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An update on Drug Repurposing: Re-written saga of the drug's fate

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ABSTRACT

Drug repurposing is an unconventional drug discovery approach to explore new therapeutic benefits of existing, shelved and the drugs in clinical trials. This approach is currently emerging to overcome the bottleneck constraints faced during traditional drug discovery in grounds of financial support, timeline and resources. In this direction, several efforts were made for the construction of stratagems based on bioinformatics and computational tools to intensify the repurposing process off-late. Further, advanced research has succeeded in widening its boundaries in identification of gene targets and subsequent molecular interactions of the drugs depending on available omics data. Currently, the advent of data repositories like Connectivity Map (CMap), Library Integrated Network based Cellular Signatures (LINCS), Genome Wide Association Studies (GWAS), Side Effect Resource (SIDER), and Directionality Map (DMAP) has bestowed great oppurtunity to the researchers in improving their drug repurposing research exponentially. On the otherhand, *in silico* approaches like pharmacophore modelling and docking techniques circumvent the routine tedious *in vitro* and *in vivo* techniques involved in former screening phases of the drugs and disease specific targets. This review elaborates on currently designed contemporary tools, databases and strategies with relevant case studies.

1. Introduction

Traditional drug development process consumes time and resources immensely before a molecule is labored into the open market. Despite huge investments, the chances of a lead molecule to enter open market are often minimal. The itinerary of the research molecule remains unpredictable, throughout its lifecycle. This situation makes newer pharma companies to give upon dreams on novel drug discovery. One of the viable options for newcomers in the field of new drug research is drug repurposing.

Drug repurposing refers to "Studying the drugs that are already approved to treat one disease or condition to see if they are safe and effective for treating other diseases" according to National Centre for Advancing Translational Sciences (NCATS) [1]. NCATS, a component of National Institute of Health (NIH) in the United States of America (USA), launched a program "Discovery of New Therapeutic Uses for Existing Molecules" in May 2012. This program focused on identification of new therapeutic indications and treatment options for the compounds that have been already marketed or the New Chemical Entities (NCE) that are in the loop of drug development pathway and offer financial support [2].

The concept of re-profiling of a drug is deep-rooted in the grounds of poly pharmacology that reveals the details on on-target and off-target proteins through which a drug exhibits both anticipated and untoward effects. A single target protein can trigger multiple responses. Protein with abnormal function in one cascade of network may be responsible for development of a disease. The same protein may also mislead other physiological process resulting in the altered response via entirely new cascade of reactions. Drugs designed for targeting such altered protein as a target in a particular indication are also considered to show effect in a different indication where the same protein is the key factor for triggering another set of responses expressed in entirely different disease [3].

2. Strategies for drug repurposing

Repurposing is carried out in two steps. The first step being the *in silico* screening of approved or marketed drugs against a particular therapeutic target and the shortlisted are further processed for investigation in specific pathophysiological pathways of the disease of interest using *in vitro* and *in vivo* methodologies [4]. The second stage of drug repurposing is to enter the clinical trials for respective indication [5].

Drugs may be repurposed at any stage of their evolution right from

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their discovery. Few examples of the repurposed drugs on different backgrounds are mentioned below:

- An approved drug with specific side effect which is serendipitously found to possess better therapeutic potential towards other indication. For example: Sildenafil originally developed for treatment of hypertension and angina pectoris has currently banked a huge market for managing erectile dysfunction [6]
- Screening of Food and Drug Administration (FDA) approved drugs for their off-label use. Itraconazole primarily approved as an antifungal agent [7] is found to possess an additional anti-angiogenesis property. Exploration of the above property directed the compound's clearance through Phase 2 trials for investigating its efficacy as a second line therapeutic agent in the treatment of lung cancer [8] (NCT02357836) [9] and prostate cancer (NCT00887458) [10].
- Implication of failed investigational drugs for new therapeutic areas. Saracatinib, a failure drug developed by AstraZeneca as an anticancer agent exhibited substantial reversal of symptoms in Alzheimer's Disease (AD) mice model [11], and passed through Phase 1 (NCT01864655) [12] trials against AD.

Drug repurposing demands an extensive literature research regarding the drug profile and targeted disease ultra-mechanisms as well. Researchers are required to be equipped with the latest reliable data pertaining to unexplored or novel pathways related to the development and progression of the disease and specific biomarkers associated with different stages of the disease. Any research focusing on genetic disorders mandates the requirement of literature supporting the potential manipulating effect of the drug at the genomic level i.e., gene expression profiles, transcriptional analysis etc. Selection of suitable approach for repurposing of the drug is a crucial step in this process. All the technologies like genomics, proteomics, transcriptomics and databases which include drug omics data, disease omics data are interconnected in designing strategy for a proposal of a drug to be repurposed. The successful proposals are further screened through traditional drug development pipeline (Fig. 1).

Advancement in genomics have led to establishment of genomic and transcriptomic data of wide variety of samples using technologies like next generation sequencing, microarray data and transcriptomics etc. Gene expression profiles, cell lines, animal models (knock out models) and tissue samples are well established for almost all known genetic disorders. Various databases (Fig. 2) and softwares (Table 1) are available publicly for genomics, proteomics and pathway analysis. Several computational strategies are developed to increase the ease of the repurposing process.

2.1. Signature based strategies

Signature based strategies focus on the identification of the genetic factors like gene expression profiles, gene regulatory profiles, transcription factors involved in the pathogenesis of the disease. These strategies unravel the molecular mechanisms behind the disease pathogenesis. This paves a path to discover the hidden mechanisms of the drug and target. There are several computational tools which are developed to explore the genetic messengers.

2.1.1. CMap

CMap is a web based tool that establishes similarities among the drugs based on genetic expression profiles observed in the cancer cell lines. First generation gene expression signatures (a total of 564) were generated using 164 small molecule perturbagens across three cancer cell lines: MCF7 (breast cancer epithelial cell line), PC3 (Prostrate cancer epithelial cell line) and HL60 (non-epithelial leukemia cell line) and SKMEL5 (non-epithelial melanoma cell line) at 10 μM concentration [57]. Next generation CMap data (Library of Integrated Network-based Cellular Signatures consortium) include a total of 476,251 gene expression profiles generated using 27,927 perturbagens across nine cancer cell lines [60]. This tool is used to analyze the differentially

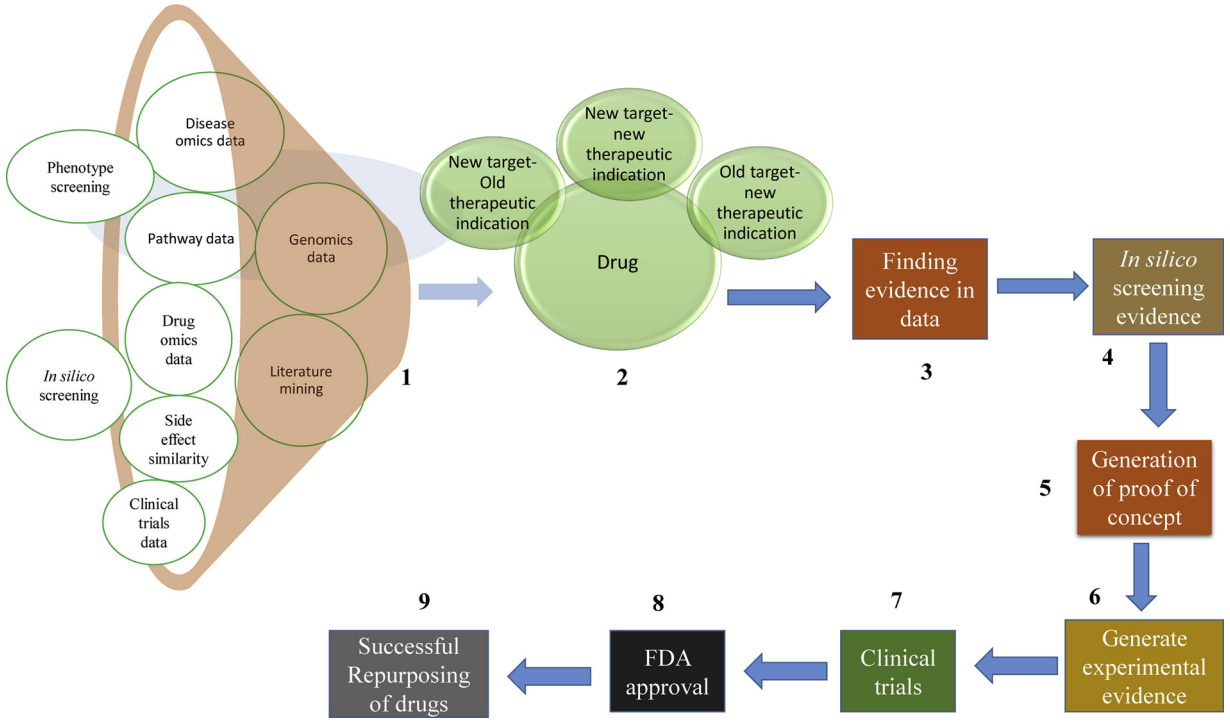

Fig. 1. Route map for drug repurposing. Drug repurposing starts with collection of raw data related to disease-drug-targets (1) followed by establishment of drug-target-disease relationship (2). With an extensive support of potential evidence (3) and *in silico* screening techniques (4) proof of concept is generated (5) which is then experimentally proved (6). Further the shortlisted compounds pass through clinical trials (7) for the new indication and enter the market with FDA labelling (8 and 9).

