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NMSDR: Drug Repurposing Approach Based on Transcriptome Data and Network Module Similarity

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Abstract: Computational drug repurposing aims to discover new treatment regimens by analyzing approved drugs on the market. This study proposes previously approved compounds that can change the expression profile of disease-causing proteins by developing a network theory-based drug repurposing approach. The novelty of the proposed approach is an exploration of module similarity between a disease-causing network and a compound-specific interaction network; thus, such an association leads to more realistic modeling of molecular cell responses at a system biology level. The overlap of the disease network and each compound-specific network is calculated based on a shortest-path similarity of networks by accounting for all protein pairs between networks. A higher similarity score indicates a significant potential of a compound. The approach was validated for breast and lung cancers. When all compounds are sorted by their normalized-similarity scores, 36 and 16 drugs are proposed as new candidates for breast and lung cancer treatment, respectively. A literature survey on candidate compounds revealed that some of our predictions have been clinically investigated in phase II/III trials for the treatment of two cancer types. As a summary, the proposed approach has provided promising initial results by modeling biochemical cell responses in a network-level data representation.

Keywords: drug repurposing, functional interaction network, module similarity, breast cancer, lung cancer

1 Introduction

Finding new and accomplished drug designs for cancer treatment is an important issue all over the world. Drug development is a costly, time-consuming, and risky investment. However, with the development of computational methods, such as drug repositioning (DR), the investigation of existing drugs as new cancer therapy candidates can be implemented in a shorter time and at a low cost.

A DR method is based on four steps: i) compound identification, ii) compound acquisition, iii) development, and iv) Food and Drug Administration (FDA) post-market safety monitoring. Therefore, it reduces development time and cost.

One of the significant advantages of DR is discovering new anti-cancer drugs from existing drugs. There are many clinical trials for DR studies and some of them have achieved success previously. Duloxetine, blocking reuptake of serotonin and norepinephrine, was used in depression therapy at the end of the '80s. Researchers observed that duloxetine increased urethral retraction.[1] Pre-clinical studies demonstrated that it could be used in stress urinary incontinence (SUI) treatment. Nowadays, it is used in both depression and SUI treatment. Sunitinib inhibits multiple receptor tyrosine kinases (RTKs), it is a small molecule and FDA approved for gastrointestinal stromal tumors, advanced renal cell carcinoma and progressive well-differentiated pancreatic neuroendocrine tumors treatment. Sunitinib efficiency was approved in a bleomycin-induced mouse model for treatment for idiopathic pulmonary fibrosis (IPF). In the same study, dabrafenib, a FDA approved drug for unresectable or metastatic melanoma treatments, is also proposed for IPF treatment.[2,3] Ibrutinib was primarily developed as Bruton's tyrosine kinase (BTK) inhibitor, also targeting the inducible tyrosine kinase (ITK) and the epithelial growth factor receptor (EGFR). Another study showed that ibrutinib treatment reduces breast tumor progression and tumor weight.[4] Phenoxybenzamine, approved for reducing blood pressure, was repositioned as an analgesic and antinociceptive by an in-silico method based on reversing of the disease and gene expression profiles.[5] The other in-silico study detected that sulconazole, which is a topical antifungal, and vinburnine, which is a vasodilator, as cancer therapeutic agents that inhibit the cell cycle.[6]

Computational DR approaches get more attention with the advent of machine learning methods. A deep learning approach suggested pimozide, which was used as an anti-dyskinesia agent, to be a new candidate drug for treatment of non-small cell lung cancer (NSCLC).[7] Quinacrine (QA) was approved by the FDA for anti-malarial drug. Another study demonstrated that QA is a therapeutic candidate for NSCLC.[8] Binimetinib (known as Mektovi or ARRY-162) was approved by the FDA for

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treatment of patients with unresectable or metastatic BRAF V600E/V600K mutation-positive melanoma.[9] Mirdametinib was approved for treating type 1 neurofibromatosis (NF1) and solid tumors. Selumetinib, trametinib, AZD-8330, CI-1040, and RO4987655 are also MEK inhibitors like mirdametinib; results showed that mentioned drugs can be therapeutic candidates to improve treatment efficacy for Cholangiocarcinoma (CCA) patients.[10]

There are different technologies to measure the effect of drug treatment on living organisms. Gene expression represents mRNA expression changes of genes after applying a perturbation (e.g., drug treatment) to a cell. Several studies used differential gene expressions as fundamental data to prioritize potential drug targets.[11,12,13,14] In another study, transcriptome profiles of disease-related genes, target proteins of drugs, and drug treated gene expression profiles of cells are used in the training of a logistic regression-based classification model. With this method, 14 repositioned candidate drugs were determined, which have the highest score for three different cancer types (glioblastoma, lung, and breast cancer). The most remarkable result is that the highest accuracy of classification was achieved by using gene expression profiles of drugs, which simply measure the effect of drugs at the transcriptome level.[15]

1.1 Network-Based Drug Repositioning in Cancer

Biological network analysis is a promising approach for DR. In recent years, the frequently used data-driven DR approaches are based on network modeling.[16] A biological network system considers molecular components as nodes (e.g., genes, proteins, metabolites, physical interactions, drugs), and their relationships or interactions (e.g., metabolic coupling, transcriptional activation, functional and direct physical interactions) as edges.[17]

There are several studies that perform DR using network-based methods. A recent study collected 10005 subject-specific samples (both cancer and control) and 26 tissue-specific models from the TCGA database. Then these data were reconstructed by rFASTCORMICS on colorectal cancer (CRC) samples and three drugs (ketoconazole, naftifine, mimosine) were identified as candidate drugs for CRC treatment which are not approved by FDA.[18]

Another network-based drug repositioning called MNBDR based on a module network to screen drugs. To predict drug candidates, it is integrated with a protein-protein interaction (PPI) network and gene expression profiles of humans. Expression data was downloaded from the LINCS database together with the z-score of genes. Five cell lines (PC3, A375, HALE, MCF7 and HT29) were used. Three candidate drugs (romidepsin, colchicine, ciclopirox) were proposed for breast cancer treatment.[19]

PharmOmics tool uses tissue- and species-specific transcriptome data to develop a gene- and network-based drug repositioning method.[20] They performed several analyses on known drugs of various diseases to show performance of their drug repositioning tool.

