# A network-based drug repositioning infrastructure for precision cancer medicine through targeting significantly mutated genes in the human cancer genomes

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## ABSTRACT

**Objective** Development of computational approaches and tools to effectively integrate multidomain data is urgently needed for the development of newly targeted cancer therapeutics.

**Methods** We proposed an integrative network-based infrastructure to identify new druggable targets and anticancer indications for existing drugs through targeting significantly mutated genes (SMGs) discovered in the human cancer genomes. The underlying assumption is that a drug would have a high potential for anticancer indication if its up-/down-regulated genes from the Connectivity Map tended to be SMGs or their neighbors in the human protein interaction network.

**Results** We assembled and curated 693 SMGs in 29 cancer types and found 121 proteins currently targeted by known anticancer or noncancer (repurposed) drugs. We found that the approved or experimental cancer drugs could potentially target these SMGs in 33.3% of the mutated cancer samples, and this number increased to 68.0% by drug repositioning through surveying exome-sequencing data in approximately 5000 normal-tumor pairs from The Cancer Genome Atlas. Furthermore, we identified 284 potential new indications connecting 28 cancer types and 48 existing drugs (adjusted $P < .05$), with a 66.7% success rate validated by literature data. Several existing drugs (e.g., niclosamide, valproic acid, captopril, and resveratrol) were predicted to have potential indications for multiple cancer types. Finally, we used integrative analysis to showcase a potential mechanism-of-action for resveratrol in breast and lung cancer treatment whereby it targets several SMGs (*ARNTL*, *ASPM, CTTN, EIF4G1, FOXP1,* and *STIP1*).

**Conclusions** In summary, we demonstrated that our integrative network-based infrastructure is a promising strategy to identify potential druggable targets and uncover new indications for existing drugs to speed up molecularly targeted cancer therapeutics.

**Keywords**: cancer genome, significantly mutated genes, network-based drug repositioning, drug-gene signatures, precision cancer medicine.

RESEARCH AND APPLICATIONS

## INTRODUCTION

The past several decades have seen massive advances in many scientific, technological, and managerial efforts to raise the efficiency of drug discovery and development. However, the number of new United States Food and Drug Administration (US FDA) approved drugs has nearly halved since 1950, and the cost of drug discovery and development has grown fairly steadily.[1] On the other hand, massive amounts of drug and biological data have been released to the community. Hence, scientists are seeking innovative technologies and approaches to reduce the cost and raise the efficiency of drug discovery and development. Recent advances in biotechnologies, such as massively parallel high-throughput sequencing and genome-wide association studies (GWAS), provide unprecedented opportunities in identifying potential targets for drug discovery and development. A recent study revealed that human genetic data generated from GWAS provide a valuable resource to select the best drug targets and indications in the development of new drugs.[2]

Several national and international cancer genomics projects, such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium, have enabled investigators to comprehensively characterize the somatic mutational landscape and mutational signatures in a large number of tumor samples.[3–5] These abundant data not only allow investigators to identify significantly mutated genes (SMGs) in cancer through both computational and experimental approaches,[3,6,7] but also provide novel druggable targets and opportunities for developing new molecularly targeted cancer therapies that target SMGs.[8] Griffith et al.[9] developed a Drug-Gene Interaction database to mine existing resources to generate hypotheses for the mutated genes that might be therapeutic targets or prioritized for anticancer drug development. The development of new computational methods that efficiently integrate massive amounts of next-generation sequencing data from TCGA and International Cancer Genome Consortium projects provides new opportunities for the timely development of molecularly targeted treatments, one of the major areas in cancer precision medicine.

Tumor initiation, progression, and resistance to certain therapeutic agents are often attributed to the accumulation of one or multiple driver or actionable mutations that activate oncogenes or inactivate tumor suppressor genes (TSGs), as well as their signaling pathways.[10] Traditional cytotoxic agents targeting cell division and DNA replication have achieved great success in molecular cancer therapy, but they often have severe side effects. Molecularly targeted agents (e.g., kinase inhibitors) that target oncogenes or oncoproteins that drive tumor initiation or progression show high selectivity for tumor cells, hence reducing side effects. Therefore, targeting driver mutations (e.g., oncogenic mutations) is expected to provide valuable opportunities for

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precision cancer medicine.[11] For example, p.Arg132His on isocitrate dehydrogenase type I is the most frequent mutation in a subgroup of gliomas and various other types of cancer.[12] Schumacher et al.[13] identified a new vaccine that selectively targets mutant isocitrate dehydrogenase type I (p.Arg132His), induces antitumor immunity, and further halts growth of gliomas. Another example is somatic mutation BRAF p.Val600Glu, which occurs in ~50% of melanoma; vemurafenib can effectively target this specific mutation.[14] However, most targeted agents often have some pitfalls; for example, all kinase inhibitors eventually lead to drug resistance.[15] Moreover, the attempt to activate TSGs that have been inactivated by somatic mutations or deletions is more challenging than targeting oncogenes. One of the reasons is that mutations in TSGs are often truncated mutations scattered in the coding regions but with low recurrence. An alternative approach is to target pathways or subnetworks (e.g., neighbors in the protein interaction network [PIN]) perturbed by the inactivation mutations in TSGs.[16] The development of innovative computational methods via targeting oncogenic mutations in oncoproteins or proteins regulated by inactivation mutations in TSGs is urgently needed for the development of targeted therapeutic agents.