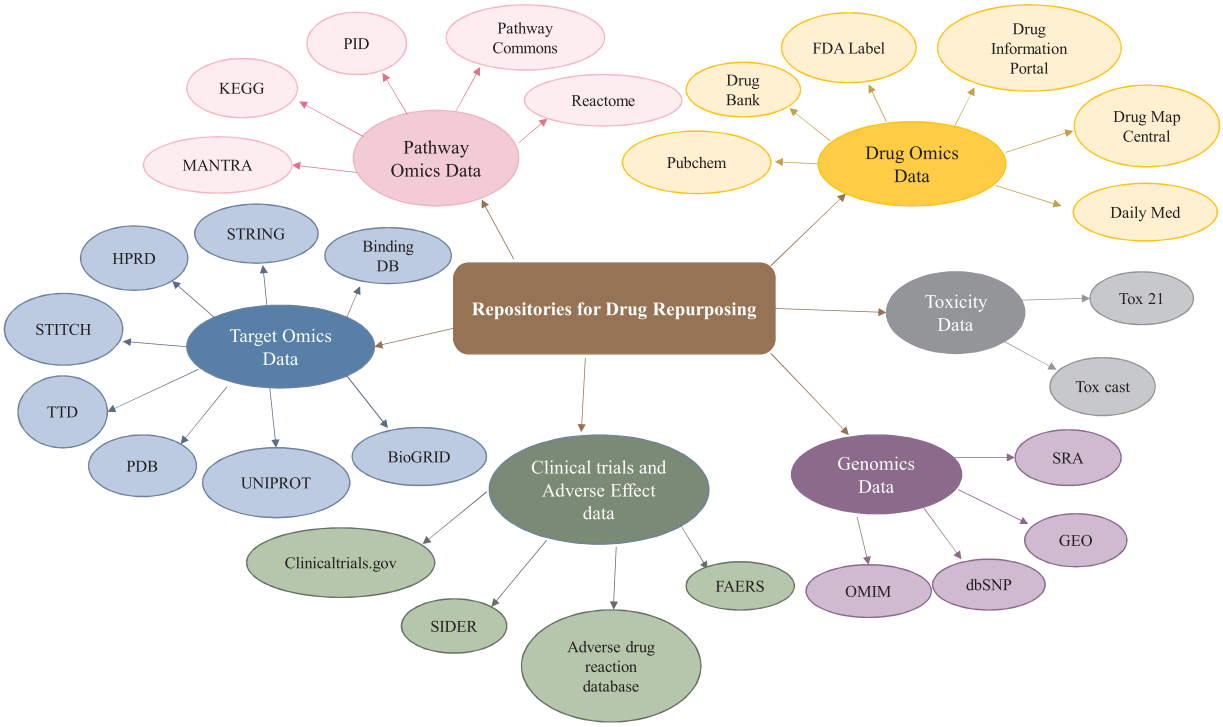

Fig. 2. List of databases used in drug repurposing. Drug omics data [13–18], Toxicity data [19,20], Genomics data [21–24]: SRA: Sequence Read Archive, GEO: Gene Expression Omnibus, dbSNP: Single Nucelotide Polymorphism database, OMIM: One Mendelian Inheritance in Man, Clinical trials and Adverse Effect data [25–27]: FAERS: FDA Adverse Event Reporting System, SIDER: Side Effect Resource, Target omics data [28–35]:BioGRID: Biological General Repository Interaction Dataset, UNIPROT: Universal Protein Knowledgebase, PDB: Protein Data Bank, STRING: Search Tool for Retrieval of Interacting Genes/Proteins, STITCH: Search Tool for Interaction of Chemicals, TTD: Therapeutic Target Database, Pathway omics data [36–40]: MANTRA: Mode of Action by NeTwork Analysis, KEGG: Kyoto Encyclopedia for Genes and Genomes, PID: Pathway Interaction Database.

Table 1
Tools used in drug repurposing.

Purpose	Tools (a- Commercial, b- Openware)
Docking [41,42,43,44,45,46,47]	Glide[a], Auto dock[b], Tar Fish Dock[b], Flare™ [a], Induced Fit[a], CovDoc-VS[a], Lead Finder™ [a]
Binding site prediction [48,49,50,51,52]	Sitemap[a], Computed Atlas of Surface Topography of proteins (Castp)[b], Findsite[b], LigASite[b], PDBeMotif[b]
Pathway analysis [53]	Therapeutic Performance Mapping System (TPMS)[a]
Drug Design [54]	Blaze[a], Forge™ [a], Spark™ [a]
Phamacokinetic parameters [55]	SwissADME[b]
Genomics [56,57,58,59]	Genome Wide Association Studies (GWAS)[b], Connectivity map (CMap)[b], Directionality map (DMAP)[b], Phenome Wide Association Studies (PheWAS)[b]

expressed genes in *in vitro* disease models and compare the same with the normal physiological states, assess the loss of function (LOF) or gain of function (GOF) of a gene and also predict the effect of a drug on the gene. The approach starts with input of query signature consisting of list of genes of interest. The analysis starts with investigation of the query signatures with the reference dataset containing expression profiles of the drug across cell lines. The connectivity between the query signatures and the reference set is represented between +1 to -1. Perturbagens with score +1 represents high connectivity with the respective genes in query signatures and -1 represent the negative correlation.

2.1.1.1. Reversal of Dexamethasone resistance using gene signatures. Dexamethasone resistance, a bottleneck in the treatment of Acute Lymphoid Leukemia (ALL), was found to be overturned when treated with Sirolimus. The gene signatures of Dexamethasone resistance and sensitivity of both control and diseased patients were constructed from bone marrow leukemic cells and used as a query signatures in CMap. The query signatures of Dexamethasone sensitivity exhibited high correlation with Sirolimus (mTOR inhibitor). Further, Gene Set Enrichment Analysis (GSEA) revealed high degree of correlation between the down regulated genes of the Sirolimus treated lymphoid cells and up regulated genes of Glucocorticoid resistant cells. Also, *in vitro* analysis using lymphoid cell line CEM-c1 revealed that pretreatment with 10 nM Sirolimus increased the sensitivity of Dexamethasone in Dexamethasone resistant ALL cells and non-resistant ALL cells [61]. Based on promising results from computational and *in vitro* analysis, proof of concept was established to support the current status of Siroliums in phase I trials in combination with Dexamethasone for relapsed ALL (NCT01403415) [62].

2.1.1.2. Construction of mode of action by NeTwork analysis (MANTRA). Diego di Bernardo *et.al* [63], designed a tool revealing the molecular mechanisms of drugs and their connectivity using transcriptional responses. In this process, 6100 gene signatures were extracted from 1309 drugs in various cell lines at different doses based on CMap analysis. The transcriptional responses were retrieved from each expressed gene and Prototype Ranked List (PRL) was designed to represent the rank along with list of differentially expressed genes for each drug. Every two drugs from the above mentioned 1309 drugs were compared in terms of the distance based on PRL of the drugs. The optimal gene signatures of the two drugs were retrieved by comparing

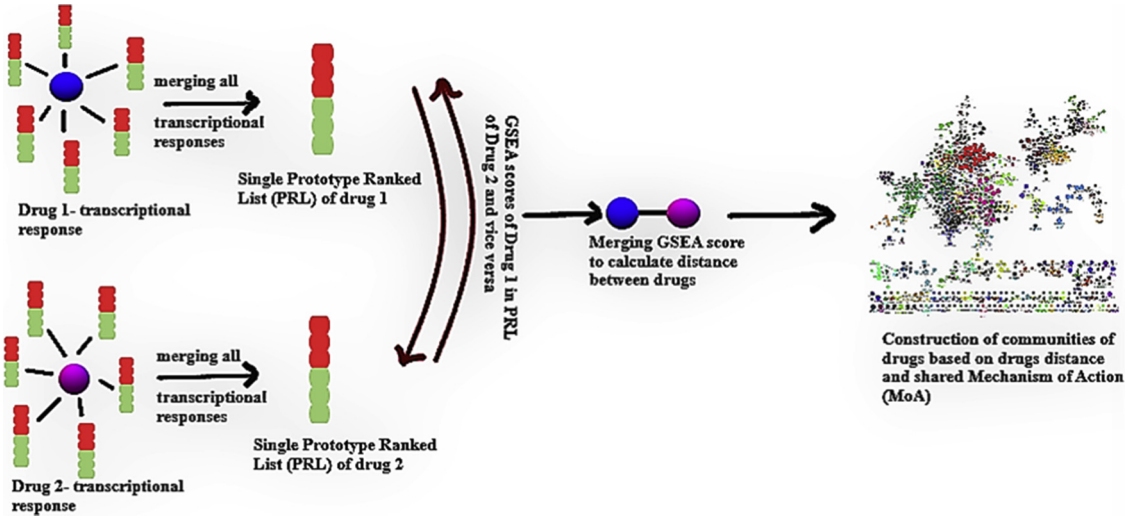

Fig. 3. Workflow in the development of MANTRA.