A gene network was used for drug repositioning applied for colorectal cancer.[21] Data was obtained from multiple databases (STRING, DrugBank, Therapeutic Target Database, ClinicalTrials.gov) for integrative analyses of a genetically-driven DR for CRC. The Connectivity Map database was used to prioritize the most promising repurposed candidates. As a result, 18 drugs - showed high potentials for CRC treatment.

1.2. Proposed Network-Based Drug Repositioning

Our DR approach has experimented on two cancer types: breast cancer and non-small cell lung cancer. Breast cancer has the highest incidence and the second-highest mortality rates in the world. Treatments can be designed based on the mutated genes of a patient with breast cancer. Lung cancer ranks at the first place in cancer-related deaths in both women and men. In suitable patients, tumor tissue can be removed by surgical methods. For other patients, chemotherapy can also be applied. Breast and lung cancers were chosen in this study due to their high indices and mortality rates.

This study aims to identify drug candidates for the treatment of breast and lung cancers by proposing a network-based drug repositioning approach. The novelty of our approach is calculating a module similarity between a disease-causing network and a compound-specific interaction network by incorporating transcriptome level gene responses. Thus, such an association leads to more realistic modeling of molecular responses of compounds in living cells. The drugs proposed as new treatment candidates were predicted by choosing out of the top-normalized module similarity scores. We also provided in-silico validations of our predictions by collecting clinical trials running on the predicted drugs and checking findings of competing studies. The support in literature and clinical trials expose potentials of the proposed drug repositioning model that presents new treatment candidates by more effectively modeling biochemical cell responses.

2 Materials and Methods

The proposed drug repositioning method computes a module similarity between a disease-causing network and a compound-specific PPI network. These networks are built on a functional interaction network (Figure 1). The module similarity of two networks (i.e., DCGN and DAGN) is represented by taking average shortest-path distances of each pair of nodes across two networks. As a novel contribution, the mRNA expression (fold change) measurement of each gene is included into the similarity calculation to incorporate biochemical reactions observed at transcriptome level.

2.1 Data Analysis

We use a functional protein-protein interaction network called Functional Linkage Network (FLN) to represent the main topology of the biological network model. In the FLN network, nodes represent human proteins, and edges that connect two proteins (nodes) represent functional similarity values of two proteins. FLN was downloaded from http://visant.bu.edu/misi/fln/.[22] FLN has 20790 unique proteins and 21952150 edges (interactions). Edge weights give functional similarities and take a value between 0 and 1. When analyzing the distribution of edge weights, most of them

accumulated between 0 and 0.1. So, we removed the edges whose weight is lower than 0.1 due to representing very low functional similarity. The final FLN contains 15002 proteins and 334255 interactions.

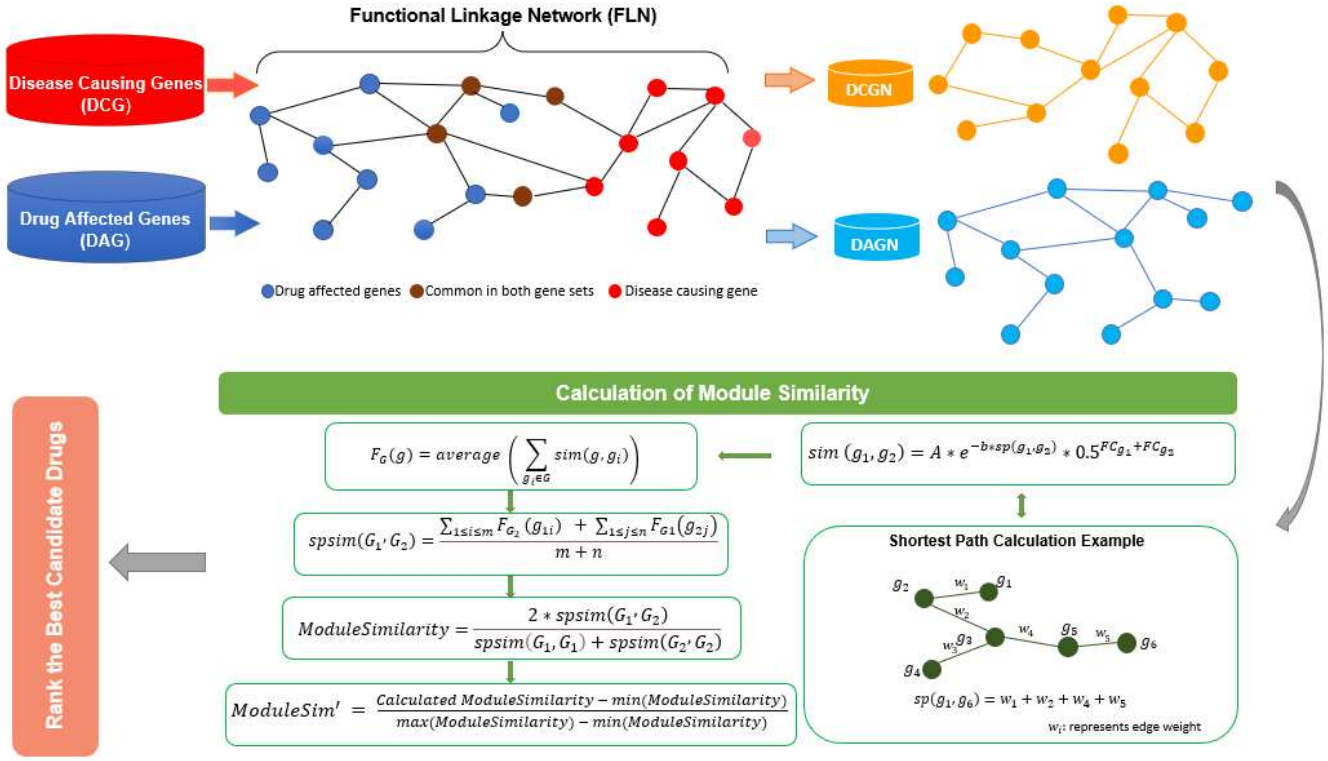

Figure 1: Workflow or proposed method.

For breast and non-small cell lung cancer (NSCLC) cell lines, we used the level-5 transcriptome profiles of each compound from the LINCS L1000 project. [23] The z-score of raw data represents the standardized mRNA expression values. Selected drug experiments have 24-hours applications on breast (MCF7) and lung cancer cell lines (A549). For each drug, there are six doses (0.04, 0.12, 0.37, 1.11, 3.33 and 10 μm). We decided to filter these concentrations based on their known IC_{50} doses on the specified cell lines. The IC_{50} value shows the necessary compound concentration that reduces the activity or number of cells by 50% for the given time period. Genomics of Drug Sensitivity in Cancer (GDSC) and Chembl databases were used for identifying optimal IC_{50} dosages.[24,25] If the IC_{50} dosage of a drug is higher than 20 μm, it is marked as NA, and its dosage is set as 10 μm. After this elimination, 345 and 95 drugs remained for breast and lung cancer, respectively.