In this study, we developed an integrative network-based infrastructure to prioritize druggable targets or potential new indications for existing drugs by directly targeting SMGs or their neighbors in the PIN. Specifically, we assembled and curated 693 SMGs derived from large-scale cancer genomics datasets in public domains and TCGA projects for 29 cancer types. We found that 121 druggable proteins encoded by SMGs were targeted by known anticancer drugs or repurposed drugs through integrating the drug-target interactions from 3 well-known public databases. Furthermore, we developed a network-based statistical approach to prioritize new anticancer indications for the existing drugs by integrating SMGs discovered in TCGA and public domains and drug-gene signatures from the Connectivity Map (Figure 1). Collectively, this study highlights the potential value of network-based approaches in identifying potential druggable targets and new anticancer indications for existing drugs by utilizing large-scale cancer somatic mutations to aid in the timely development of precision cancer medicine.

## MATERIALS AND METHODS

### Manual curation of the significantly mutated genes discovered in cancer genomes

We manually curated and assembled high-quality SMGs from 15 large-scale TCGA cancer genome analysis projects and other public domains as briefly described below.[11,17–29] For example, Lawrence et al.[19] identified 224 SMGs from 4742 human cancer genomes across 21 cancer types using the MutSig method. Rubio-Perez et al.[11] identified 459 SMGs acting in one or multiple cancer types by analyzing 6792 tumor samples. The details are provided in online Supplementary Table S1. We annotated all SMGs using gene Entrez ID, chromosome location, and the official gene symbols from the National Center for Biotechnology Information database.[30] This process resulted in a total of 693 manually curated, unique SMGs across 29 cancer types (online Supplementary Table S1).

### Creation of protein interaction network

We used a large-scale, high-quality PIN by integrating 4 types of protein-protein interactions (PPIs) with complementary biological information: physical interactions, PPIs with 3-dimensional structural data, innate immunity-related PPIs, and kinase-substrate interactions mediating phosphorylation reactions, as described in our previous studies.[31,32] In total, we compiled 29 475 PPIs connecting 625 proteins encoded by SMGs and 7249 neighbors of SMG-encoded proteins in PIN.

### Construction of drug-target interaction network

We collected drug-target interactions from 3 public databases: Therapeutic Target Database,[33] DrugBank (v3.0),[34] and PharmGKB.[35] Drugs are grouped using Anatomical Therapeutic Chemical classification system codes[36–38] and are further annotated using the Unified Medical Language System and Medical Subject Headings vocabularies.[39] All protein coding genes were mapped and annotated using the gene Entrez ID and official gene symbol from the National Center for Biotechnology Information database.[30] In total, we obtained 17 490 drug-target interactions connecting 4059 US FDA-approved or experimental drugs and 2746 human proteins. After mapping the proteins encoded by 693 SMGs into the above global network, we found 1396 drug-target interactions connecting 121 proteins encoded by SMGs and 636 drugs, including 342 US FDA-approved drugs and 267 experimental drugs under preclinical or clinical studies (online Supplementary Table S2).

### Collection and preparation of drug-gene signatures

We compiled drug-gene signatures from the Connectivity Map (CMap, build 02).[40] The CMap was composed of over 7000 gene expression profiles from 4 human cultured cancer cell lines treated with 1309 bioactive compounds covering 6100 individual instances at different concentrations. A measure amplitude ($a$) of the extent of differential expression for a given probe set was defined in the original study,[40] briefly described below:

$$a = \frac{t - c}{(t + c)/2}$$

where $t$ is the scaled and thresholded average difference value (a measure of the relative level of a transcript monitored by a particular probe set) for a compound treatment group and $c$ is the thresholded average difference value for the matching control group. The average difference values were scaled and thresholded based on an MAS 5.0 (Affymetrix) approach.[40] Based on the above definition, $a = 0$ indicates no differential expression, $a > 0$ indicates increased expression (up-regulation) by the drug treatment, and $a < 0$ indicates decreased expression (down-regulation) by the treatment. In CMap, an amplitude of 0.67 denotes a 2-fold induction. According to the original study,[40] we defined a gene signature as being up-regulated if its amplitude was greater than 0.67 and down-regulated if its amplitude was less than −0.67. Finally, we mapped probe sets into SMGs. In total, we compiled 380 969 up- or down-regulated drug-gene pairs from the CMap connecting 6092 instances and 7476 SMGs (170) or their coding protein neighbors (7306) in PIN.

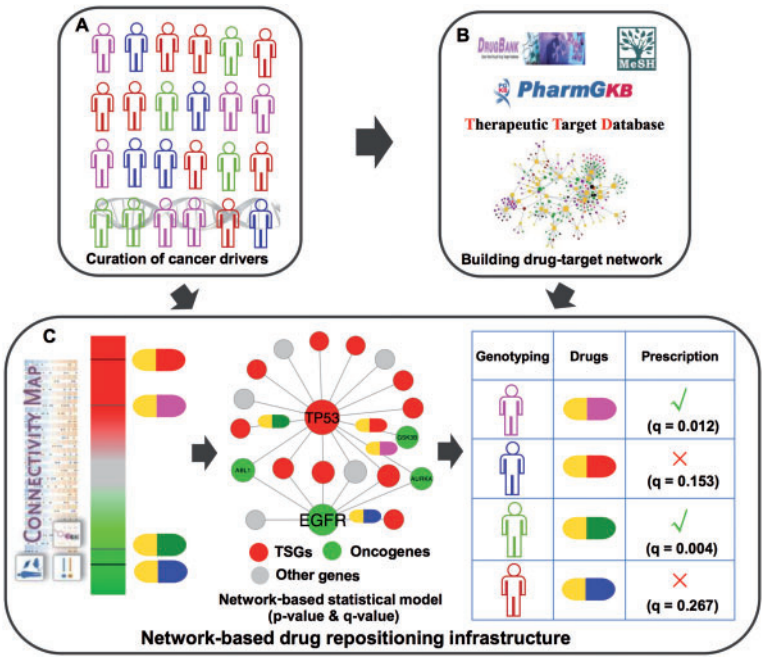
### Predicting new anticancer indications for existing drugs