the top 250 genes that are over expressed and down regulated in the PRL of the 2 drugs. Further, GSEA score was assigned based on the optimal gene signature of drug A in PRL of B and vice versa. Later, the two scores were merged to define the distance between drugs, wherein shorter distance represents high degree of similarity between the drugs (Fig. 3). Drug Network (DN) was constructed using nodes that represent drugs and edges i.e., distance between the drugs. In this study, 1302 drugs of CMap were studied to possess 41,047 edges based on PRL. The highly connected nodes were categorized as communities (106 communities among 1302 drugs) based on the developed clustering algorithm. Further analysis, based on the direct targets and Anatomical Therapeutic Chemical Classification (ATC) codes revealed that 52 communities were highly enriched with similar Mechanism of Action (MoA). Gene Ontology (GO) enrichment analysis was done to identify the commonly up and down regulated genes among the communities. Based on the above technique, Flavopiridol, previously known as Cyclin Dependent Kinase (CDK) inhibitor was found to be closely related to Topoisomerase inhibitors. Similarly, a Rho-kinase inhibitor, Fasudil was identified as cellular autophagy promoting agent due to its close distance with known autophagy inducer (2-deoxyD-glucose). Immunohistochemistry revealed the increase of microtubule associated protein 1 A/1B-light chain 3 (LC3)-phosphatidylethanolamine conjugate (LC3-II) levels (an autophagy marker) in human wild type fibroblasts when treated with Fasudil [64]. This concept lead to development of MANTRA. Luca Cardone et.al, applied the principles of MANTRA to identify the inhibitory potential of two anthelmintic drugs Niclosamide and Pyrivinium Pamoate (PP) against PI3K pathway [65]. This laid foundation to investigate the above drugs as inhibitors of PI3K pathway in mutated Human Mammary Epithelial cells (HME). Western blot analysis revealed that the inhibition of PI3K pathway is due to inhibition of P70S6K phosphorylation and down regulation of downstream messengers responsible for the pathway.

2.1.2. GWAS [56]

GWAS is a database developed by the National Human Genome Research Institute (NHGRI), a component of NIH, in 2008 which consist of reported Single Nucleotide Polymorphisms (SNP, a single DNA nucleotide change that corresponds to the change in genetic makeup) and their associated genetic trait expressions. Later NHGRI has collaborated with European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) to identify the risk loci and their association with disease development including the phenotypic expressions through GWAS. Currently, GWAS compiles 62,652 SNP-traits and their respective disease associations. The following section narrates the drug repurposing projects based on the identification of genetic risk loci and disease associations using GWAS.

2.1.2.1. Identification of potential drugs for Rheumatoid Arthritis (RA) based on GWAS data. Rong Xu *et.al*, on the basis of GWAS data, identified drugs to treat RA based on the assumption that RA related diseases may have overlapped genes either directly or indirectly with RA genes. The drug that has efficacy in treating most of the RA related diseases can offer promising cure for RA than the drug that is effective in few RA related diseases. Based on this assumption, the Genetic Disease Network (GDN) was constructed using the disease associated genes from GWAS catalog. A total of 22,470 diseases and their associated traits were obtained symbolizing 881 diseases and 8689 responsible genes. Cosine distance and Jaccard similarity approaches (used to identify the similarity between two sets of attributes based on overlapping attributes) were used to identify the overlapping genes among the diseases. GDN was constructed using Search Tool for Interacting Genes/Proteins (STRING) consisting of 882 nodes and 200,758 edges among the diseases. Disease ranking algorithm was developed to rank and identify the most RA related disease from GDN. Based on GDN analysis, immune and autoimmune diseases represent the most related disease classes for RA (with 2.45 and 5.59 fold enrichment respectively). Drug repositioning algorithm was developed to identify and prioritize the drugs based on their efficacy on the number of RA related diseases. This approach revealed the potential of 74 drugs belonging to immune and autoimmune categories against RA [66].

2.1.2.2. Use of Weighted Gene Co-expression Network Analysis (WGCNA) to scrutinize the GWAS data for Parkinson's Disease (PD). Jake Y. Chen *et.al* [67], used WGCNA, a package in R language which constructs the network based on correlation between datasets, to repurpose the drugs against PD using the GWAS data specific for PD and their co-expression details. This approach filters the genes which are co-expressed along with the candidate genes (PD specific genes) and retrieves their protein-protein interactions along with the regulatory pathways. The methodology identifies differentially expressed genes using limma package in R. The candidate genes were identified using GWAS and were further analyzed for retrieving the clusters of highly correlated genes using WGCNA. Pathways Annotated lists and Gene-sets Electronic Repository (PAGER) database (a collection of gene sets, pathways and networks for various diseases) was used for identification of specific gene regulatory network. Enrichment analysis was executed for the

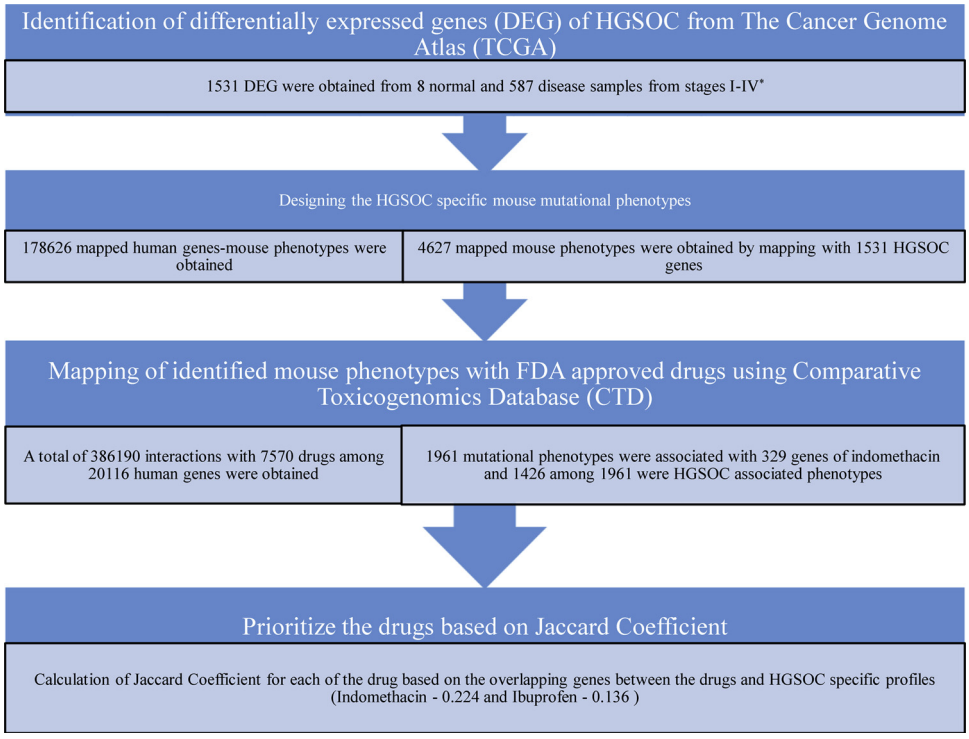

Fig. 4. Work flow for the Identification and prioritization of the drugs for HGSOC genes.
*Stage I: Cancer confined to one or both ovaries. Stage II: Cancer cells expand to other pelvis regions. Stage III: Cancer cell spreads to upper abdomen. Stage IV: Extension of cancer cells to other regions of body.

overlapped genes between co-expressed genes and GWAS candidate genes. Gene interactions were mapped in Human Annotated and Predicted Protein-Protein Interaction (HAPPI-2) database and PD specific network module was created. Further, p-score was calculated using Directionality map (DMAP) and Relevant Protein score (RP) was analyzed for each PD network module. Drug effect sum score of each drug in all PD modules was calculated to identify potential drugs. WGCNA analysis retrieved 5 co-expression modules with 50 out of 2895 genes which were found to be overlapped with the candidate genes acknowledged through GWAS data. The results were subjected to ClueGO analysis which revealed that the recognized candidate genes were involved in 4 biological processes and 2 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Finally, 12 among 1201 drugs were shortlisted based on their interactions with the proteins and co-expression profiles. Among the shortlisted drugs, Estradiol that regulates the menstrual cycle, scored the highest and was reported to protect dopaminergic neurons in PD. *In vivo* studies identified the protective role of both forms: 17α-Estradiol [68] and 17β-Estradiol [69] in PD rat model. Isotretinoin designed to treat *Acne vulgaris* was found to have efficacy in later onset PD. Sirolimus previously known to treat the Age related Macular Degeneration (AMD), was reported to be effective against cognitive deficits in PD. The protective effect of Sirolimus against cognitive deficit was reported *in vitro* in 1-methyl-4-phenylpyridinium (MPP) treated astrocyte cell lines and the potential mechanism elucidated was through the activation of JAK/STAT and PI3K pathway. Sirolimus also was observed to reduce the Glutamate/Aspartate transporter (GLAST) in mid brain and Glutamate Transporter Expression (GLT) in straitum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD model [70]. *In vivo* immunohistochemistry studies in the rat PD model revealed that pretreatment with Sirolimus exhibited significant decrease in the loss of Tyrosine Hydroxylase positive (TH^+) dopaminergic neurons, Bcl-2 up regulation and Bax down regulation supporting the potential anti-parkinsons effect [71].

