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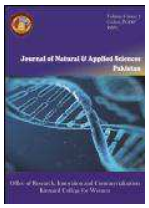

DRUG REPOSITIONING, AN APPROACH FOR IDENTIFICATION OF NEW THERAPEUTICS

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Abstract

Drug repositioning of FDA approved drugs is an innovation stream of drug discovery. This review elaborates the advantages, approaches and challenges of drug repositioning. This low cost, less risky and less time consuming drug repositioning includes effective computational and experimental approaches to identify new indications for existing drugs. Similar to drug discovery, there are various challenges to repurpose a drug such as selection of an appropriate approach and target population, intellectual property (IP) protection and etc. This review also focuses on history and success stories of drug repositioning. An alternative approach, drug combination of two or multiple drugs has dramatically increased the success rate of drug repositioning. This synergistic drug repositioning is a promising strategy to combat various diseases such as infectious diseases, cancer, neurological disorders and many rare diseases. Various examples of successful synergistic drug repositioning have been described in this review. Finally, we can say that synergistic drug repositioning provides a new way to drug discovery research.

Keywords

Drug repositioning, drug repurposing, drug re PROFILING, de novo drug discovery, computational approach, experimental approach, synergistic drug repositioning.

1. Introduction

In the 1870s, Paul Ehrlich introduced the idea of "chemoreceptor". Since that time, scientists are searching for those compounds that interact with specific targets to treat various diseases. These medications proved beneficial but this one-drug-one-target model has some limitations (Kola and Landis 2004). Now, our current challenge is to find "master keys" that can interact with multiple targets (Medina-Franco, Giulianotti *et al*. 2013). One strategy to win the challenge is drug repositioning (also known as drug repurposing and drug reprofiling). Drug repositioning is defined as identifying and developing new uses for existing drugs (Ashburn and Thor 2004). It also involves exploitation of established drugs that have already been approved for treatment.

1.1 Stages Of De Novo Drug Discovery And Development And Drug Repositioning

Denovo drug discovery includes six stages that are target discovery, discovery & screening, lead optimization, ADMET (absorption, distribution, metabolism, excretion and toxicity), development and registration. While repositioning process of a drug includes four stages are compound identification, compound acquisition, development and registration as illustrated in figure 3 (Ashburn and Thor 2004).

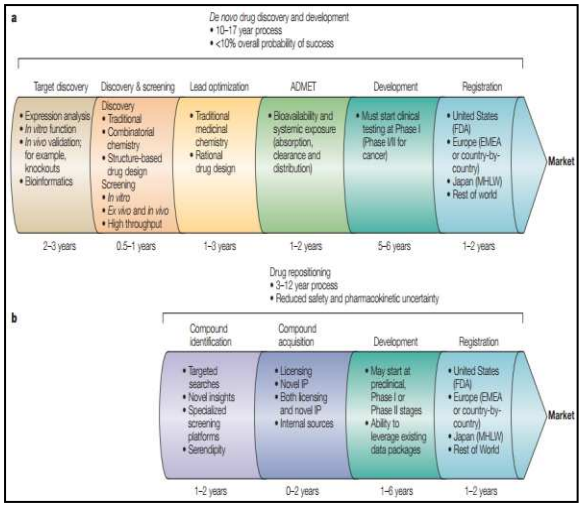

Figure 3: Stages of de novo drug discovery and development and drug repositioning. a) de novo drug discovery and development b) drug repositioning. EMEA, European Medicines Agency; FDA, Food and Drug Administration; IP, intellectual property; MHLW, Ministry of Health, Labour and Welfare (Ashburn and Thor 2004) .

1.2 Advantages Of Drug Repositioning

Drug repositioning is an effective strategy. Development of a new drug requires time, cost, effort and it is a highly risky process (Xue, Li *et al*., 2018). Drug repositioning has following advantages

- Drug repositioning requires less time for development as compared to development of a new drug. According to a report of Eastern Research Group (ERG), development process of a new drug requires 10-15 years. On average, 1-2years are required for the identifications of new targets and 8 years are required for the development of repositioned drug (Sertkaya *et al*., 2014; Xue, Li *et al*., 2018).