For each cancer type, we manually curated its SMGs from TCGA and public domains. We also collected the up-/down-regulated genes for each drug from CMap. Then, we assumed that a drug would have a high potential for a specific anticancer indication if its up-/down-regulated genes from CMap tended to be SMGs or their neighbors in protein interaction network. For each drug-cancer pair, we conducted Fisher's exact test to calculate the significance of the enrichment of SMGs in up-/down-regulated genes. The $p$-values were corrected by using Benjamini-Hochberg false discovery rate[41] for each drug-cancer pair. Finally, we used the adjusted $P$-value ($q$) threshold $< 0.05$ to identify the significant drug-cancer pairs and prioritize new potential anticancer indications for existing drugs.

### Collection and preparation of somatic mutations

We downloaded the somatic mutation data from 3 sources: (1) Sanger website: (ftp://ftp.sanger.ac.uk/pub/cancer/AlexandrovEtAl, December

**Figure 1:** Diagram of network-based infrastructure for prioritizing potential druggable targets and searching for the existing drugs targeting significantly mutated genes (SMGs) in cancer genomes. This infrastructure is designed for better development of newly targeted cancer therapies. (**A**) Manual curation of SMGs discovered in large-scale cancer genome data generated from The Cancer Genome Atlas and public domains (see the Materials and Methods section). (**B**) Searching for new potential druggable anticancer targets via mapping the curated SMGs in A into drug-target interaction network collected from public databases: DrugBank, PharmGKB, and Therapeutic Target Database. (**C**) Development of a network-based drug repositioning approach to prioritize new potential anticancer indications for existing drugs. It incorporates drug-gene signatures from the Connectivity Map, the manually curated SMGs, and the protein-protein interaction network using a statistical approach (see the Materials and Methods section). TSGs: tumor suppressor genes.




16, 2013); (2) Elledge's Laboratory website at Harvard University (http://elledgelab.med.harvard.edu/?page_id=689; accessed in March 2014)[42]; and (3) COSMIC: Catalogue of Somatic Mutations in Cancer (v69) database (http://cancer.sanger.ac.uk/cosmic). Here, we only used nonsynonymous somatic mutations with TCGA tumor-normal matched sample IDs to ensure the quality of somatic mutation data. In total, we compiled 808 042 nonsynonymous mutations, including missense mutations and small inserts/deletions (indels) in 5003 cancer genomes for 16 cancer types. The detailed descriptions of somatic data collection and preparation are provided in our previous study.[43]

### Kaplan-Meier survival analysis

We used the online tool PREdiction of Clinical Outcomes from Genomic profiles (https://precog.stanford.edu) to investigate the relationship between gene expression and survival rate. Currently, PREdiction of Clinical Outcomes from Genomic consists of the overall survival data of approximately 18 000 patients and measures the relationship between gene expression and survival rate using a univariate Cox regression. The statistical associations were calculated using *z*-scores, where a $|z - \text{score}| > 1.96$ corresponds to a 2-sided $P < .05$. The details are provided in a previous study.[44]

### Network and statistical analysis

All statistical analysis was performed using the R platform (v3.01, http://www.r-project.org/). Networks were visualized using Cytoscape (v2.8.1).[45]

## RESULTS

### Overview of a network-based infrastructure

We proposed a network-based infrastructure to prioritize potential druggable targets and identify potential new indications of existing drugs for precision cancer medicine by identifying those that may specifically target SMGs or their neighbors in PIN. Previous studies have revealed that most drugs have polypharmacological features.[36,37,46] The hypothesis of our network-based drug repositioning approach is that if a set of up- or down-regulated genes perturbed by a drug of interest is overrepresented as SMGs or their neighbors in the PIN for a particular cancer type, this drug has a high potential of having a new indication for this cancer type. As shown in Figure 1, for oncogenes or oncoproteins, we proposed to prioritize potential drugs (e.g., inhibitors) to inhibit the function or expression of oncogenes. For TSGs, we proposed to prioritize potential drugs targeting their neighbors in the PIN. We defined a significant drug-cancer pair as one with a $q < .05$. Finally, we performed network analysis and survival rate analysis to explore the potential mechanism-of-action (MOA) of new predicted anticancer indications for existing drugs.

### A global drug-target interaction network through targeting SMGs

In this study, we curated and assembled 693 SMGs across 29 cancer types (online Supplementary Table S1): acute lymphocytic leukemia (ALL), bladder carcinoma (BLCA), breast carcinoma (BRCA), chronic lymphocytic leukemia (CLL), colon or rectum adenocarcinoma (COAD/READ), diffuse large B-cell lymphoma (DLBCL), esophageal carcinoma (ESCA),

glioblastoma multiforme (GBM), hepatocarcinoma (HC), head and neck squamous carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), acute myeloid leukemia (LAML), low grade glioma (LGG), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), medulloblastoma (MB), melanoma (MEL), multiple myeloma (MM), neuroblastoma (NB), non-small cell lung cancer (NSCLC), ovarian serous cystadenocarcinoma (OV), pilocytic astrocytoma (PA), pancreatic adenocarcinoma, prostate adenocarcinoma (PAAD), small cell lung cancer (SCLC, cutaneous melanoma (SKCM or CM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrioid (UCEC). Figure 2A shows that the average number of SMGs per cancer type is 90, with ranges from 2 SMGs observed in PA to 250 SMGs observed in SKCM. We next examined how many US FDA-approved drugs or experimental drugs may target SMGs by integrating drug pharmacological data from 3 public databases: DrugBank,[34] Therapeutic Target Database,[33] and PharmGKB[35] (see the Materials and Methods section). As shown in Figure 2B, we found 64 FDA-approved anticancer agents (drugs with Anatomical Therapeutic Chemical first class code "L") targeting 66 SMGs, 267 experimental anticancer agents under preclinical or clinical studies targeting 75 SMGs, and 278 FDA approved noncancer drugs targeting 54 SMGs. In total, there are 121 unique SMGs targeted by US FDA-approved drugs or experimental drugs, indicating a potential for cancer genomics data to aid in the development of molecularly targeted cancer therapeutics. The detailed lists of approved or experimental drugs targeting SMGs in different cancer types are provided in online Supplemental Table S2.