We represented the transcriptome profile of each drug on the FLN; eventually, we constructed drug-specific networks. Each drug-specific network contains genes affected by the treatment of that drug in the corresponding cell line. For drug-specific network construction, differentially expressed genes of a drug were marked on the FLN. All drug samples were analyzed based on several thresholds, which are z-score values higher than |1|, |1.5|, or |2|. We decided to apply the z-score threshold as higher than |1|, since z-score distributions of other thresholds cover a very limited range of differentially expressed genes.

After extracting differentially expressed genes of each drug by filtering based on a z-score threshold, the remaining drug affected genes (DAG) were mapped on the FLN individually. Since our aim is to construct an independent interaction network specific to a given drug, such a network can more effectively express the functional interactions between genes which were affected due to the treatment of this drug. For this purpose, we considered mapping using two different neighboring schemes on the FLN: direct neighboring and via 1-intermediate neighboring. The direct neighboring scheme only keeps DAG and their immediate interactions in between. This scheme considers genes and interactions under this condition: if both A and B are members of DAG, and they have a direct interaction. The 1-intermediate neighboring scheme extends the neighbor definition by including one more gene between the DAG members. For example, if C is a neighbor of both A and B, C is not a member of DAG, then three genes and their interactions are kept in the final network based on the 1-intermediate neighboring scheme. Drug-specific networks are constructed by applying both neighboring schemes on the FLN. This mapping was also applied on two cancer types. We compared both schemes by analyzing the total number of nodes, interactions, and connected components found in the constructed drug-specific networks (data is not shown). After these analyses, it was decided to select the direct neighbor mapping to construct drug-specific networks (Figure 2). The drug-specific networks should not reach the coverage of the original FLN, since they are specialized for a better observation of the drug-treatment effect in the biological network. When we analyze the coverage of the direct-neighbor method, it serves this biological hypothesis much better.The proposed drug

repositioning approach calculates a similarity score between a disease network and a drug-specific FLNs. 1139 and 1360 genes were used as disease-causing genes (DCG) for breast and lung cancer, respectively. To obtain DCG, RNA-sequencing samples of lung and breast cancer patients (LUAD and BRCA) were downloaded from the TCGA database[26] using the TCGABiolinks[27] package. The *p*-value (FDR-corrected) threshold value of 0.01 and the absolute log-fold-change values of 2.0 were used to identify statistically significant genes. The number of obtained genes was mapped on the FLN after name-Entrez identifier conversion. After the mapping of DCG on the FLN, this scheme constructs a breast cancer DCG network (i.e., disease network) which has 770 genes and 3698 interactions. In lung cancer, the DCG network has 906 genes and 4173 interactions.

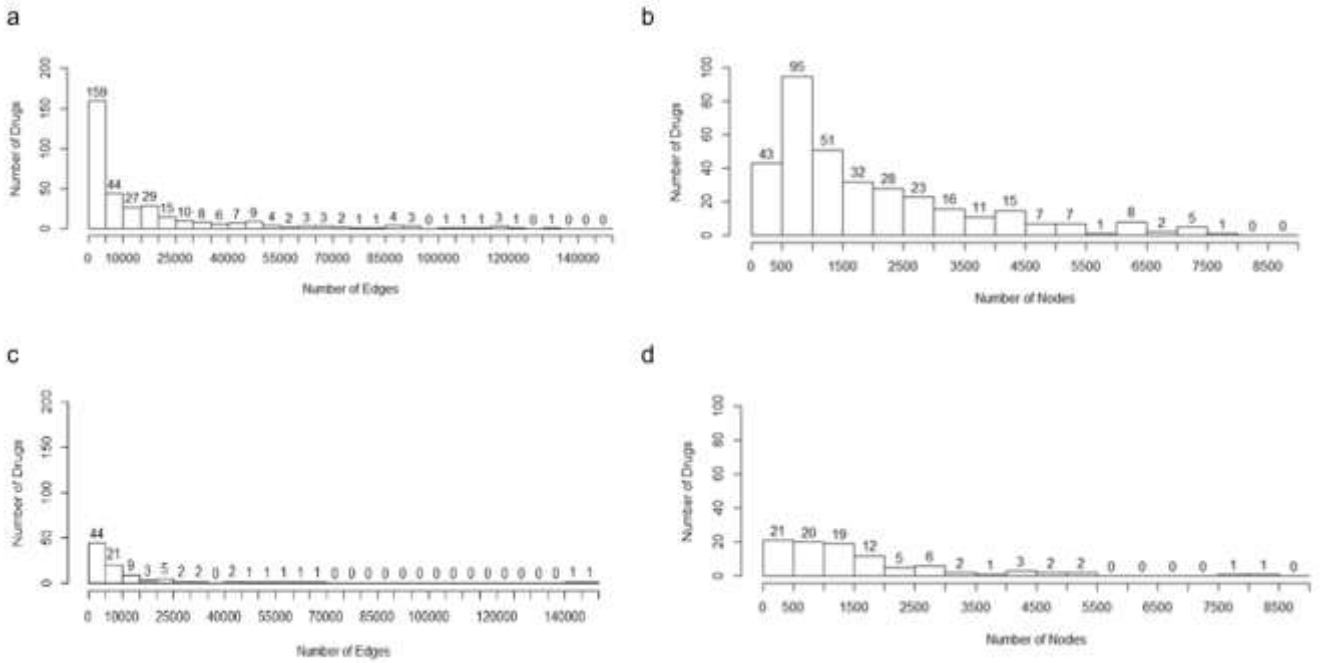

Figure 2: Distribution of network members. a) Edge counts of drugs after applying the direct-neighbor schemes for breast cancer. b) Node counts of drugs after applying the direct-neighbor schemes for breast cancer. c) Edge counts of drugs after applying the direct-neighbor schemes for lung cancer. d) Node counts of drugs after applying the direct-neighbor schemes for lung cancer**.**

2.2 Algorithm

The proposed algorithm aims to find the optimal DAG network (i.e., compound) that effectively inhibits the given DCG network (i.e., disease). For this purpose, a module overlap between the given DAG and DCG networks is calculated. A module overlap is represented as a similarity score that is based on a shortest-path distance of two networks.[28] We adapted the original module similarity in a better representation of a network similarity for a drug-repositioning approach. A higher similarity score should indicate a better candidate compound for inhibiting disease-causing genes.