- According to a report of Food and Drug Administration (FDA), expenditure on the development of new drugs is increasing continuously while the number of approved drugs by FDA is decreasing as depicted in figure 1 (Ashburn and Thor 2004). Expenditure on conventional drug development method is high as compared to that on drug repositioning. The cost for the development of new drug by conventional methods is $12billion and that for repositioning is $1.6billion (Deotarse *et al*., 2015; Xue, Li *et al*., 2018).

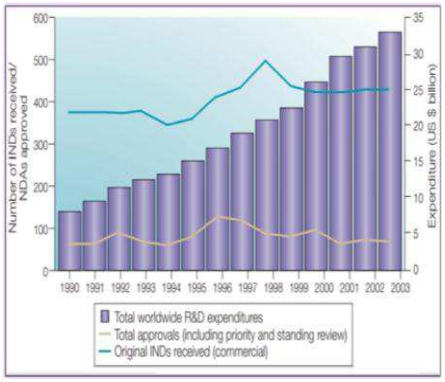

Figure 1: The growing productivity gap in the biopharmaceutical industry (Ashburn and Thor 2004).

- Reduction in timelines and cost for pharmaceutical research results in the increase in risk. Drug repositioning has reduced this risk because repurposed drugs have passed all phases of clinical development and their safety profiles are known. A reward-risk diagram is often used to compare repositioning and conventional process of drug development (Ashburn and Thor 2004). Repositioned drugs have high reward and low risk as shown in figure 2 (Xue, Li *et al*., 2018).

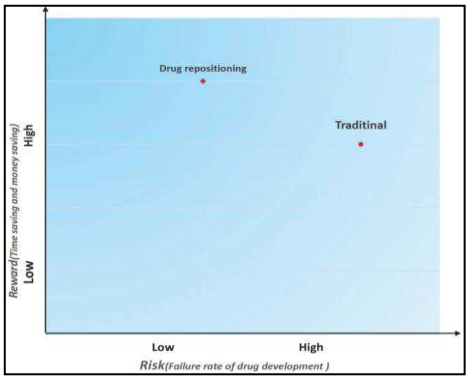

Figure 2: The risk-versus-reward trade-off between different drug development strategies (Xue, Li *et al*., 2018).

- Drug repositioning is less laborious. Much effort is required for the development of new drug by conventional method. Success rate of the development of new drug is only 2.1% (Yeu *et al.*,2015; Xue, Li *et al*., 2018).

1.3 Approaches Of Drug Repositioning

Historically many repositioned drug candidates have been discovered serendipitously. There are various approaches of drug repositioning as depicted in figure 4 (Grinnan, Trankle *et al*. 2019). Computational approach includes Drug combination prediction, EHR based drug repositioning, Fragment-based drug repositioning, Genome-based drug repositioning, Network-based drug repositioning, Neural network-based drug repositioning, Off-targeting data driven repositioning, Pathway based repositioning, Protein-protein interaction driven prioritization, Protein-small molecule interactions, Structure based drug repositioning, Systematic drug repositioning, Text-mining driven drug repositioning and Topic modeling. Experimental approach includes Chemical genomics, Chemical systems biology, Clinical side-effects, Genome-wide association studies (GWAS), Medical genetics, MicroRNA (miRNA) expression, Network medicine, Serological marker, Small interference RNA (SiRNA) screening and Transcriptomic response.

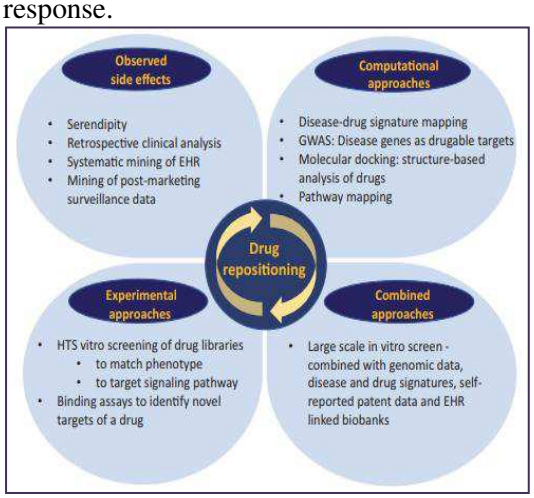

Figure 4: Current and future approaches for drug repositioning. HTS, high-throughput screening; EHR, electronic health record (Grinnan, Trankle *et al*. 2019).