We further built a bipartite drug-target interaction network to visualize the MOA of 64 approved anticancer drugs and 278 approved noncancer drugs targeting 113 SMGs (Figure 3). We found that approved anticancer drugs, such as pazopanib, dasatinib, vandetanib, and bosutinib, target multiple SMGs, consistent with the polypharmacological profiles of targeted agents (e.g., kinase inhibitors).[47] In addition, we found that several SMGs (such as *TLR4* and *SCN9A*) can potentially be targeted by FDA-approved noncancer drugs. For example, *TLR4*, encoding toll-like receptor 4, was significantly mutated in the ER+/HER2− subgroup of breast cancer,[18] and recent studies further revealed that *TLR4* plays critical roles in breast cancer.[48] Figure 3 reveals that a specific opiate drug (naloxone) inhibits *TLR4* signaling,[49] which may be potentially useful in breast cancer chemoprevention and treatment. In addition, *SCN9A*, encoding sodium channel protein type 9 subunit alpha, was previously reported to be mutated in glioblastoma.[21] Figure 3 reveals that several approved noncancer drugs, such as ranolazine[50] and zonisamide,[51] block SCN9A function. This suggests that ranolazine and zonisamide may provide potential indications for glioblastoma chemoprevention and targeted therapy.

### Surveying benefit rates by the currently targeted cancer therapy

We examined how many cancer patients could potentially benefit from the currently targeted molecular therapies by surveying 5003 tumor genomes with unique TCGA sample IDs harboring nonsynonymous mutations in specific SMGs across 16 cancer types. Figure 4 reveals that the approved or experimental cancer drugs could potentially target SMGs in 33.3% mutated cancer samples. For example, breast cancer and lung cancer have a large number of SMGs, such as *EGFR* and *ERBB2*, that are targeted by many available US FDA-approved targeted agents (e.g., afatinib, lapatinib, gefitinib), as shown in Figure 3. The number would increase to 68.0% if we utilized drug repositioning by including noncancer (repurposed) drugs. Collectively, this information makes the currently targeted agents more promising for cancer therapeutics. It is worth noting that our analysis of simply using percentage patients for benefit rate in targeted cancer therapies may be biased toward the frequency of SMGs and cancer types due to data incompleteness.

**Figure 2:** Survey of significantly mutated genes (SMGs) and druggable targets in various types of cancer. (**A**) Distribution of the number of SMGs across 29 cancer types (see online Supplementary Table S1). (**B**) Venn diagram showing the druggable proteins encoded by SMGs targeted by US FDA-approved anticancer drugs, experimental anticancer drugs, and noncancer drugs. Due to space limitations, when the number of genes is more than 10 in Venn diagram, only the top 10 cancer targets with the largest number of approved drugs are listed. The detailed lists of genes shown in Figure 2 are provided in online Supplementary Table S2. The abbreviations for cancer types are provided in the main text.