2.1.3. Drug repurposing using Library of Integrated Network-based Cellular Signatures (LINCS) [72]

LINCS is a data portal initiated by NIH in 2011 with a goal to provide complex data in the form of organized databases integrating the cell based information like gene and protein expression signatures, gene ontologies and drug-disease pathways validated through standardized cell models using perturbagens. This metadata is generated through six Data and Signature Generation Centres (DSGCs) and one Data Coordination and Integration Centre (DCIC). L1000 database developed by LINCS has observed the effect of treatment with 20,000 compounds on 72 cell lines and narrowed down to the gene expression profiles of 978 candidate genes that were widely influenced in response to the above compounds.

Xiaochen Bo *et.al*, mapped the transcriptomic data extracted from L1000 database with 480 FDA approved drugs and reported the re-purposing potential of 98 drugs. Notably, Zonisamide (anti-epileptic drug) and Brinzolamide (approved for open-angle glaucoma and ocular hypertension) were studied in-depth and were proposed to possess capability to get repurposed for cardiovascular diseases with high probabilities 0.945 and 0.935 using LINCS L1000 database. Sodium voltage-gated channels like SCN1B, SCN2B, SCN3B, and SCN5A were found to be potential targets of Zonisamide and further analysis revealed that Zonisamide was found to interact significantly with other 81 proteins using Search Tool for Interactions of Chemicals (STITCH), a database containing the collection of small molecules and their phenotypic expressions and cellular mechanisms. The down regulated genes of Zonisamide were found to be enriched in ventricular tachycardia. Drug-Target Network constructed based on Side effect data (DTN-SE) revealed the similarity among Zonisamide, Doxazosin (Hypertension) and Bumetanide (Congestive Heart failure and Nephrotic Syndrome) in sharing targets. Drug-Target Network based on Transcriptome data (DTN-T) network revealed four direct targets and thirty one further interacting proteins. The gene expression studies of Brinzolamide revealed that the down regulated genes of this drug were involved in cardiomyopathy and cardiac myofibril assembly formation.

On the other hand, up regulated genes were associated with the regulation of blood pressure. The drug exhibited similarity with Hydrochlorthiazide based on side effect profiles and Nifedipine based on structure [73].

2.1.4. Phenotype based drug repositioning for High Grade Serious Ovarian Cancer (HGSOC)

DrugPredict, profile based drug repurposing tool develops the mouse mutational phenotypes using Mouse Genome Informatics (MGI) for a given set of genes associated with a specific disease and then correlates these phenotypic expressions to screen drugs/chemicals. As a pilot phase of the study the tool was tested for repositioning drugs for HGSOC (The work flow for the study is depicted in Fig. 4). Among 6996 drugs screened, 31 classes (including anti-cancer drugs) of the drugs were found to be best ranked. Next to anti-cancer drugs, the best ranked class was Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) with a mean ranking of 23.55%. Among the NSAIDS, two selective Cyclooxygenase (COX) inhibitors (Celecoxib, Nimesulide) and one non-selective COX inhibitor (Indomethacin) inhibitor were the top ranked compounds. With the controversies of selective COX inhibitors, Indomethacin, a non-selective COX inhibitor was further evaluated for molecular mechanisms and was found to be effective even in Platinum/Cisplatin resistant cells. Indomethacin was found to decrease the cell viability in all ovarian cancer cell lines and even in cisplatin resistant cell lines (OV81.2/CP10 and CP70). This effect was in accordance with rise in apoptosis associated with drastic increase in levels of cleaved Poly (ADP-Ribose) Polymerase (PARP) and Histone variant (γ-H2AX), the hallmark apoptotic factors. In contrast to cisplatin sensitive cells and normal ovarian cells, Indomethacin treated cisplatin resistant cells exhibited significant decrease in the levels of β-catenin (a protein involved in Wnt signaling pathway) supporting the evidence that antitumor effects of NSAIDS are independent of COX. Other transcriptional mediators of Wnt signaling like Aldehyde dehydrogenase 1 (ALDH1), Transcription Factor 7 (TCF7), Leucine rich repeat G protein coupled Receptor 5 (LGR5), Cluster of Differentiation 24 (CD24) and Epithelial Cell Adhesion Molecule (EpCAM) were also decreased [74]. The current success status of Indomethacin against ovarian cancer paved its way to reach phase 1 studies for its efficacy in combination with platinum therapy (NCT01719926) [75].

2.2. Target based strategies

Target based methods involve the study of a particular protein/biomarker involved in pathophysiology. A protein plays a multi pathophysiological role in a diseased condition and in normal healthy conditions. A dysfunctional protein or mutated protein or a misfolded protein may lead to pathological cross-talks triggering the disease development. Target based approaches are direct approaches with the proteins. This method explores a new target in an old indication or an old target in a new indication. Few target based strategies developed are described below with the case studies.

2.2.1. Directionality map (DMAP) [58]

DMAP, a drug directionality map, establishes the effect of drugs on gene (either activation or inhibition) via protein interactions in computational approach. DMAP is designed based on 24,121 PubChem compounds mapped with 5196 Uniprot proteins establishing a total of 4, 38, 004 drug-protein relationships. In this process, similarity index between drug-drug and drug-protein interactions were studied and ranked accordingly. Based on the scores obtained, the proposed drugs may be considered for repurposing for any particular disease. DMAP's repurposing tool utilizes two approaches for drug repurposing predictions: Drug similarity search by Tanimoto Coefficient (calculated based on the common features in two sets of selected drugs) and Kolmogorov-Smirnov approach (non-parametric statistical test used to compare the sample and reference drugs). As a pilot phase of the study, this tool was used for breast cancer and retrieved Testosterone, Testolactone and Progesterone as potential therapeutic agents. Among the three, Progesterone was known to decrease the metastatic potential of breast cancer cells through dephoshorylation of kinases (STAT3, FAK, Akt and p70S6) and anti-metastatic potential was found to be independent of the Progesterone Receptor (PR) activation and dependent on Glucocorticid Receptor (GR) [76]. Progesterone has completed its phase 2 trials in combination with cyclophosphamide and methotrexate with PR negative Recurrent/Metastatic breast cancer patients (NCT00577122) [77].

2.2.2. Docking techniques

Docking techniques are used to study the drug and receptor interactions to predict the potentiality of a drug or a ligand using mathematical calculations. Numerous drugs can be evaluated against a set of targets in a very short span. There are several docking techniques available based on the types of datasets to be studied. Cross docking is one of the technique widely used to predict the interactions in large set of proteins like antigen-antibody interactions [78] [79]. Induced Fit Docking (IFD) permits the movement of side chains of binding site residues during the docking process by repetitively sampling the pose of the ligand [80].

Inverse virtual docking, a new principle of docking approach wherein set of ligands will be screened against a panel of target proteins. This applied strategy has potential to shed lights on unknown pharmacological actions of both known and unknown compounds. This technique is useful in identifying the cellular mechanisms of the compounds which were known to elicit potential activity against a particular disease with a dearth in target information.

2.2.2.1. Investigation of eccentric anti-cancer target for orphan compounds of sulfonamide class. Simone Di Micco *et.al*, designed orphan compounds belonging to trifluoromethyl benzene sulfonamide family to target Jumonji domain-containing protein 3 (JMJD3), a histone H3K27 demethylase (overexpressed in cancers). But the purpose was not met due to poor efficacy led to identify the potential targets of the compounds through Inverse Virtual Docking (IVD) technique by screening against a dataset of potential anticancer targets (Fig. 5). Some compounds were found to be potential against erb4, pdk1 and epha3 protein kinases with 90%, 60% and 50% frequency respectively. Among the synthesized compounds, the fluorophenylethyl group of five compounds were found to be super imposable with fluorophenylmethoxy group of co-crystallized ligand of erb4 with higher degree of inhibition (> 50%), IC_{50} being 4.41–8.05 μM. Among the five compounds, one potential compound exhibited significant cytotoxicity (9 μM IC_{50}) in MCF7 breast cancer cell line with the cell cycle arrest at G2/M phase. Other three compounds were found to significantly inhibit (~67%) the radiometric kinase activity associated with pdk1 [81].

2.2.2.2. Repurposing of a phytoconstituent of Salvia miltiorrhiza, a Chinese medicinal plant for leukemia. Shao-Jun Chen studied the molecular targets of Tanshinone IIA, an active constituent extracted and characterized from *Salvia miltiorrhiza* commonly called as Danshen (Chinese medicinal plant). The compound was reported to possess antitumor effects at different phases like inhibition of invasion, prevention of metastasis and activation of apoptotic pathway in colorectal, hepatocellular and breast cancer. Despite its multiple effects reported, direct targets and molecular mechanisms were yet to get elucidated to determine its anti-tumor effects. The molecular targets of the Tanishone IIA were identified using PharmMapper and these identified targets were further validated using docking studies by Autodock Vina. The repurposing studies were carried out using Drug Repositioning and Adverse drug Reaction via Chemical Protein Interactome (DRAR-CPI) servers. PharmMapper screened the possible targets and offered best fit scores of 4.315 and 3.736 in the favor of Retinoid Receptor Alpha

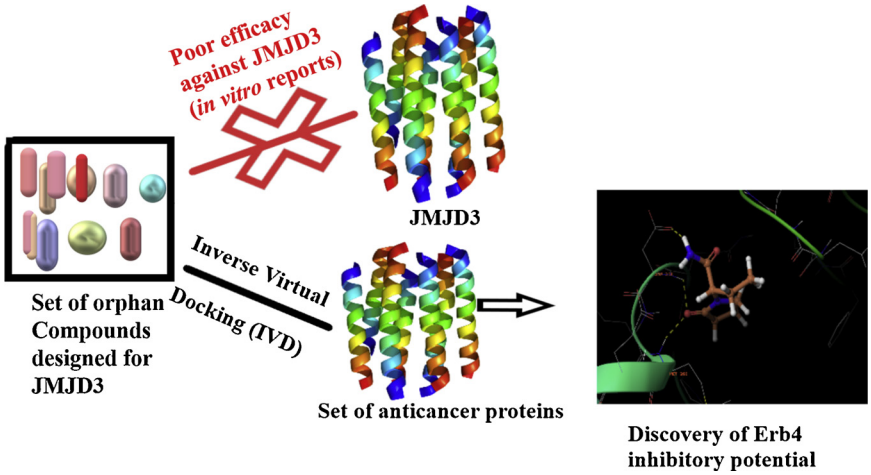

Fig. 5. IVD of orphan compounds of sulfonamide class.