1.4 Challenges To Drug Repositioning

During drug repositioning, researchers encounter a large number of challenges. Some of these challenges have been described here.

1.4.1 Selection Of An Appropriate Approach

Different approaches that may be experimental or computational are used. Selection of an appropriate approach is required (Novac 2013). Many researchers have introduced mixed approaches, in which both computational and experimental approaches are included (Ai *et al*., 2015; Amelio *et al*., 2014; Lee et al., 2016; Xue, Li *et al*., 2018). During mixed approaches, outcomes of computational methods are confirmed by clinical tests and experiments.

1.4.2 Selection Of Target Population

Target group selection is an important task in drug repositioning. Incorrect selection of target population results in outcomes other than expected one (Matthews and Mccoy, 2003; Gns, Gr et al. 2019). For example, thalidomide was developed to treat morning sickness in pregnant women but it was found to treat multiple myeloma when this group was removed (Novac, 2013).

1.4.3 Intellectual Property (IP) Protection

Intellectual property (IP) issues are issues related to the commercialization. If the drug candidate has never approved for marketing, IP can enhance value of repositioner. However, repositioned candidate already exists in the scientific community, pre-existing patents might block the commercialization of candidate. To defend drugs against competitors, two general cases are considered. First is that composition of matter (COM) IP is held by other party and deals to license. Second case is that compound is off patent. In this case, the repositioner depends on method of use (MOU) patent or new substantial barriers are provided using new drug formulations, dosage forms or drug combinations (Ashburn and Thor 2004).

1.4.4 Overlapping Of Models

For drug repositioning, different models must be used. Because overlapping of models results in overlapping of investment issues.

1.4.5 Achievement Within Timeline

When an old drug is repositioned for a new indication, appropriate dosage of drug and route of administration must be chosen. Drug formulation is optimized in such a way that it does not destabilize drug (Novac 2013).

1.4.6 Unexpected Adverse Events

Drug repositioning involves different groups of patients. Each group has a characteristic set of physiological conditions so each group might

response in different way. This may result in different adverse effects (Gns, Gr et al. 2019).

1.4.7 Demands Prerequisite Data

Repositioning requires data about drug, its structure, its target, pharmacodynamics and pharmacokinetics. This will result in outcomes that are expected (Gns, Gr et al. 2019).

1.5 Brief History Of Drug Repositioning

In 1942 during II World War, people of France suffered from typhoid. Marcell Johnson treated patients with sulfonamide antibiotics. However, he noticed that blood sugar has dropped in some patients and few of them died after a hypoglycemic coma. Complementary researches were carried out about this phenomenon in 1946 and sulfonamides were introduced as anti-diabetic agent. Other members of this family such as chlorpropamide and acetohexamide were released in market to cure diabetes in 1950s (Mohammad Jafari, Sheibani *et al*. 2018). Since that time, a large number of drug candidates are under consideration to reposition. The origin of repositioned drugs belongs to one of following groups: i) Non-commercialized compounds from academic and public sector labs; ii) Compounds under clinical development that exhibited polypharmocology; iii) Shelved compounds that demonstrated inefficiency; iv) Drugs that were abandoned due to safety reasons; v) Drugs proximal to patent expiry or competition from generics; vi) Drugs with incremental new indications referred to as Line Extension (Naylor 2015). Sildenfil is an example of shelved candidate. This compound was being examined to treat angina in clinical trials in 1998. During trials, some unexpected side effects were found that resulted in the development and approval of this drug candidate to cure erectile dysfunction (ED) (Novac 2013). Later on, this compound (marketed under the brand name REVATIO) was approved to treat pulmonary arterial hypertension in USA in 2005. Other examples of drugs repurposed during clinical trials include Canakinumab, Pertuzumab and Finasteride (Naylor 2015). Thalidomide is one of the drugs that have failed after post-market launch. It was originally developed to treat morning sickness that was common in pregnant women. In 1962, this drug was withdrawn from market. This drug was repositioned to treat leprosy in 1998 and to treat multiple myeloma in 2006. This drug is currently under examination for new uses (Naylor 2015). Other such off-targets drugs include Crizotinib and Imatinib. Everolimus is an on target repositioned drug. It was originally developed as immunosuppressant and involved in the inhibition of mammalian target of rapamycin. The approval of this drug was accepted to treat kidney cell cancer in 2009, astrocytoma in 2010, metastatic pancreatic neuroendocrine tumour in 2011 and HER-2(-) breast cancer in 2012 (Naylor 2015). Other examples of on-targets repositioned drugs include Duloxetine, Sunitinib and the first anti-retroviral Zidovudine. During the past several years, the interest in repositioning has greatly increased and efforts are being done to find new indications for already approved FDA drugs.