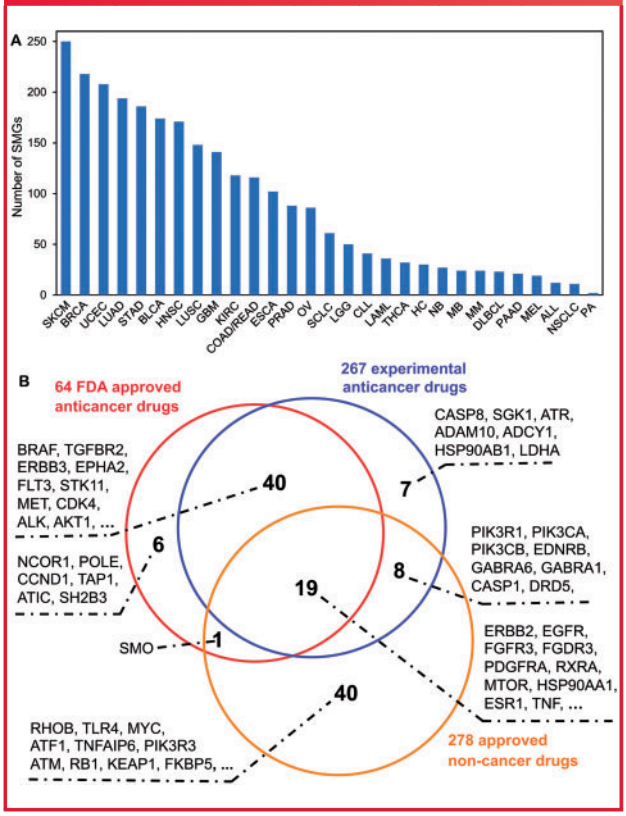
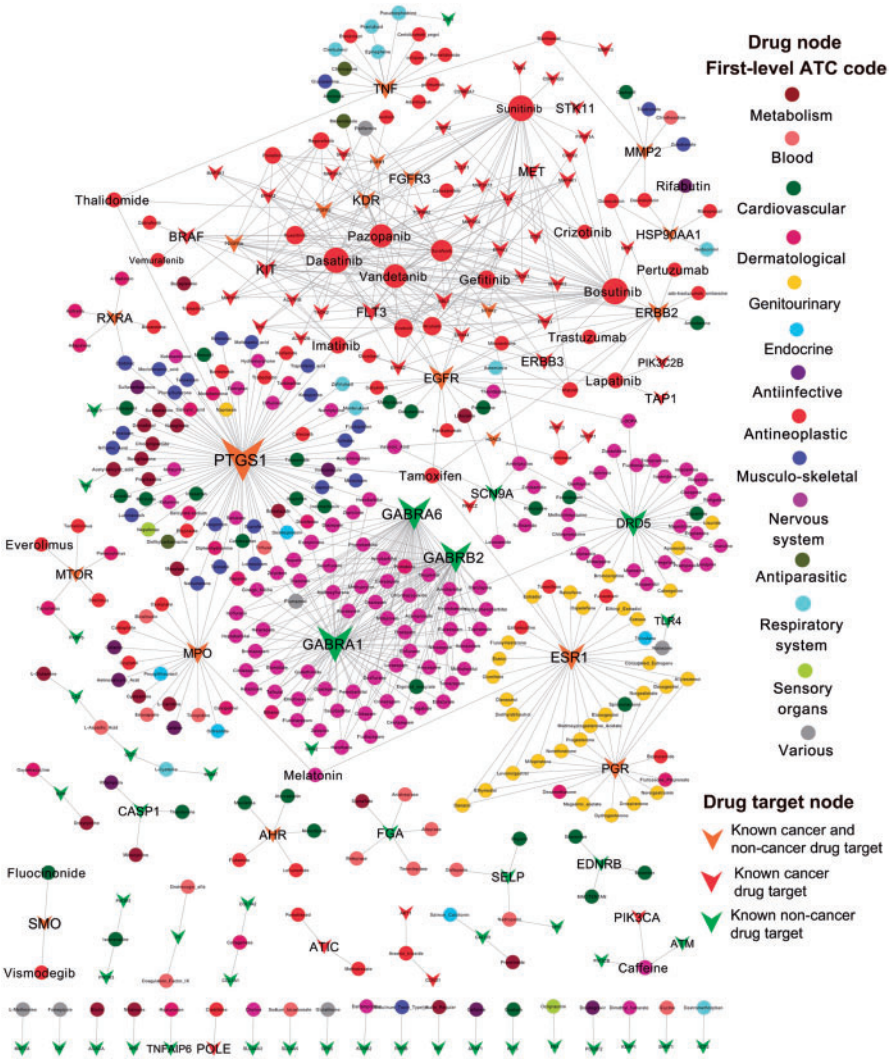



Figure 5 shows the nonsynonymous mutation spectrum for 66 druggable SMGs targeted by US FDA-approved anticancer agents. Those 66 SMGs were selected for presentation based on their high frequencies in the total samples. The 5 most commonly mutated druggable SMGs are *BRAF*, *EGFR*, *MTOR*, *EPHA3*, and *KIT*. For example, 45% of the patients (117/259) in SKCM harbored a mutation in BRAF. Specifically, among 325 patients, 183 (56%) TCHA patients had the mutation at the BRAF V600 site. Although there are only a few targeted agents available for thyroid carcinoma (Figure 4), some approved drugs (e.g., vemurafenib) targeting BRAF V600E may provide new off-label indications for thyroid cancer therapy.[52] In addition, several new SMGs, such as *SMO*, have been investigated or used for targeted cancer therapy. For example, vismodegib, developed by Genentech, an SMO inhibitor halting the hedgehog signaling pathway (Figure 3), was approved for adult basal cell carcinoma treatment on January 30, 2012.[34] Collectively, Figure 5 may provide useful information

**Figure 3:** Global drug-target interaction network connecting 113 proteins (denoted by Vee shape) encoded by SMGs and 342 US FDA-approved drugs (denoted by circle, 64 anticancer drugs and 278 noncancer drugs). All drugs were grouped using the Anatomical Therapeutic Chemical classification system codes, as described in a previous study.[35] Drug targets are denoted by Vee shapes: (i) orange Vees denote both known cancer and repurposed (noncancer) drug targets (SMGs targeted by both known anticancer drugs and noncancer drugs); (ii) red Vees denotes known cancer drug targets (SMGs targeted by known anticancer drugs); (iii) green Vees denote repurposed (noncancer) cancer drug targets (SMGs targeted by known noncancer drugs). The size of nodes (circles and Vees) reflects their degree (connectivity) in the network.




for the development and application of off-label uses for cancer therapies.

### Prioritizing new anticancer indications through targeting SMGs

Here, we proposed a network-based statistical approach to prioritize new potential indications for existing drugs by incorporating drug-gene signatures from CMap into 693 manually curated SMGs and their neighbors in the PIN across 29 cancer types (Figure 1). Based on the notion of systems pharmacology, our network-based statistical approach prioritizes existing agents that target oncoproteins or network neighbors regulated by TSGs for further development of targeted cancer therapy (Figure 1). Using the threshold of $q < .05$, we identified 284 significant drug-cancer pairs connecting 28 cancer types (gold squares) with 48 existing drugs (circles) in Figure 6 and online Supplementary Table S3. We did not find any significant drug-cancer pairs for 1 rare cancer type (pilocytic astrocytoma), which had only 2 SMGs (Figure 2B). To verify the performance of predicted results, we systematically searched existing literature for the predicted indications on these 48 drugs. Among the 48 predicted drugs, 32 drugs ($32/48 = 66.7\%$ success rate) were previously reported to have potential anticancer indications in literature data (online Supplementary Table S4). In the next paragraphs, we selected 4 drugs (niclosamide, valproic acid, captopril, and resveratrol) that have more experimentally validated data in the literature as example drugs to illustrate their anticancer profiles and MOA.