(RARα) and Proto-oncogene tyrosine protein kinase Lck (Lymophocyte-specific kinase) respectively as molecular targets for Tanishone IIA in Acute Promeylocytic Leukemia (APL). Five hydrophobic and three H-acceptor interactions with RARα were responsible to elicit the anti-tumor action. Among the hydrophobic interactions with RARα, the common interacting residues were Phe238, Ser232, Phe302 for BMS614 (a co-crystallized ligand of RARα) and Tanishone IIA. The residue specific interacting property of Tanishone IIA with Ser232 revealed the isotype selectivity of RARα among other RAR's. Since the target RARα is associated with the development of leukemia, Tanishone IIA was considered for repurposing against leukemia and this proposal has successfully gone through *in silico* process [82]. The antileukemic potential of Tanishone IIA through cell line studies revealed the activation of Raf/ERK/P90RSK and AMPK/mTOR/p70S6K in human chronic myelogenous leukemia cells (KBM-5) [83]. *In vivo* studies of Tanishone IIA (100 mg/kg) in NOD/scid mice, showed significant level of apoptosis through extrinsic pathway mediated via caspase 8 and intrinsic pathway via Caspase 3 [84].

2.2.3. Molecular dynamics (MD)

Molecular dynamics is a simulation technique used to study the dynamics between the macromolecular structure and a ligand. Characterization of binding interactions, thermodynamic aspects, kinetics and dynamics for target protein-ligand complex by experimental data are very much essential for therapeutic validation [85]. Molecular dynamic simulations with enormous applications is an alternative approach to perform similar type of calculations computationally. This intensive tool should be a part of drug discovery to gain invaluable information about the conformational changes and energy settings which are not accessible by sophisticated experimental techniques.

2.2.3.1. Identification of potential candidates against wild type and mutant forms of BCR-ABL fusion protein. Hassan Aryapour *et.al*, applied the molecular dynamics approach to identify the potential drugs for repurposing against leukemia through inhibition of the wild type and mutated BCR-ABL fusion protein (T315I - responsible for kinase inhibitor resistance) that is involved in pathogenesis of Chronic Myeloid Leukemia (CML). Previously the kinase inhibitors were proven to offer promising therapeutic outcomes through inhibition of BCR-ABL target. The instances of delayed response to kinase inhibitors were linked with the development of resistance against the drug and T315I mutation was identified as the key factor involved. Based on the molecular weight, 1590 FDA compounds were shortlisted and were subjected to docking studies against both wild type and mutant forms of BCR-ABL target and rated based on Ledock docking score. The compounds within -10.5 to -13 were considered for the further validation with MD studies and the results were compared with Ponatinib, the reference standard. Root Mean Square Deviation (RMSD) and Root Mean Square Function (RMSF) values were used to validate the stability of proteins and flexibility of the complexes respectively. Based on the stability achieved after 30 ns of simulation, one of the test drugs, Cangrelor was excluded because of its detachment from the protein after simulation. In the wild type protein, Ponatinib and Paromomycin exhibited low flexibility, while the rest of the drugs were of desired flexibility in both the form of targets. Paromomycin was excluded due to low binding energy than the reference drug. Finally, Chlorhexidine (−729 kJ/mol) and Deferoxamine (-1095.6 kJ/mol) were observed with higher binding energies than the Ponatinib (-686.6 kJ/mol) in the wild type, whereas in case of mutant form, Chlorhexidine (-1686.5 kJ/mol) exhibited the highest binding energy than Ponatinib (-265 kJ/mol) [86]. The workflow is depicted in Fig. 6.

2.2.3.2. Retrieval of potential drugs against targets of Ebola virus using simulational studies. Lie Xie *et.al* [87], conducted drug repurposing studies to identify the potential drugs against Ebola targets. Lack of effective therapeutic agents for the Ebola virus and financial crisis in developing new drug mandated drug repurposing research. One of the potential targets identified in the virus was Ebola Viral Protein 24 (VP24) which crucially interacted with human Karyopherin alpha that disables immune system and eventually responsible for survival of the virus [88,89]. Another important one is Ribose Nucleic Acid (RNA) directed RNA polymerase that is involved in replication of the virus. Homology modelling and MD simulational techniques were employed

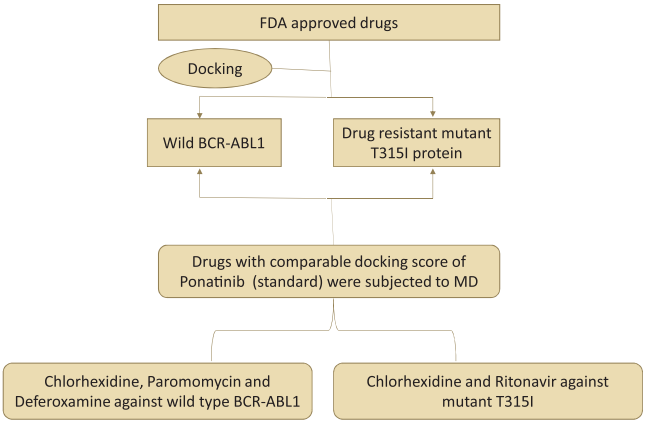

Fig. 6. Workflow to identify the potential inhibitors of wild type and mutant forms of Bcr-Abl.

to explore flexibility and druggable sites for the targets. Based on binding site similarities, targets were analyzed using SMAP software and the sites possessing co-crystallized compounds with p-value < 0.05 were selected for further analysis. SMAP and MD simulations revealed a small binding site at the Protein-Protein Interaction (PPI) interface of VP24 and human Karyopherin alpha complex which showed significant similarity with the binding site of HIV protease. 1766 FDA approved drugs from ZINC database and 259 additional drugs from DrugBank database were selected for the docking studies using 4 docking tools: Surflex, Autodock Vina, Autodock and Protein Ligand ANT System (PLANTS). Post-docking analysis shortlisted 20 potent inhibitors of VP24, among which Indinavir, a protease inhibitor exhibited binding affinity towards VP24 with 3 hydrogen bonds and 11 hydrophobic interactions. Interactions of Indinavir with Gln94 of VP24 in the binding site and with Ala28, Asp29, Asp25 (Chain A) and Asp25 (Chain B) of HIV protease are crucial for anti-retroviral ability against Ebola. The other identified 19 inhibitors also interacted with Gln94 amino acid in the binding site of VP24 similar to Indinavir. Montelukast (Leukotrine receptor antagonist), Iloprost (Prostacyclin receptor antagonist), hSalmeterol Xinafoate (beta-2 adrenergic receptor agonist), Travoprost and Latanoprost (Prostagladin F2 receptor antagonists) were the compounds other than Indinavir with the significant scores. Among the 4 domains in the RNA directed RNA polymerase of the virus, the structure of O'-2-Methyl Tranferase (MTase) domain was predicted due to the non-availability of confirmed structure [90]. The protein was modelled by PROCHECK revealed that 80% of druggable sites fall in the allowed regions of Ramchandran Plot. Binding site similarity studies revealed that MTase domain of RNA polymerase of Ebola virus was superimposable with the human CAP specific mRNA 2′ O-MTase domain. Sinefungin, an anti-fungal agent was found to exhibit significant docking score when compared with aza-S-adenosyl L-methionine (SAM: known binding ligand of the target). Other drugs reported to have significant interactions were Maraviroc (Chemokine receptor antagonist), Abacavir (Reverse transcriptase inhibitor), Telbivudine (nucleoside analog against Hepatitis B) and Cidofovir (anti-viral).

2.3. Drug based strategies

Drug based strategies are gaining importance due to huge availability of the computational approaches developed which are useful in exploring the hidden mechanisms of drugs. Development of techniques like pharmacophore modelling, drug-drug similarities and side effects based approaches led to identify the drug signatures on the several targets. Some drug based strategies are described below.

2.3.1. Pharmacophore modelling

A pharmacophore denotes the significant interactional features of a ligand responsible for agonistic or antagonistic biological response. 3D-Pharmacophore models label the 3D geometry of the features of bioactive compound. Thus constructed models can be screened for bioactive molecules virtually to support the lead identification [91].