Due to availability of large volume of omics data, use of bioinformatics and various computational methods is increasing in drug repositioning. Drug repositioning has various applications. One of applications is discovery of anti-cancer drugs. Pessetto *et al*. (2013) repositioned Auranofin for the treatment of gastrointestinal stromal tumors (GIST) using high throughput screening. This drug was approved in 1985 by FDA to treat rheumatoid arthritis. It was also suggested that this drug is effective in imatinib-resistant patients of GIST. Irinotecan is a top1 inhibitor. It was approved by FDA to treat colorectal cancer (CRC). Biomarker-guided repositioning reveals that this is also effective against breast cancer (Stenvang *et al.* 2013; Li, Zheng *et al*. 2015). Mebendazole is an antihelmintic drug. It is repurposed to treat colon cancer using *in silico* drug screening (Nygren *et al*. 2013; Li, Zheng *et al*. 2015). Researchers are focusing on the discovery of novel cancer indications for already approved FDA approved drug candidates.

Drug repositioning is also being utilized to discover more drugs against microbial infections. Statins are lipid-lowering agents and its derivatives such as Simvastatin and Atorvastatin are used to treat cardiovascular diseases. Graziano *et al.* (2015) performed cell viability and biofilm assays and found that Simvastatin has pronounced activity against *Pseudomonas aeruginosa* (Serafin and Horner 2018). Toremifene is FDA approved anticancer agent. A time kill study reveals that this drug

has bactericidal effect against oral bacteria *Streptococcus mutans*. Macromolecular synthesis assays and Biophysical studies using fluorescent probes and fluorescence microscopy indicated membrane-damaging activity of this drug (Gerits *et al*., 2018).

Research is also being carried out to reposition approved drugs to treat neurological disorders. Friedreich's ataxia is a disease during which level of frataxin decreases. There is no drug candidate available to treat this disease. To find therapy, Alfedi, Luffarelli *et al.* (2019) performed a high throughput screening of 853 FDA approved drug candidates. They performed cell-based reporter assay to determine the level of frataxin. During screening, it was found that an anti-viral drug Etravirine increased the level of frataxin. This examination resulted in the repositioning of Etravirine for Friedeich's ataxia (Alfedi, Luffarelli *et al*. 2019). Epilepsy is a chronic disease. To identify drugs to treat epilepsy, researchers performed transcriptomic analysis in six epilepsy patient brain biopsy specimens. Out of 184 drug candidates, only four drugs (doxycycline, metformin, nifedipine, and pyrantel tartrate) were tested *in vivo* in zebrafish PTZ-induced seizure model. Doxycycline is an antibiotic, metformin is an anti-diabetic agent, nifedpine is antihypertensive medication and pyrantel tartrate is an anti-parasitic agent. Except doxycycline, rest three drug candidates represented novel anti-seizer activity to treat epilepsy (Brueggeman, Sturgeon *et al*. 2019).

So, drug repositioning is a promising strategy to find new uses of already approved drugs and rapid advances have been made in the development of computational drug repositioning approaches. To use available data efficiently, researchers proposed an efficient approach, RWHNDR (Random Walk on a Heterogeneous Network for Drug Repositioning) (Luo, Wang *et al.* 2018). This approach achieved well performance to prioritize drugs for diseases. All these efforts results in the improvement in drug discovery and development. Much more effort is being done to reposition more drug candidates.

2. Synergistic Drug Repositioning

Synergistic drug repositioning refers to identifying new indications for already approved drugs when used in combination. Since the past five years, the number of repurposed drugs has increased. However, many newly developed drug candidates have low potential and many diseases involves various mechanisms. So, combination of two or more drugs is best approach to increase drug potency and to target multiple components of a mechanism.