Firstly, the length of a shortest path is used to calculate the distance between two genes, as:

$$sim\ (g_1, g_2) = A * e^{-b*sp(g_1,g_2)} * 0.5^{FC_1+FC_2} \quad (1)$$

In the Equation 1, $sp(g_1, g_2)$ represent the length of shortest path between g_1 and g_2 nodes in the protein-protein interaction network; *A* and *b* represent constants that are determined by users. $sim(g_1, g_2)$ measures the transformed distance between two nodes, it was proposed by Perlman et al. and Paik et al.[29, 30] We introduced the fold change (FC) measure in the $sim(g_1, g_2)$ equation as a novel contribution to incorporate effects of gene expression change in the module similarity calculation. If the summation of FC_1 and FC_2 is close to zero, the expression value of two nodes counteracts each other, i.e., providing a good matching as a pair of nodes and biologically higher similarity. A higher $sim(g_1, g_2)$ value shows a biologically close relationship between g_1 and g_2 nodes. After testing various values (0.25, 0.5, 0.75 and 1) for b constant, we applied 1 and 0.5 for A and b constants, respectively. We used as $A = 1$ to keep the value of $sim(g_1, g_2)$ in the range of [0-1].

G is a biological module (i.e., a subset of FLN) that indicates a set of genes associated with a disease (DCG) or affected by a compound treatment (DAG). The relevance of genes can be measured by Equation (2). The gene's relevance score is calculated as the average transformed distance between g_i and all genes in G.

$$F_G(g) = average\ \left(\sum_{g_i \in G} sim(g, g_i)\right) \quad (2)$$

Suppose that G_1 is a module with m genes, and G_2 is another module with n genes. Equation (3) calculates a relation between two modules by considering all gene pairs in two modules.

$$spsim(G_1, G_2) = \frac{\sum_{1 \le i \le m} F_{G_2}(g_{1i}) + \sum_{1 \le j \le n} F_{G1}(g_{2j})}{m+n} \quad (3)$$

For a consistent estimate of module similarity, a normalization is applied on the relatedness score between G_1 and G_2 as given in Equation (4), which shows the final similarity (i.e., overlap) between $G_1$and G_2modules.

$$ModuleSim = \frac{2*spsim(G_1,G_2)}{spsim(G_1,G_1)+spsim(G_2,G_2)} \quad (4)$$

In all equations, G_1 and G_2 represent the DCG network (breast or lung cancer) and a single DAG network, respectively.

2.3 Statistical Analysis

Three hundred forty-five and ninety-five different module similarity scores were calculated for breast cancer and lung cancer, respectively. Firstly, the distribution of these values was examined, and the module similarity scores did not show a normal distribution in two cancer types. A min-max normalization was applied to make a more accurate interpretation of the data without changing the original score distributions. Because there would be other similarity metrics to compare with this method. The min-max normalization, also called min-max scaling, is a naïve method for rescaling values into [0-1] range.[31] The formula for min-max normalization is given as Equation 5. Among the normalized results obtained, those with values greater than 0.5 were selected as the candidate or predicted drugs.

$$ModuleSim' = \frac{Calculated\ ModuleSim - min(ModuleSim)}{max(ModuleSim) - min(ModuleSim)} \quad (5)$$

2.4 Drug-Target Protein Analysis

The STITCH database was used to search proteins targeted by a given drug.[32] As an input, each prediction of our model was provided and performed a search for its human protein targets. STITCH provides an interaction score which represents the strength of biological evidence between a protein and a compound. We only used experiments and databases as biological evidence. Minimum required interaction score was set to 0.7 or higher values. Maximum number of interactions of a given drug was selected as “no more than 20 interactions”. Then the created drug-target network was downloaded and visualized by the Cytoscape tool. Target proteins are colored based on whether they are up-regulated or down-regulated in the related disease-network. In addition, the inhibition or activation biochemical relations between a protein and a drug are indicated by a truncated arrow or arrow, respectively.

2.5 Other Methods

To compare the proposed method with other DR methods, we considered two methods: Module Network Based Drug Repositioning (MNBDR) and PharmOmics.

MNBDR identifies dense modules in a PPI network and significant modules are chosen based on the high number of cross-talks among modules.[19] The PageRank algorithm is used to choose the important modules of a disease. The gene expression data of drug treated samples are combined into important modules to compute a DR score for a pair of drug-disease. To make a fair evaluation, we used the original FLN and drug-treated samples for breast and lung cancers. MNBDR operated over the same interaction network (FLN) and gene expression data of drug-targeted cells of this study. The DR score of MNBDR is also scaled between 0-1 to set 0.5 threshold as the selection criteria of repositioned drugs.

PharmOmics provides a web-platform for drug repositioning and toxicity prediction.[20] Its DR approach computes a similarity between a drug and a gene signature in their platform. In a Bayesian gene regulatory network, the distance between the drug and disease signatures is the average shortest path lengths of each pair across signatures. The tool ranks candidate drugs based on the distance between drugs and the given disease genes. We used the pre-computed drug signatures for homo sapiens provided in the PharmOmics server. We uploaded the original FLN as the regulatory network of the DR approach. Original disease genes breast and lung cancers are provided as input genes of the tool. The results of the PharmOmics server are ordered by z-scores and normalized score (z-score rank), which ranges between 0-1. We applied a 0.5 threshold on this normalized score as the selection criteria of successfully repositioned drugs.

3 Results

We will report proposed candidates by NMSDR for both cancers in this section.

3.1 Breast Cancer

The selection strategy of new repositioned candidates is critical for designing more effective wet-lab experiments. Therefore, we focused on the module similarity scores, which should be higher than the normalized score of 0.5. In total, 38 of 345 drugs have a normalized module similarity score greater than 0.5 for breast cancer. Two of these are already FDA-approved drugs (doxorubicin, epirubicin) used in the clinical treatment of breast cancer. These 38 drugs and their normalized similarity scores are listed in Table 1. The remaining FDA-approved treatments with module similarity scores less than 0.5 are also listed at the end of Table 1.