2.3.1.1. Drug repurposing project on selective Glycogen Synthase Kinase-3 (GSK-3) inhibitors. Liliana Pacureanu *et.al* [92], developed a pharmacophore model to identify the potential Glycogen Synthase Kinase-3 (GSK-3) inhibitors. GSK-3 belongs to the family of serine/threonine kinases, involved in multiple physiological (purine metabolism, cell cycle and insulin signalling) and pathological signalling pathways (pancreatic cancer [93], AD [94] and diabetes [95]). Potentiality of GSK-3 signalling in cellular mechanisms mandates the search for GSK-3 inhibitors. Literature mining revealed the potential of Maleimides to possess GSK-3 inhibitor activity at picomolar and nanomolar concentrations [96]. Among the 203 identified maleimides, 10 compounds with least nanomolar range inhibitory activity were used to build the pharmacophore model. Validation set from ChemBL and Binding DB drug repositories was designed based on selectivity ratio (SR) against CDK-2, CDK-4 and GSK-3, and categorized 149 drugs as selectives and 88 as non-selectives. Around 2106 compounds were chosen from PubChem assays as decoy set (inactive set). Pharmacophore model was constructed using PHASE of Schrodinger using 10 most active ($pIC_{50} > 9$) and 16 inactive ($pIC_{50} < 6$) drugs. The best alignment was observed with RMSD of 1.2 Å. Few hypotheses were created based on 5–7 point features, among them 5 point feature (Hypothesis-1) was considered to be significant which mandates the requirement of 1 H-acceptor, 1 H-donor, 1 hydrophobic interaction and 2 aromatic rings to arrive at best survival score. Interactions with Asp133 and Val135 were found to impart affinity for GSK3. Atom based Quantitative Structural Activity Relationship (QSAR) was performed using 143 selectives and 60 non-selective compounds as training set. Among the 355 five-point pharmacophore hypotheses, 238 validated hypotheses were generated by Atom based 3D QSAR and further analysis revealed a total of 180 confirmed hypotheses. Virtual screening yielded 1510 approved drugs, among them 30 drugs were found to be aligned with the Hypotheis-1. The nucleoside analogues, Fudarabine, Nelarabine, Clofarabine and Cladribine were found to be the best hit with Hypothesis-1 features and were proposed to be repositioned for GSK-3 inhibitory activity.

2.3.1.2. Development of pharmacophore model to screen the potential drugs for African sleeping sickness. Recently, D Swati and Manyank Rashmi designed a point feature pharmacophore model to screen the potential drugs against GlcNAc-PI de-*N*-acetylase (enzyme required for protozoan for anchoring on the host plasma membrane) in *Trypanosoma brucei gambiense*, a protozoan causing African sleeping sickness in humans. This enzyme was found commonly in *Trypanosoma* and *Leishmania* species. The target sequence was retrieved from Therapeutic Target Database (TTD) and Blast Local Alignment Search Tool for protein (BLASTp) search revealed the non-availability of significant homologues. Glycosyl Phosphotidyl Inositol (GPI) domain (useful in anchoring) of the enzyme found to contain 126 amino acid residues with 20 transmembrane residues. The protein model was built up by Iterative Threading ASSembly Refinement (I-TASSER) (Fig. 7) modelling tool and further refined and analyzed by the Ramachandran Plot. Structural alignment of the protein was done by PyMol and active site was analyzed by four tools and arrived at one common active site with 13 residues. As the target lacks natural ligand, the ligand was modelled by PRODRG server and docked using Autodock Vina to identify the significant interactions. Based on these interactions the pharmcophore model was constructed with five point features. Ligandscout algorithm revealed 2 H-acceptors and 4 H-donors at binding site. Structure based virtual screening yielded 220 compounds and 23 previously reported inhibitors with best pharmacophore fitness score. Molecular docking further refined the process and shortlisted 10 drugs with negative binding energies, among them Ethambutol and Metaraminol showed the best interactions similar to the known inhibitors and are proposed for the treatment of African Sleeping Sickness [97].

2.3.2. Repurposing based on the clinical side effects

Clinical side effects are provided by the web based tools SIDER [26] and clinicaltrials.gov [27]. The approach is based on the two assumptions: drugs with similar side effects exerts similar mechanisms and drugs reported to have same side effect might be acting through same receptor which may be target for new indication.

2.3.2.1. Retrieval of potential anticancer drugs. Recently, Su and Sanger [98] utilized the 12E (Literature mining tool for the specific adverse effect of the drugs) and PolyAnalyst (analyses the data and prioritize the drugs based on Odd Ratios and z-score) tools to analyze the data obtained by mining the clinicaltrials.gov for cancer as Serious Adverse Events (SAE) negotiating the existing anti-cancer drugs and those in the

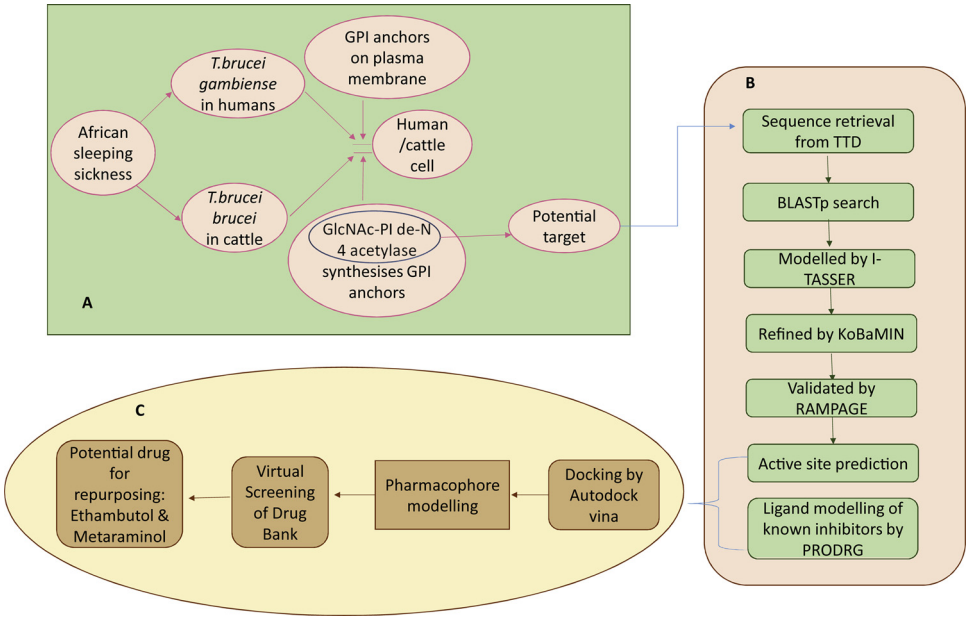

Fig. 7. Workflow employed in identification of potential drug for repurposing for African sleeping sickness. A: Background work for the selection of target through literature. B and C: *In silico* process employed in the modelling of target and virtual screening of drugs respectively.

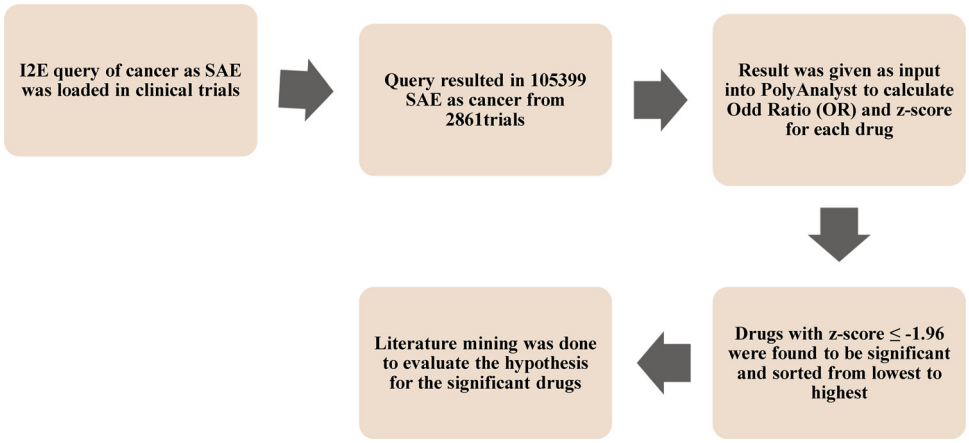

Fig. 8. Flow chart depicting the methodology for identifying the drugs for cancer using data mining from clinical trials.

clinical trials (Fig. 8). The results revealed that Telmisartan (angiotensin II receptor antagonist) might be effective against colon cancer through activation of Peroxisome Proliferator activated receptor-γ (PPAR-γ). Cell line studies of Telmisartan in human colon cancer cell lines (HT-29, SW-480 and SW-620) exhibited the dose-dependent reduction in cell viability and induced apoptosis via caspase 3 activation [99]. Phylloquinone (vitamin-K dietary supplement) was reported to lower the risk of colon cancer with increased dietary intake. Further validation through human adenocarcinoma cell lines (Caco-2, HT-29 and SW-480), with Phylloquinone led to increase in the Bax/Bcl-2 ratio suggesting the induction of apoptosis. The induction of apoptosis was found to be caspase independent and dependent on activation of MAPK/ERK pathway at high concentrations (100 μM). V501 vaccine was reported to have less risk for cervical dysplasia, an initial stage for cervical cancer [100].

The role of renin in activating the notch signaling (through notch1 and KRT6B-keratin 6) in cancer cell proliferation [101] was explored through literature mining. Aliskiren being a renin inhibitor, apart from its anti-hypertensive potential, was observed to inhibit the cell proliferation in renal carcinoma cell lines (786-O and ACHN) *in vitro*.

