friendly formats by more 'non-experts'. Second, improved access to industry-generated pre- clinical and clinical compounds is needed.

CONCLUSION:

Technologfurther accelerate the process of drug purposing in the drug discov-ery program. In the era of precision medicine, the drug repositioning strategy hasbecome very much useful to establish the unknown mechanism of action of drugs through exploration of novel disease/metabolic/signaling pathways, or off-targets and target-specific mechanisms/ genetic expression profile for even genetdisorders.Advancement in genomics have provided us with genomic and transcriptomicdata in huge quantities using technologies like next generation sequencing, microar-ray data and transcriptomics, etc. Network biology and systems biology approaches through insights into drug-target interaction profile at molecular/genetic leve

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