2.1 Potential For Drug–Drug Interaction

During synergistic drug repositioning, the potential of adverse drug-drug interactions (DDI) must be considered. Pharmacodynamics (PD) and pharmacokinetics (PK) are adverse DDIs (Sun, Sanderson *et al.* 2016). In 1990s, selective serotonin reuptake inhibitor (SSRI) paroxetine was prescribed to treat depression and side effects of tamoxifen in patients of breast cancer. Paroxetine inhibited the metabolism of tamoxifen. A study in 2010 reported that this DDI resulted in increased death rate (Kelly *et al*., 2010). Therefore, much more care is required during synergistic drug repurposing.

2.2 Approaches Of Synergistic Drug Repositioning

Many computational models including bioinformatics and other databases are useful in synergistic drug repositioning. Nowak-Sliwinska *et al*., (2016) introduced a feedback system control (FSC) to screen various drug combinations. In this method, dose–response curves are plotted after phenotypic cell viability assay for each drug and a differential evolution (DE) algorithm predicts drug combination. Low Daugherty *et al*., (2016) suggested that to discover drug pairs with synergistic repurposing potential, use of electronic health records (EHRs) is also very effective.

2.3. Success Stories Of Synergistic Drug Repositioning

Efficient synergistic drug repositioning resulted in the understanding of complex disease physio-pathology and designing of better treatments. Synergistic drug repositioning has been applied to treat cancer, microbial infections, neurological disorders, diabetes and a large number of rare diseases. Synergistic drug repositioning approach is effective to discover more drug candidates against infectious agents (viruses, bacteria, protozoans and etc.), neurological disorders and cancer.

2.3.1 Synergistic Drug Repositioning Against Bacteria

Synergistic drug repositioning is beneficial against infectious diseases. First advantage of combination of drugs is that it broadens the spectrum of antibiotics (Zilberberg *et al*., 2014). Secondly, drug combination helps to overcome drug resistance (Fleisher *et al*., 1983; Houang *et al*., 1984; Qin *et al*., 2017). Bacteria are developing resistance against antibiotics and there is a current need to develop more antibiotics using synergistic drug repositioning. Out of 1,163 FDA approved drug candidates, three compounds in combination with Tigecycline exhibited activity against carbapenem resistant *Enterobacteriaceae* (CRE) when tested using a time-kill assay and a checkerboard assays. Combination of lead compound Zidovudine (an oral anti-viral drug) and Tigecycline was proposed as a promising treatment against CRE infections (Ng, Sioson *et al.,* 2018). Nanoliter-scale droplets have been introduced that automatically constructs different drug combination. This platform was utilized to find drug pairs against pathogenic E. coli. This platform is simple, cost effective and can be developed to other phenotypic assays (Kulesa, Kehe *et al*., 2018). An in vitro antimicrobial activity was carried out to check combined effect of polymyxin B and mitotane against carbapenem resistant *Acinetobacter baumannii.* Time kill study showed that combination of polymyxin B and mitotane exhibited bactericidal activity (Tran, Wang *et al.,* 2018). A combination activity of colistin and azidothymidine was evaluated using mini checkerboard broth microdilution assay. This study showed that this synergy is effective to treat infections of colistin-resistant *Klebsiella pneumoniae* (Falagas, Voulgaris *et al.*, 2019).

2.3.2 Synergistic Drug Repositioning Against Protozoans

Repositioning of many drugs resulted in the discovery of many drugs against protozoans. In 2015, approximately 3.2 billion people were suffered in malaria. Resistance to newly developed drugs has become a global problem. Researchers utilized synergistic drug repositioning approach to develop new anti-malarial drugs. They combined gene-expression data and large-scale anti-malarial screening data to repurpose more drugs against three *Plasmodium falciparum* strains (3D7, DD2 and HB3). Results of their experiments were represented in a network as shown in figure (KalantarMotamedi, Eastman et al. 2018). This research wok showed that machine learning model along with gene expression data is effective to predict new drug combination therapies.