Niclosamide is approved for the treatment of tapeworm infection and acts by inhibiting oxidative phosphorylation, glucose uptake, and

**Figure 4:** Distribution of the available agents targeting significantly mutated genes (SMGs) and the number of mutated patients harboring nonsynonymous mutations in drug-targeting SMGs across 15 cancer types from The Cancer Genome Atlas. One cancer type (PAAD: pancreatic adenocarcinoma) did not have any SMGs targeted by approved drugs, so it was not included. All drugs were grouped using the Anatomical Therapeutic Chemical (ATC) classification system codes, as labeled in the figure. The *X*-axis shows the type of cancer. The abbreviations for cancer types are provided in the main text.

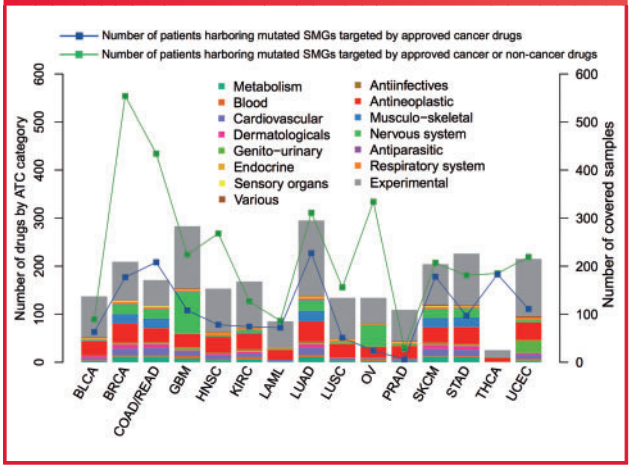
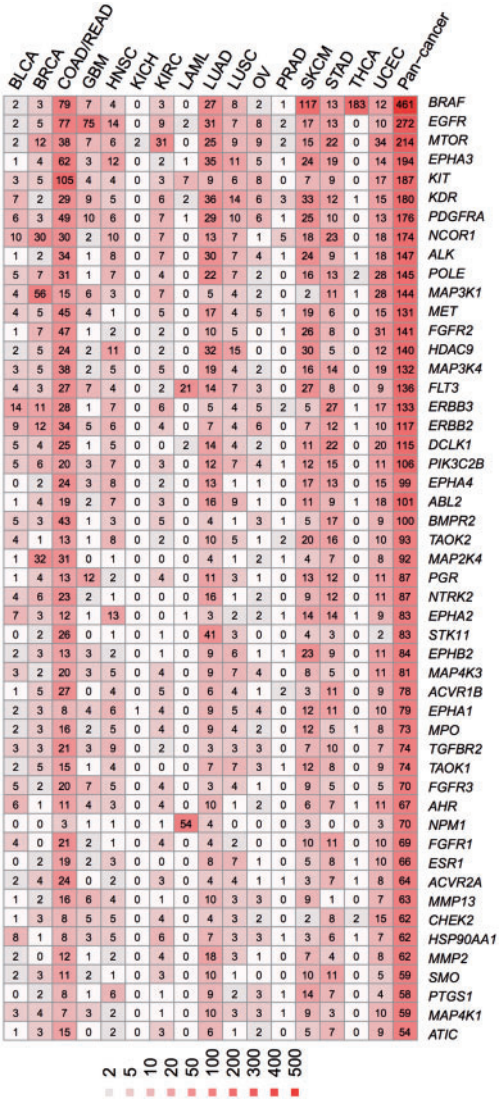



**Figure 5:** Frequencies of nonsynonymous mutations in 66 selected significantly mutated genes (SMGs) targeted by US FDA-approved anticancer agents across 15 cancer types in approximately 5000 cancer genomes. These 66 SMGs were presented here because they harbored the largest number of nonsynonymous somatic mutations in pancancer samples collected from The Cancer Genome Atlas. One cancer type (PAAD: pancreatic adenocarcinoma) was not included, as explained in Figure 4's legend. The color keys (heatmap) reflect the number of nonsynonymous somatic mutations counted in each cancer type or pancancer samples. The abbreviations for cancer types are provided in the main text.