2.3.2.2. Recognition of new drug-target pairs based on similarities in side effects. Monica Campillos *et.al* [102], predicted the new drug-target pairs based on the side effect similarity for chemically dissimilar compounds. This study was based on the assumption that drugs with identical protein binding profiles tend to correlate with similar off-target effects. In the study, a reference set of 502 compounds were collected from the different drug databases with 4857 known human targets based on the correlation between side effect similarity and sharing of similar targets. Based on the similarity calculations using Tanimoto index of the obtained reference set, only 35 pairs were found to possess both side effect similarity (198 pairs) and chemical similarity (301 pairs). Among 746 marketed drugs for human and non-human targets with established side effect profiles, 946 drug pairs were observed to share the targets. Of these, the probability of 424 drug pairs to share the targets was 25% and 261 of 424 pairs were found to be structurally dissimilar. Further analysis revealed that Rabeprazole had properties of sharing targets between Peroglide (Parkinson's disease), Paroxetine (Obssesive Compulsive Disorder), Fluoxetine (Depression) and Zolmitriptan (Migraine). Further *in vitro* analysis revealed that Rabeprazole was found to inhibit dopamine receptor (DPD3) and possess high affinity towards seretonergic receptor

(HTR1D).

2.4. Literature based strategies

Literature mining is one of the platforms to retrieve potential data that enriches the researchers to establish hypotheses on repurposing. Numerous hidden indirect relations between drug-target-disease can be extracted through extensive scientific literature. Literature mining is carried out through Medical Subject Heading (MeSH) terms to retrieve relevant information [103] [104].

2.4.1. Design of MeSHDD, a drug-drug similarity database based on MeSH terms

Chirag and Adam 2017 [105], critically analyzed the MeSH terms enriched similarity between the FDA approved drugs and disease and developed a database, MeSHDD, through literature mining. In this process, MeSH terms (234,030,670) and drug terms (5,223,226) were identified from the MEDLINE repository and FDA approved drugs (2142) were chosen from drug bank. The above elements were mapped and the data were narrowed to a total of 81,474,709 MeSH-Drug pairs. Analysis of these pairs through hypergeometric Boniferroni enrichment test confined the research with 251,594 drug-MeSH term pairs based on their significance. The distance between the MeSH-drug pairs was calculated in terms of binary digits i.e, drugs with high similarity is close to 0 and dissimilar ones are near to 1. Based on the distance, 33 drug-drug with shared indications were clustered and matched for 482 diseases. This novel approach bestowed the path to identify the drug Metformin, an anti-diabetic, in the cluster enriched for Cystic Fibrosis (CF) due to its potentiality to activate Adenosine Mono Phosphate Kinase (AMPK), that is deregulated in CF.

2.4.2. Development of ABC model, an indirect drug repurposing approach

Jung-Hsien Chiang *et.al* 2016 [106], explored an indirect drug repurposing pathway based on literature from MEDLINE database by developing ABC model in which the relation between A (disease) to B (gene) and B (gene) to C (drug) is investigated to derive an indirect relation between A (disease) to C (drug). The research team, used TTD to study the association between 2723 diseases with 3188 targets, after which they extracted 20,043 drugs from TTD and filtered them through MEDLINE data. Another approach to derive the disease-drug relationship was through Apache Lucene, a search engine which was used to collate 5.3 million diseases, 7.1 million genes and 5.5 million drug related information. Finally, 5.4 million disease-gene-drug related documents were retained to retrieve the data for disease-drug relationship. The correlation from filtered data was analyzed in three different approaches: (1) Named Entity Recognition (NER) approach to retrieve the information based on the terms: disease, gene and drug (2) Dependency tree parsing method to identify significant information based on presence of paired entities among disease, gene and drug (3) Trigger word learning technique based on the root part of the sentence (like sensitive, agonist and antagonist) from the data obtained from Comparative Toxicogenomics Database (CTD). Among the 94,513 documents in CTD, 11,000 trigger words were shortlisted based on this approach. This approach led to the retrieval of 1,812,775 indirect disease-drug relationships from 114,381 direct disease–gene relationships and 176,219 direct gene-drug relationships. The obtained data were further processed by two methodologies for drug repurposing predictions: (1) Drug vector space which calculates the similarity between two drugs based on their effect on genes. If two drugs act on the similar genes, this implies the similarity between the mechanisms of the drugs (2) Drug target similarity ranking based on the Jaccard index based on their similarities. Thus, indirect drug-disease relationship was derived, based on which, Celecoxib was predicted to be repurposed for ovarian and breast cancer, Raloxifene for prostate cancer, Erlotinib for colorectal cancer and Rapamycin for leukemia.

3. Challenges in repurposing process

- Optimization of inclusion and exclusion criteria in selection of target population: Selection of the treatment groups is the main task to evaluate the expected outcome of the drug. Incorrect subject selection leads to disaster effects of the drugs instead of expected ones. For example, Thalidomide when prescribed for pregnant women in first trimester for managing morning sickness resulted in amelia and phocomelia. Exclusion of this special population criteria in selecting the subjects led to discovery of its new application against multiple myeloma [107].
- Achievement within timeline: Repurposing of old drug for new indication involves lot of considerations like dosing regimen and route of administration for achieving the significant benefit for the novel indication. Optimizing the formulation without destabilizing the drug is a bottleneck [108].
- Repurposing of drug aims different group of patients with different set of physiological conditions, therefore unexpected adverse events may be anticipated, mandating careful investigation of every response.
- Repurposing drug combinations demand prerequisite data on drug-drug interactions, pharmacodynamics and pharmacokinetics of the

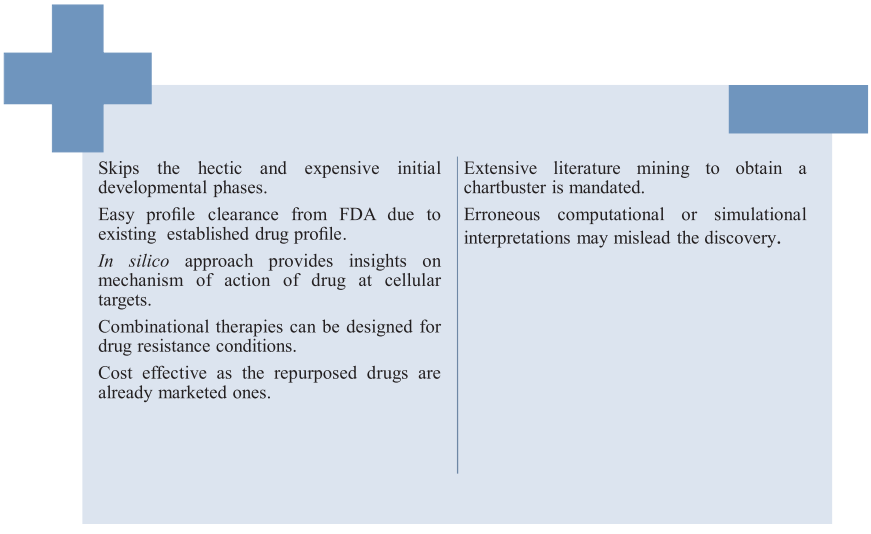

Fig. 9. Pros and Cons [109] [110].

Table 2
Current status of repurposed drugs in clinical trials.