Alvocidib (flavopiridol) achieved the highest module similarity score and it is not a FDA-approved treatment for breast cancer. Therefore, we performed a drug-target protein analysis by using the STITCH database. The associated target proteins of flavopiridol were filtered by setting the interaction confidence score to 0.7 or higher values. Out of 20 direct interactors of flavopiridol, the CDK1 protein is available in the disease-causing genes (DCG) network of breast cancer (Figure 3). CDK1 has a high gene expression in the breast cancer cohort, hence inhibitory effect of flavopiridol on CDK1 may induce its down-regulation and it may work as a treatment on breast cancer.

3.2 Lung Cancer

The same selection strategy has been applied to lung cancer experiments. In total, 14 of 95 drugs have a normalized module similarity score greater than 0.5. Two of these are FDA-approved drugs (crizotinib, trametinib) used in treatment of lung cancer. Fourteen drugs and their normalized similarity scores are listed in Table 2. The remaining FDA-approved treatments with module similarity scores less than 0.5 are also listed at the end of Table 2.

Table 1: Drugs that have a normalized module similarity score greater than 0.5 for breast cancer (FDA-approved drugs are marked with blue color).

Rank	Drug_Name (Dosage/μm)	Similarity Score (ModuleSim)	Normalize Similarity Score
1	Alvocidib (0.12)	1.7811	1
2	BMS-387032 (1.11)	1.7258	0.9678
3	AT-7519 (10.0)	1.7198	0.9643
4	BMS-345541 (10.0)	1.7001	0.9528
5	QL-XI-92 (10.0)	1.6924	0.9483
6	JW-7-24-1 (10.0)	1.6667	0.9333
7	AZD-5438 (10.0)	1.6504	0.9237
8	Mitoxantrone (10.0)	1.6470	0.9218
9	Ponatinib (10.0)	1.5362	0.8572
10	MK-1775 (10.0)	1.4514	0.8077
11	PF-562271 (10.0)	1.3869	0.7700
12	Doxorubicin (1.11)	1.3847	0.7687
13	ZM-447439 (10.0)	1.3710	0.7607
14	Triptolide (0.04)	1.3455	0.7459
15	Dinaciclib (0.37)	1.3454	0.7458
16	PHA-793887 (10.0)	1.3347	0.7396
17	Mocetinostat (3.33)	1.3120	0.7263
18	Calcitriol (10.0)	1.3077	0.7238
19	Epirubicin (1.11)	1.2340	0.6843
20	Alvocidib (0.04)	1.2333	0.6804
21	Lestaurtinib (3.33)	1.2061	0.6646
22	Entinostat (10.0)	1.1823	0.6506
23	Dinaciclib (0.04)	1.1662	0.6413
24	Linifanib (10.0)	1.1637	0.6398
25	QL-XII-47 (10.0)	1.1454	0.6291
26	Rucaparib (10.0)	1.1451	0.6290
27	Defactinib (10.0)	1.1336	0.6222
28	LCL-161 (10.0)	1.0777	0.5896
29	AST-1306 (10.0)	1.0439	0.5699
30	Camptothecin (0.12)	1.0172	0.5543
31	Belinostat (0.12)	0.9891	0.5379
32	Sunitinib (10.0)	0.9820	0.5338
33	Amsacrine (10.0)	0.9814	0.5334
34	Inosine (10.0)	0.9515	0.5160
35	Mepacrine (3.33)	0.9461	0.5129
36	GSK-1059615 (10.0)	0.9283	0.5024
37	Lasalocid (3.33)	0.9233	0.4995
38	Vandetanib (10.0)	0.9201	0.4976
52	Epirubicin (3.33)	0.7702	0.4102
65	Fulvestrant (1.11)	0.6597	0.3457
67	Fluorouracil (10.0)	0.6575	0.3445
85	Gemcitabine (1.11)	0.5754	0.2966
95	Fulvestrant (0.04)	0.5393	0.2755
112	Gemcitabine (0.12)	0.4795	0.2406
113	Doxorubicin (0.04)	0.4781	0.2398
129	Palbociclib (3.33)	0.4293	0.2113
153	Docetaxel (0.37)	0.3728	0.1784
158	Palbociclib (1.11)	0.3682	0.1757
164	Toremifene (10.0)	0.3525	0.1665
173	Docetaxel (0.04)	0.3333	0.1553
222	Talazoparib (3.33)	0.2578	0.1113
231	Paclitaxel (0.04)	0.2480	0.1056
238	Alpelisib (10.0)	0.2386	0.1001
250	Olaparib (10.0)	0.2167	0.0873
256	Letrozole (0.04)	0.2019	0.0786

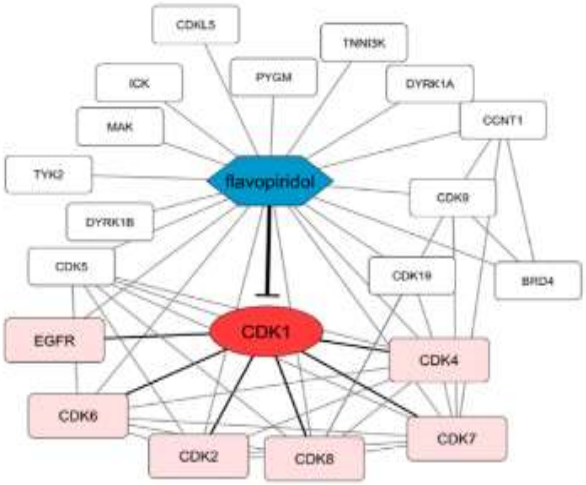

Figure 3: Drug-target protein interaction network for alvocidib (flavopiridol). Flavopiridol inhibits the CDK1 protein which is upregulated in the breast cancer cohort. Thick black lines indicate direct associations of CDK1. Gray lines show protein-protein / protein-drug interactions.

Drug Repositioning Approach

Table 2: Drugs that have a normalized module similarity score greater than 0.5 for lung cancer (FDA-approved drugs are marked with blue color).