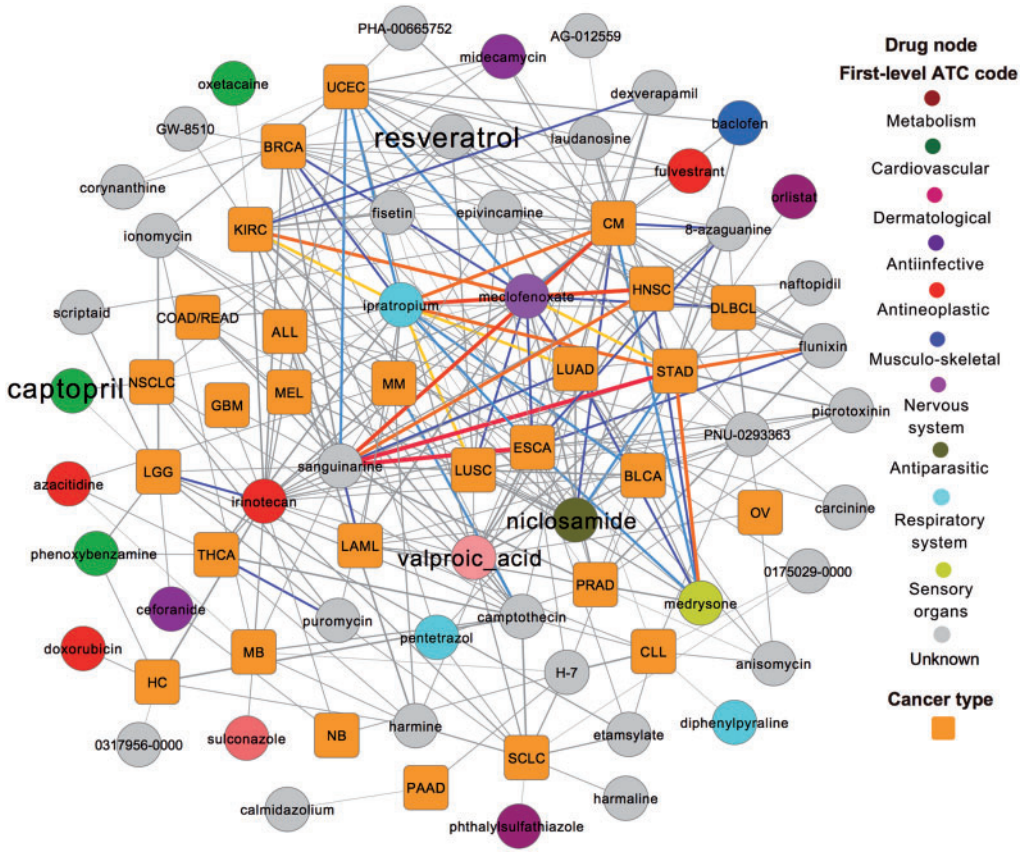



anaerobic metabolism.[53] Figure 6 shows that niclosamide is predicted to have potential indications for multiple cancer types, such as STAD ($q = 1.1 \times 10^{-6}$), SKCM ($q = 2.1 \times 10^{-5}$), LUAD ($q = 4.1 \times 10^{-5}$), and colon or rectum adenocarcinoma ($q = 6.9 \times 10^{-3}$). Two recent studies suggested that niclosamide had high tumor suppression activity in human sporadic colorectal cancer[54] and glioblastoma[55] by inhibiting the Wnt/beta-catenin pathway. Valproic acid, a fatty acid with an anticonvulsant profile, is approved for the treatment of epilepsy. Figure 6 shows that valproic acid is predicted to have potential anticancer indications for several cancer types, such as esophageal carcinoma ($q = 1.1 \times 10^{-4}$), bladder carcinoma ($q = 1.8 \times 10^{-4}$), and LUAD ($q = 2.7 \times 10^{-4}$). Recent studies reported that valproic acid has anticancer (e.g., lung cancer) and anti-virus properties as an HDAC inhibitor.[56–58] Captopril, a competitive inhibitor of angiotension-converting enzyme, was approved for the treatment of hypertension. In our analysis, we predicted that captopril has new indications for NSCLC ($q = 0.021$) and low-grade glioma ($q = 0.048$). A recent study reported that captopril is a promising agent for inhibiting lung tumor growth and metastasis in a xenografts model.[59] Collectively, our network-based approach demonstrated high performance in prioritizing new anticancer indications for existing drugs (e.g., niclosamide, valproic acid, and captopril), as indicated in Figure 6.

### Case study: discovery of potential anticancer mechanism-of-action for resveratrol

We next investigated the potential MOA of resveratrol for its predicted anticancer indications. Resveratrol is a naturally derived stilbene that exists in various foods and beverages. In 1997, Jang et al.[60] first reported the possible anticancer indication of resveratrol in a 2-stage mouse skin model. Now, resveratrol has been widely used and tested in preclinical or clinical studies to examine its chemopreventive and antitumor activities.[61,62] So far, the molecular mechanism of resveratrol's anticancer indication remains to be elucidated prior to its clinical approval as an anticancer agent.[62] In this study, resveratrol was predicted to have multiple anticancer indications (Figure 6): SKCM ($q = 4.2 \times 10^{-4}$), LUSC ($q = 1.2 \times 10^{-3}$), STAD ($q = 1.5 \times 10^{-3}$), kidney renal clear cell carcinoma ($q = 2.0 \times 10^{-3}$), head and neck squamous carcinoma ($q = 4.0 \times 10^{-3}$), uterine corpus endometrioid ($q = 5.8 \times 10^{-3}$), BRCA ($q = 8.9 \times 10^{-3}$), acute

**Figure 6:** Drug-cancer indication network for 284 predicted drug-cancer pairs (adjusted *P*-value [*q*] < .05, online Supplementary Table S3) connecting 48 existing drugs (circles) and 28 cancer types (orange squares). The thickness of links represents the *q* values calculated by our statistical approach (online Supplementary Table S3). The color of links represents significance categories of *q* values: red, $q < 10^{-9}$; orange, $10^{-9} < q < 10^{-8}$; yellow, $10^{-8} < q < 10^{-7}$; blue, $10^{-7} < q < 10^{-6}$; cyan, $10^{-6} < q < 10^{-5}$; and gray, $10^{-5} < q < 0.05$. The text size of 4 drugs (resveratrol, captopril, niclosamide, and valproic acid) was made larger because they were selected as example drugs and discussed in the main text. The abbreviations for cancer types are provided in the main text.