Drug	Mechanism of action	FDA indication	Repurposed indication	Clinical Trials
Malaria				
Rosiglitazone	Agonist of Peroxisome Proliferator Activated Receptor gamma (PPARγ)	Anti-diabetic	Malaria as an adjunct therapy	Initial Phases (NCT02694874) [116]
Fosidomycin & Clindamycin	Clindamycin- inhibits bacterial protein synthesis	Broad spectrum antibiotic	Malaria in children	Completed Phase 2 (NCT01464138) [117]
Paracetamol	COX inhibitor, Inhibitor of heme protein catalyzed lipid peroxidation	Analgesic, Anti-pyretic	AKI in *Plasmodium knowlesi* Malaria	Phase 3 (NCT03056391) [118]
Imatinib	Non-specific Protein Tyrosine Kinase Inhibitor	Leukemia	Uncomplicated malaria	Phase 1 (NCT02614404) [119]
Chloroproguanil, Dapsone and Artesunate	Dapsone is Dihydro Folate Reductase (DHFR) inhibitor	Leprosy	Uncomplicated malaria	Completed Phase 3 (NCT00371735) [120]
Tinidazole	Disruption of Ferredoxin mediated electron transport system	Trichomoniasis	Radial cure of *Plasmodium vivax*	Completed Phase 2 (NCT00811096) [121]
Tuberculosis				
Linezolid	Inhibits bacterial protein synthesis	Antibiotic for Vancomycin resistant strain	Multi Drug Resistant Tuberculosis (MDR-TB)	Recruiting Phase (NCT02778828) [122]
Ibuprofen	Non selective inhibitor of COX	Juvenile rheumatoid arthritis, osteoarthritis	Extensive Drug Resistant Tuberculosis (XDR-TB)	Phase 2 (NCT02781909) [123]
Azithromycin	Inhibitor of bacterial protein synthesis	Broad spectrum antibiotic	Pulmonary Tuberculosis	Phase 2 (NCT03160638) [124]
Doxycycline	Inhibitor of bacterial protein synthesis	Respiratory tract infection	Pulmonary tuberculosis	Completed Phase 2 (NCT02774993) [125]
Moxifloxacin in combination with Bedaquiline and Pyrazinamide	Moxifloxacin inhibits DNA gyrase	Respiratory infections and conjunctivitis	MDR-TB	Phase 2 (NCT02193776) [126]
Metranidazole	Inhibits bacterial nucleic acid synthesis	Amoebic infections	Pulmonary tuberculosis	Phase 2 (NCT00425113) [127]
Prednisolone as adjunctive therapy	Inhibits Phospholipase A2	Anti-inflammatory and Immunosuppressant	Tuberculosis Pericarditis	Phase 3 (NCT00810849) [128]
Cancer				
Oxycodone	Agonist of μ type opoid receptor	Diarrhoea, severe pain	Pain management in cancer	Completed Phase-3 (NCT01675622) [129]
Enzalutamide	Competitive androgen receptor inhibitor	Prostate cancer	In combination with Fulvestrant in Advanced Breast Cancer	Phase 2 (NCT02953860) [130]
Cetuximab + Bevacizumab	Cetiximab binds to Epidermal Growth Factor Receptor (EGFR) and Bevacizumab binds to Vascular Endothelial Growth Factor (VEGF)	Colorectal cancer	Head and Neck Cancer	Completed Phase 2 (NCT00703976) [131]
Itraconazole	Inhibits 14 α-demethylase	Anti-fungal	Prostate Cancer	Completed Phase 2 (NCT00887458) [10]
Nelfinavir	Protease inhibitors	Anti-viral	Kaposi Sarcoma	Phase 2 (NCT03077451) [132]
Disulfiram with copper	Irreversable inhibitor of aldehyde dehyrogenase	Management of alcoholism	Metastatic Breast Cancer	Phase 2 (NCT03323346) [133]
Digoxin	Inhibtis Na^+-K^+ ATPase pump	Congestive Heart Failure (CHF)	Prostate Cancer	Completed Phase 2 (NCT01162135) [134]
Diabetes				
Dextromethrophan	Antagonist of NMDA glutamergic receptor	Dry Cough	Type 2 Diabetes Mellitus (T2DM)	Completed Phase 2 (NCT01936025) [135]
Ranolazine	P-Glycoprotein inhibitor	Anti-anginal	T2DM	Completed Phase 3 (NCT01472185) [136]
Rilonacept	Prevents the activation of Interleukin 1 (IL-1) receptors by binding to IL-1	Cryopirin associated periodic syndrome	Type 1 Diabetes Mellitus (T1DM)	Completed Phase 1 (NCT00962026) [137]
Neuropsychiatric Disorders				
Acetritin	Acts on retinoid receptors (RXR and RAR)	Severe Psoriasis	AD	Completed Phase 2 (NCT01078168) [138]
Bexarotene	Activates RXR receptors	T-cell lymphoma	AD	Completed Phase 2 (NCT01782742) [139]
Riluzole	Inhibition of glutamate release Inactivation of voltage dependent sodium channels	ALS	Mild AD	Phase 2 (NCT01703117) [140]

(*continued on next page*)

Table 2 (*continued*)

Drug	Mechanism of action	FDA indication	Repurposed indication	Clinical Trials
Rilapladip	Inhibitor of Lipoprotein associated Phospholipase A2 (PLA2) inhibitor	Atherosclerosis	AD	Phase 2 (NCT01428453) [141]
Carvedilol	Beta Adrenergic Blockers	Mild to moderate heart failure	AD	Completed Phase 4 (NCT01354444) [142]
Isradipine	Inhibits the L-type calcium channel	Hypertension	PD	Phase 3 (NCT02168842) [143]
Nilotinib	Tyrosine kinase inhibitor	CML	PD	Phase 2 (NCT03205488) [144]
Pioglitazone	Agonist of PPAR-γ	T2DM	PD	Phase 2 (NCT01280123) [145]
Minocycline	Inhibitor of bacterial protein synthesis	Upper respiratory tract infections	HD	Phase 3 (NCT00277355) [146]
Fenofibrate	Agonist of PPAR-α	Anti-Hyperlipidemia	HD	Phase 2 (NCT03515213) [147]
Diphenhydramine	Competitive inhibitor of histamine receptors	Anti-emetic	Migraine	Completed Phase 4 (NCT01825941) [148]
Propranolol and Venlafaxine	Non selective beta blocker	CVS diseases	Vestibular migraine	Completed Phase 4 (NCT02350985) [149]
Mifepristone	Competitive inhibitor of progesterone receptor	Termination of pregnancy	Bipolar Depression	Completed Phase 2 (NCT00043654) [150]
Ofatumumab	Cell mediated cytotoxicity	CML	Relapsing remitting Multiple sclerosis	Completed Phase 2 (NCT00640328) [151]
Others				
Isotretinoin	Decreases Sebum production	Recalcitrant Nodular acne	Relapsed/ Refractory neuroblastoma	Completed Phase 2 (NCT01334515) [152]
Propronalol	Beta adrenergic blocker	CVS diseases	Age related osteoporosis	Early Phase 1 (NCT02467400) [153]
Lansaprozole	Proton pump inhibitor	Anti-ulcer	Chronic idiopathic thrombocytopenic purpura	Completed Phase 4 (NCT00467571) [154]
Eflornithine	Suicidal inhibitor of ornithine decarboxylase	Facial hirsutism	African Trypanasomiasis	Phase 4 (NCT00906880) [155]

drugs with special emphasis on toxicity profile.

The pros and cons are illustrated in Fig. 9.

4. Current scenario

Owing to massive failures in unearthing novel chemical entities and lack of financial support for the traditional drug discovery and development pipeline, pharma industries diverted their focus towards drug repositioning. With advancement in the technologies and computational methods, screening of drugs for off-label indication has become much easier. Several organizations like NCATS, MRC-AstraZeneca, World Health Organization (WHO) and other public, private sectors are sponsoring conceptual proposals on drug repurposing with huge expectations towards solutions for unanswered issues in existing medical conditions and therapy failures [111]. The drugs under different phases of drug repurposing clinical trials are listed in Table 2.

The concept of drug repurposing as alternative for drug discovery brought a number of databases into lime light for a smart way out. These databases can be categorized as resources for drug information (Drugbank, Pubchem), 3D structures of targets (PDB), Adverse effects (SIDER) and clinical trials information (clinicaltrials.gov). Development of CMap, led to discovery of the drugs which can mimic/reverse the genomic signatures providing an opportunity for repurposing the drugs with common actions on genetic products (RNA, DNA and Proteins). Using this web tool, the conserved signatures of a drug can be retrieved. Repurposing studies are carried out with this technology for the diseases like cancer and TB which are highly prone to resistance development. With the development of DMAP, a drug's response on respective genes and its subsequent relation with the protein could be retrieved. Using this approach many drugs like Paclitaxel, Testosterone, Estradiol and Doxorubicin were repositioned for breast cancer by mathematical approaches (Tanimoto Coefficient and Kolmogorov-Smirnov approaches). Using gene expression signatures of the disease and drug, the possibility of drug identification for the targeted therapeutic response has become handy.

By integrating the data in GWAS and PheWAS, one can correlate the phenotypic and genotypic expressions of a drug. GWAS technology ensures its applicability in identifying the gene response towards the drugs of interest which renders advances in research enhancing development of bio-drugs like antibodies and protein therapeutics. Drugs developed in this mode were Statins which inhibit HMG CoA reductase (3-hydroxy-3-methyl glutaryl CoA reductase) for hypercholestoremia [112]. PheWAS technology uses International Classification of Disease (ICD) codes for a disease to investigate if any SNP change is involved in the disease pathology and predicts phenotype based alterations. Implication of both of the above mentioned approaches for a specific disease condition can reveal the relationships between drug-disease-gene and trace out the underlying cross talk mechanisms [113].

Application of Next Generation Sequencing (NGS) simplifies the process of tracing out the defective mechanisms involved in the diversion of normal physiological process to the development of disease phenotype, in addition to designing of miRNAs. Construction of miRNAs for genetic disorders is a promising solution in diseases like cystic fibrosis [114]. TPMS developed by Anaxomics provide biological map for any disease by exploring multiple targets [53].

Recently the notion of repositioning also gained the focus in herbal products. Similar to antifungals like Amphotericin B and Miltefosine being repurposed against leishmaniasis, the essential antifungal oils also exhibited anti-leishmanisis activity in balb/c mice. The essential oils from *Otacanthus azureus* and *Protium heptaphyllum* known to possess strong antifungal activity also exhibited leishmanicidal properties. Further investigation in the composition of the above oils lead to identification of synergistic leishmanicidal potential of α-limonene and α-pinene. Mechanistic studies demonstrated the presence of ergosterol as a common target in *Leishmania* species and fungi which is the basis for switching of drug between the categories. Based on the above studies, futuristic approaches towards repurposing ayurvedic drugs is anticipated to fetch promising therapeutic outcomes [115].

5. Conclusion

In spite of advanced medical research, there are numerous diseases with unmet therapeutic options around globe to challenge the researchers in finding suitable therapeutic alternatives. In this regard, the concept of repurposing a drug offers a wide scope to research the hidden potential behind the drug and recycle the same. Although the researchers are fortunate enough in this era to be equipped with such computational tools, still there exists certain gap to be filled to troubleshoot the post-prediction shortcomings in optimizing the dosing regimen, formulation and selection of target population etc. Despite of the hiccups during the process, the importance of the drug repurposing using *in silico* approaches has gained importance in the drug discovery field in present pharma industries.

Conflicts of interest

There is no conflict of interest.

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