Rank	Drug_Name (Dosage/μm)	Similarity Score (ModuleSim)	Normalized Similarity Score
1	BMS-345541 (10.0)	1.2429	1
2	Dinaciclib (0.04)	1.1721	0.9423
3	Tanespimycin (10.0)	1.0801	0.8673
	Mitoxantrone (0.12)	1.0746	0.8624
5	NVP-BEZ235 (0.04)	1.0390	0.8338
6	Mitoxantrone (0.37)	0.8967	0.7177
s	NVP-AUY922 (0.04)	0.8788	0.7031
8	Foretinib (1.11)	0.8686	0.6948
9	Celastrol (3.33)	0.8233	0.6580
10	Mocetinostat (10.0)	0.7278	0.5800
11	NVP-BEZ235 (3.33)	0.7094	0.5650
12	Crizotinib (10.0)	0.7057	0.5620
13	Trametinib (10.0)	0.7047	0.5612
14	Tozasertib (10.0)	0.6855	0.5455
29	Trametinib (0.04)	0.4462	0.3505
38	Dacomitinib (10.0)	0.3519	0.2736
44	Erlotinib (10.0)	0.3091	0.2387
55	Gefitinib (10.0)	0.2532	0.1931
61	Erlotinib (3.33)	0.2169	0.1635
74	Everolimus (10.0)	0.1448	0.1048
75	Osimertinib (0.37)	0.1402	0.1010
82	Afatinib (3.33)	0.1133	0.0791
88	Dabrafenib (10.0)	0.0854	0.0564
93	Dabrafenib (3.33)	0.0564	0.0327
95	Osimertinib (3.33)	0.0388	0.0184

When we analyzed the top-scored repositioned candidates for lung cancer, we could construct drug-target protein interaction networks for dinaciclib, tanespimycin, and mitoxantrone. There are only three associations (CDK1, CDK2, CDK9) of dinaciclib with 0.7 or higher interaction score (Figure 4a). The CDK1 protein with a high-expression is available in the lung cancer cohort. Inhibitory effect of dinaciclib on CDK1 may induce a treatment on lung cancer. Tanespimycin has 13 protein neighbors in the drug-target protein interaction network; only the NQO1 protein is available in the lung cancer cohort with a high-expression value (Figure 4b). Mitoxantrone has 18 associations in the drug-target protein interaction network; the TOP2A and ABCG2 proteins are also members of the lung cancer cohort (Figure 4c). Due to inhibitory effects of mitoxantrone on these proteins, the downregulation of TOP2A might be a promising treatment option for lung cancer.

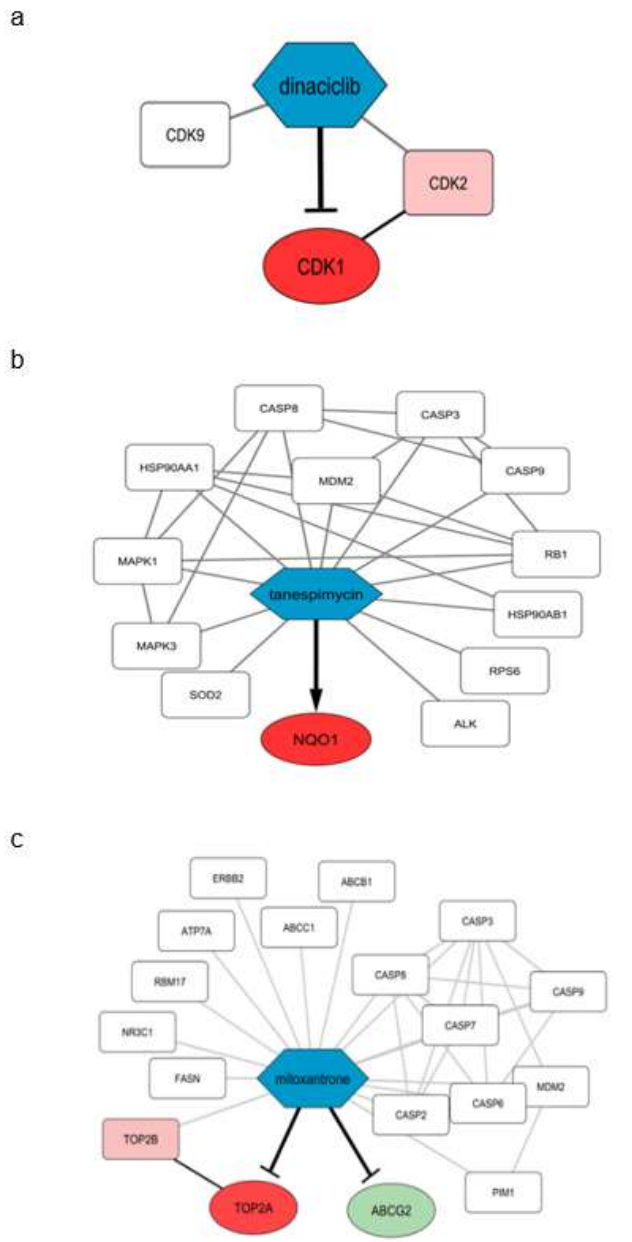

Figure 4. (a) Drug-target protein interaction network for dinaciclib, which inhibits the CDK1 protein. Thick black line indicates proteins associated with CDK1. (b) Drug-target protein interaction network for tanespimycin, which activates the NQO1 protein. (c) Drug-target protein interaction network for mitoxantrone that inhibits the TOP2A and ABCG2 proteins. In the figure gray lines show protein-protein / protein-drug interactions. Red and green colored proteins show up-regulated (positive expression) and down-regulated (negative expression) proteins in the DCG network. Pink colored proteins indicate associated proteins, which might be indirectly inhibited / activated by the candidate drugs.

4 Discussion

4.1 Literature Validation of Proposed Drugs

We will summarize the previous computational drug-repositioning studies applied for breast and lung cancers as literature validations of our results.

4.1.1 Breast Cancer

According to a study by Khanjani et al., the candidate treatment identification for breast cancer was conducted using a network analysis. Gene expression data were obtained from TCGA and differentially expressed genes were obtained by applying the limma method. These data were given as input to the L1000CDS software, and 24 drugs were identified as candidate drugs.[33] Six of these drugs (mocetinostat, alvocidib, BMS-387032, AT-7519, PF-562271, QL-XII-47) are also predicted by our method.

Another network-based approach used ten different breast cancer subtypes for repurposing. Level-5 drug expression data was downloaded from the LINCS database and only 0,04 and 10,0 μm dosages of each drug are used. As a result, 20 candidate drugs were proposed for every ten subtypes of breast cancer.[34] Three of the proposed drugs (MK-1775, PHA-793887, mocetinostat) are the same with our predictions.

Another study with network analysis proposed drug candidates for treatment of breast cancer. They downloaded gene expression data of patients from TCGA and differentially expressed genes were obtained by the limma method. Differentially expressed genes were analyzed with the STRING network and 29 possible drug candidates were predicted.[35] Four of these predictions (dinaciclib, alvocidib, AT-7519, AZD-5438) are common with our results.