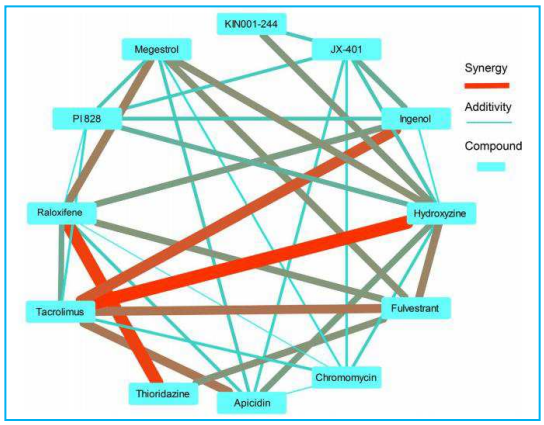

Figure 5: Network representation of compound combination synergies. Red thick edges represent high synergy and blue thin edges represent additivity. The level of thickness or redness is inversely related to gamma (calculated based on experiments) in average for the tree *P. falciparum* strains used in this study (KalantarMotamedi, Eastman et al. 2018).

2.3.3 Synergistic Drug Repositioning Against Viruses

Viruses causes many infections and are developing resistance to already developed drugs. To reposition drugs against Ebola virus (EBOV), Human immunodeficiency virus (HIV), Zika virus and other viruses is our goal. Researchers performed EBOV entry assay and screened 795 FDA approved drugs. Drugs were screened in unique three-drug combinations. Out of these 795 drugs, toremifene-mefloquine-posaconazole and toremifene-clarithromycin-posaconazole demonstrated efficacy in blocking entry of EBOV. This work proved effective to treat EBOV infection (Sun, He *et al*. 2017). Zika virus causes an infectious disease and is transmitted through mosquito. It is common in Americas. There is no therapeutic anti-viral agent against Zika virus (ZIKV). A Cytoprotection (CP) assay was performed to test combined activity of Interferon-α and Interferon-β and Sofosbuvir in a hepatic cell line (Huh7). This synergistic assay exhibited effective inhibition of ZIKV

infection (Snyder, Goebel *et al*., 2018). In 2018, mechanism-based modeling (MBM) and other experimental assays were carried out to find combined effect of favipiravir (FAV) and interferon alpha (IFN) against zika virus. This synergy proved effective to inhibit infection (de Mello, Tao *et al*., 2018).

2.3.4 Synergistic Drug Repositioning Against Neurological Diseases

Many advances has been made to develop drugs against neurological disorders. Best approach is synergistic drug repositioning. According to Shi *et al*., (2016), induced pluripotent stem cell (iPSC) technology emerged as a great revolution in drug discovery. In 2017, this technology was used to identify the synergistic anti-amyloid β combination for Alzheimer's Disease (AD). Pharmaceutical compounds were screened for amyloid β peptide (Aβ) phenotypes. Then chemical structure based clustering and combination analysis showed that synergy of bromocriptine, cromolyn, and topiramate is effective as anti-Aβ cocktail. This study showed that iPSC is an ideal platform to develop drugs against AD (Kondo, Imamura et al. 2017). Despite of major advancement in technologies, our knowledge is limited. Quantitative Systems Pharmacology (QSP) is an emerging approach. It combines computational and experimental methods. This small molecule approach has great potential in synergistic drug repurposing and was applied to develop drug candidate against Huntington's disease (HD) (Pei, Li et al. 2017). A study carried out on Glioblastoma (GBM) exhibited that combination of disease-specific gene expression signatures and Library of Integrated Network-Based Cellular Signatures (LINCS)-small molecule perturbagen-response signatures are promising to identify synergistic drugs against GMB (Stathias, Jermakowicz et al. 2018).

2.3.5 Synergistic Drug Repositioning Against Cancer

To develop a therapeutic drug candidate against Adrenocortical carcinoma (ACC), Nilubol et al. used approach of synergistic drug repositioning and high-throughput screening exhibited that the combination of flavopiridol and carfilzomib is effective to treat ACC (Nilubol, Boufraqech et al. 2018). Cancerous cells are also developing resistance against various drugs. To screen a large number of drug combinations is very laborious so use of CRISPR-based double knockout (CDKO) system is quite feasible. This system is very effective and robust. This system has been applied to create a large-scale human GI map and to identify lethal drug-target pairs. This strategy has great potential to screen synergistic drug repositioning in cancerous cells (Han, Jeng et al. 2017).

Not all drug combination efforts are successful. Some may result in unacceptable side effects. However, it has been found that drug repositioning is best approach to overcome the need of new therapeutics. Finally, we can say that drug repositioning is very effective, low cost, less risky and less time consuming approach. Further, synergistic drug repositioning has revolutionized drug discovery research and success rate of new repositioned drugs has increased due to advancement in technologies.

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