A graph neural network model (GraphRepur) was experimented on breast cancer. This model uses drug signatures and topological structure information for drug relationships, then applies machine learning approaches for repurposing. Drug signature and gene expression of breast cancer are obtained from LINCS and GEO, respectively. After developing an adaptation of the GraphSAGE network, 10 drugs were predicted as new candidates for breast cancer treatment.[36] One of them (sunitinib) is the same with our predicted drugs.

As a summary, ten proposed candidates in this study were also suggested in recent studies applied on breast cancer, some of them (alvocidib, AT-7519, mocetinostat) found repetitively. These findings from literature promote predictions of this study and empower the repositioning reliability of the proposed approach.

4.1.2 Lung Cancer

We identified two recent studies which also proposed mitoxantrone as a candidate drug for lung cancer treatment. Mitoxantrone is primarily used in acute myeloid leukemia treatment. However, a study result showed that it is a perfect ex-vivo antitumor activity for human lung adenocarcinoma. In a recent study, immune-checkpoint inhibitors and proliferation genes were used as input data. An immune-omics network was constructed by integrating immune checkpoints and their interacting partners from different data sources. Finally, they reported four compounds including mitoxantrone, as repurposed drugs for the treatment of NSCLC.[37] Based on the recent studies, one of the repositioned candidates, mitoxantrone, should be further investigated as a new treatment option in lung cancer. One of the protein targets of mitoxantrone is TOP2A (DNA topoisomerase II alpha), which shows higher expression levels and poor survival rates in lung adenocarcinoma.[38, 39] Therefore, the treatment efficacy of mitoxantrone in lung cancer should be further investigated by in-vivo experiments and clinical trials.

4.2 Phase Studies of Repositioned Candidates

We will summarize in this section the ongoing and completed clinical trials that use the proposed candidates of this study for breast and lung cancers.

4.2.1 Breast Cancer

We focused on the top-scored compounds (higher than 0.5 normalized similarity score) for breast cancer, and these compounds are chosen for clinical trial search. The FDA-approved compounds were eliminated, and the final repositioning candidates were composed of 34 unique compounds. We identified phase-I, phase-II, or phase-III studies (by searching in http://clinicaltrials.gov) which were conducted for 18 of these 34 unique drugs. However, of the clinical trial results obtained so far, only three compounds (entinostat, mitoxantrone, sunitinib) have shown promising results with less toxicity and longer survival periods in breast cancer treatment.

There are several clinical trials for entinostat (MS275), either as a single compound or combined with others for cancer treatment. Entinostat is a benzamide histone deacetylase inhibitor undergoing clinical trials to treat various cancers and inhibits class I HDAC1 and HDAC3. A phase-I trial was applied to 31 patients (two on daily and 29 on the q14-day (every-14-day) schedule) between 2001-2003 (NCT00020579). Despite there aren't any complete or partial responses, 52% of 27 patients had stable disease ranging from 62 to 309 days. The most common adverse effects were fatigue (100%), nausea (83%), and neutropenia (74%) in all grades. Study results show that entinostat can be given safely on a q14-day schedule.[40] In another randomized phase-II trial, there were 130 breast cancer patients (NCT00676663). The patients are randomly assigned to two groups: exemestane plus entinostat (EE, n=64) and exemestane plus placebo (EP, n=66). The median overall survival was 28.1- and 19.8-months EE versus EP, respectively. The most common adverse effects were fatigue (48%), nausea (40%), and neutropenia (30%) in the EE group; fatigue (26%), nausea (15%) in the EP group. As an outcome of this study, entinostat plus exemestane is generally well-tolerated and demonstrated activity in ER+ advanced breast cancer patients.[41]

Mitoxantrone is a type II topoisomerase inhibitor. A phase-III trial aimed to compare the effectiveness of mitoxantrone with or without docetaxel in treating women with metastatic breast cancer with a poor prognosis (NCT00002544). A phase-III trial was applied to 260 patients between 1992-1997. The patients are randomly assigned to two groups: FEC (fluorouracil, epirubicin, and cyclophosphamide) (n=127) and mitoxantrone (n=133). Overall survival median was 15.8 and 14.1 months for FEC and mitoxantrone groups, respectively. The most common adverse effect is alopecia, which was observed at a rate of 62% in the FEC group, while it was observed at only 5% in the mitoxantrone group. According to study results, patients would gain more from single-agent mitoxantrone than a combination chemotherapy.[42] Another randomized phase-II trial was applied to 60 patients between 2015 and 2017. The aim of this study was to evaluate the efficacy and safety of Lipo-MIT (Mitoxantrone hydrochloride liposome injection) in breast cancer patients (NCT02596373). The patients are randomly assigned to two groups: Lipo-MIT (n=30) and MIT (Mitoxantrone hydrochloride injection) (n=30). The median progression-free survival (PFS) was 1.92 months for the Lipo-MIT group and 1.85 months for the MIT group.

Common serious adverse effects were thrombocytopenia (13.3%) and leukopenia (6.7%) for the Lipo-MIT group, and thrombocytopenia (3.3%) and Pneumonitis (6.7%) for the MIT group. Study results show that conventional mitoxantrone is effective in breast cancer treatment.[43]

Sunitinib is a small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor. A phase-I trial was applied to 26 breast cancer patients between 2006 and 2009. The patients were treated with combination sunitinib, docetaxel, and trastuzumab (NCT00372424). The median PFS was reported as 58.9 weeks. The most common adverse effects were fatigue/asthenia (52%), pyrexia (48%), diarrhea (44%), and nausea (44%). As a result of this study sunitinib, docetaxel, and trastuzumab combination had acceptable toxicity and showed antitumor activity for metastatic HER2+ breast cancer.[44] Another phase-II trial was applied to 60 breast neoplasm patients between 2006 and 2008. The patients were treated with 37.5 mg sunitinib taken once daily with trastuzumab (NCT00243503). The median PFS was reported as 6.4 months. The most commonly reported adverse effects were fatigue/asthenia (75%), diarrhea (60%), and stomatitis/related oral disorders (53%). Sunitinib plus trastuzumab showed acceptable safety and antitumor activity in HER2+ advanced breast cancer patients who were treatment-naive or had only received prior adjuvant treatment.[45]

4.2.2 Lung Cancer

Similar to breast cancer investigation, we focused on the top-scored compounds for lung cancer, and these compounds are chosen for literature validation. The final repositioning candidates were composed of 12 unique compounds. Phase-I, phase-II, or phase-III studies were retrieved for 5 of these 12 compounds by a manual search over http://clinicaltrials.gov. In the clinical trial results obtained so far, only two compounds (dinaciclib, foretinib) in lung cancer treatment have shown promising results with less toxicity and longer survival times.

Dinaciclib (SCH-727965) is a small molecule inhibitor of cyclin-dependent kinases (CDKs). There was only one phase-II trial on dinaciclib for lung cancer. This trial was applied to 67 patients between 2008 and 2011 (NCT00732810). This study was designed to evaluate the efficiency and safety of dinaciclib 50 mg in patients with lung cancer, compared with standard doses of erlotinib. Patients were divided into two groups: 17 patients to dinaciclib, 50 patients to erlotinib based on randomization. The median time-to-progress was 1.49 months for dinaciclib. The most common adverse effects were diarrhea (69%), neutropenia (63%), vomiting (53%); diarrhea (43%), and rash (39%) for dinaciclib and erlotinib, respectively. Dinaciclib was well tolerated at the 50 mg dosage, but does not have activity in treated NSCLC. Evaluation of dinaciclib in combination with other agents for other indications including breast cancer and multiple myeloma is in progress.[46]

Another compound, foretinib is a multitargeted kinase inhibitor, targeting MET, RON, AXL, TIE-2, VEGF receptors, and ROS-1. There was a phase-I trial for foretinib for lung cancer. A trial was given between 2010 and 2013 with 31 lung cancer patients (NCT01068587). Twenty-eight of 31 were evaluable for the response, but 3 patients did not have repeat assessment of their disease. Five experienced a partial response (17.8%), with a median duration of response of 10.8 months. Thirteen had stable disease, median duration 4.8 months, and 10 had disease progression as their best response. Diarrhea, fatigue, anorexia, dry skin, rash, and hypertension (≥20%) were reported as the most common adverse events.[47]

4.3 Comparison with Other DR Methods

The proposed method is compared with two network-based DR methods: Module Network Based Drug Repositioning (MNBDR) and PharmOmics.

We scaled the DR score of MNBDR between 0-1 to set 0.5 threshold as the selection criteria of repositioned drugs. The results of MNBDR are listed in Supplementary Table 1 and Supplementary Table 2, which cover repositioned drugs for breast cancer and lung cancer, respectively. When the repositioned drugs are analyzed for breast cancer, the proposed method and MNBDR identified the same drugs (doxorubicin, epirubicin) that are also FDA approved for breast cancer treatment (Supplementary Table 1). MNBDR listed four unique drugs (doxorubicin, triptolide, epirubicin, lestaurtinib) with 0.5 or higher score, which are the same drugs with exact dosages predicted by our method. Total number of repositioned drugs (38) by our method is doubled compared to the results of MNBDR. The comparison for lung cancer experiment is interesting, since the number of drugs repositioned by two methods is quite close. There are 12 and 8 unique drugs repositioned by our method and MNBDR, respectively (Supplementary Table 2). One common drug (crizotinib) identified by two methods is a FDA approved treatment for lung cancer. Seven drugs (dinaciclib, mocetinostat, BMS-345541, mitoxantrone, NVP-BEZ235, celastrol, crizotinib) are commonly proposed by both methods. This result can suggest that significant modules found by MNBDR might highly cover the lung cancer disease network constructed in this study.

We applied a 0.5 threshold on the z-score rank values of the PharmOmics tool. The results of PharmOmics are listed in Supplementary Table 3 and Supplementary Table 4, which cover repositioned drugs for breast cancer and lung cancer, respectively. Due to having different drug signatures, the proposed method and PharmOmics provided quite diverse predictions. The results of PharmOmics are classified based on tissue types. We focused on the respiratory system and breast tissue for lung and breast cancer, respectively. Methods share 23 and 7 drugs in their original signature cohorts for breast and lung tissue / cancers. The only common repositioned drug by both methods is calcitriol for breast cancer (Supplementary Table 3). On the other hand, crizotinib is the single common prediction of both methods for lung cancer (Supplementary Table 4). Although we provided the original FLN as the main network, different calculation of signature similarities and variations in drug cohorts led to divergent repositioning results in comparison with PharmOmics.

MNBDR and PharmOmics do not integrate the mRNA expression (fold change) measurement of each gene into their network-based DR algorithms. The proposed method with this novel integration might provide a better modeling of biochemical reactions observed at transcriptome level.

5 Conclusions

In this study, we have developed a novel drug repositioning approach based on a functional interaction network. The novelty of this approach is computing a graph module similarity between two networks: disease-causing and

compound-specific. The new approach has been applied on breast and lung cancers to propose new treatment candidates for them.

In-silico evaluation of repositioned candidates is not a trivial task and quite important to properly design the follow-up in-vitro experiments. The validation of our approach was performed in three steps. First, we performed a literature survey for the proposed compounds whether they were recently identified by competing drug repositioning studies. For breast cancer repositioning studies, ten candidates identified in this study were also reported by other methods. Specifically, alvocidib, AT-7519, and mocetinostat were suggested in several studies, so these compounds should be further investigated by wet-lab experiments for breast cancer treatment. The recent repositioning studies and our findings on lung cancer propose that mitoxantrone should be further investigated with clinical trials as a new treatment option for lung cancer. As the second validation step, we searched our findings within on-going or completed clinical phase trials and we found some of our predictions have high potentials for further phase trials. The promising clinical trials in breast cancer cover entinostat, mitoxantrone, sunitinib compounds as either single therapy or combined with others. Clinical trials for lung cancer highlighted dinaciclib, foretinib suggested by this study with relatively lower efficiencies in patients. The final evaluation was a comparison with two network-based DR approaches. Although there are some similarities between repositioned drugs of different approaches, in terms of clinical trial search, the proposed DR approach suggests more novel predictions. Overall, the predictions of the proposed approach revealed more effective compounds in treatment of breast cancer based on results of literature studies and clinical trials. As a summary, the evidence in literature and clinical trials empower the reliability of the proposed drug repositioning model.

The proposed approach has led to promising results by effectively modeling cell responses in a network-level data representation. As a future work, we are planning to update the similarity function in this model by experimenting with other network centrality metrics. The construction of a disease network will be extended by using different data types. Based on the biological motivation in a specific disease treatment, the disease-causing genes might be a mixture of mutation and mRNA expression data.

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