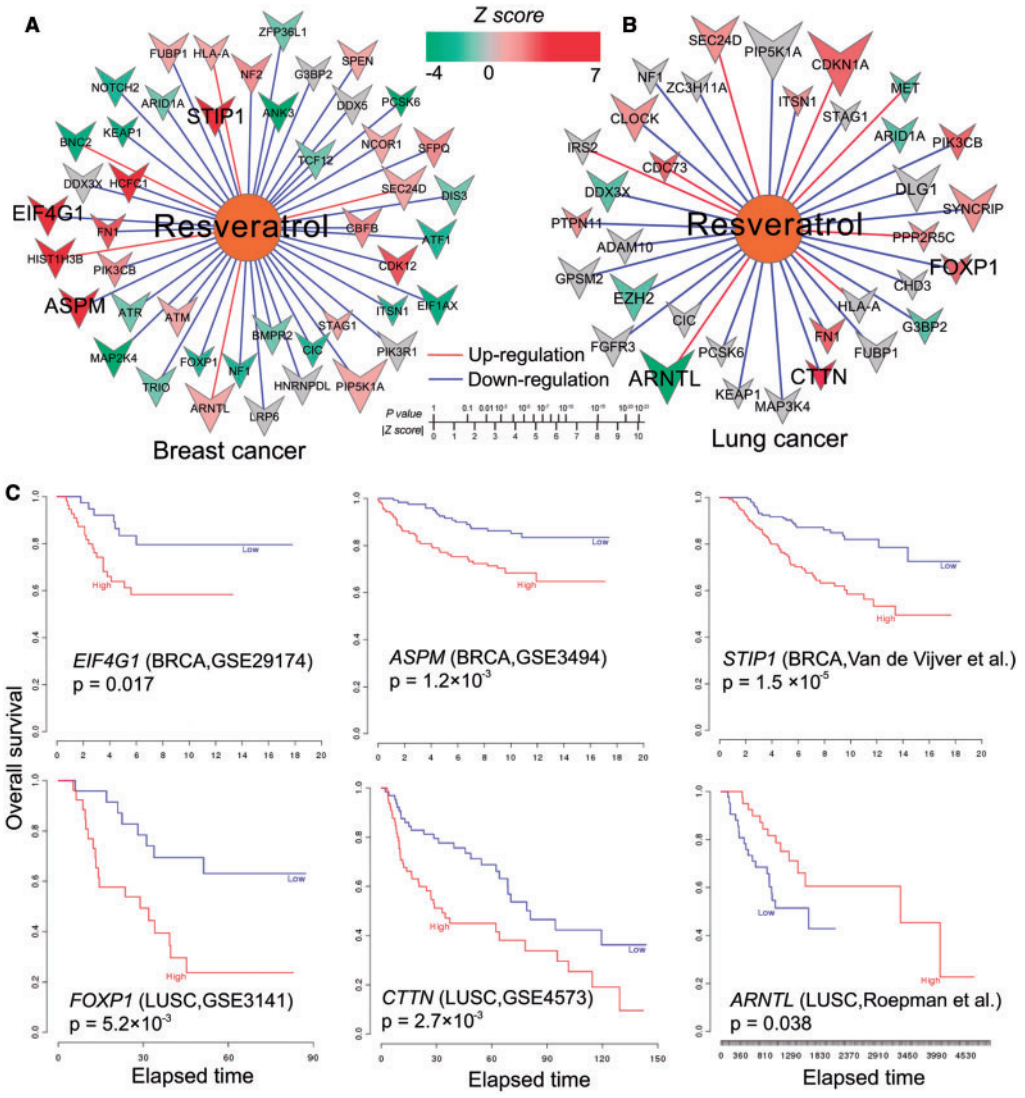



lymphocytic leukemia ($q = 0.011$), and NSCLC ($q = 0.011$). This is consistent with the previous studies,[61,62] but our work provides detailed information on a total of 9 cancer types.

We constructed a resveratrol-gene network to investigate the MOA of resveratrol targeting SMGs in beast cancer (Figure 7A) and lung cancer (Figure 7B) by integrating network analysis and survival analysis. As shown in Figure 7, the color of the node represents the *z*-score of association between gene expression and survival rate measured by univariate Cox regression, as described in a previous work.[44] The size of the gene node represents the amplitude (*a*) of differential expression with resveratrol, as described in CMap.[40] As shown in Figure 7, we asserted that if the high expression (or low expression) of a down-regulated gene (or up-regulated gene) by resveratrol was associated with poor survival rate in a particular cancer type, this gene would be more likely to explain the MOA for its anticancer therapeutic effect by resveratrol, based on a previous study.[63] Figure 7A reveals that several SMGs (such as *ASPM, EIF4G1*, and *STIP1*) in BRCA are down-regulated by resveratrol. Furthermore, high expression of *EIF4G1* ($P = .017$, log-rank test, GES29174[64]), *ASPM* ($P = 1.2 \times 10^{-3}$, GES3494[65]), and *STIP1* ($P = 1.5 \times 10^{-5}$, Van de Vijver et al.[66]) was significantly associated with poor survival rate in breast cancer. *EIF4G1*, encoding eukaryotic translation initiation factor gamma 1, plays crucial roles in breast cancer.[67] Previous studies reported that resveratrol inhibited human breast cancer cell migration and invasion,[68] so down-regulation of *EIF4G1, ASPM*, and *STIP1* by resveratrol may provide a potential MOA for its chemoprevention and therapeutic properties in breast cancer.

As shown in Figure 7B, resveratrol down-regulated *FOXP1* gene expression, while high expression of *FOXP1* was significantly associated with poor survival in LUSC ($P = 5.2 \times 10^{-3}$, GES3141[69]). *FOXP1*, encoding forkhead box P1, is a member of the forkhead box transcription factor family and plays important roles in lung cancer.[70] In addition, resveratrol up-regulated *ARNTL* expression, but down-regulated *CTTN* expression (Figure 7B). Interestingly, low expression of *ARNTL* was significantly associated with poor survival ($P = .038$, Roepman et al.[71]), while high expression of *CTTN* was significantly associated with poor survival ($P = 5.2 \times 10^{-3}$, GES4573[72]) in LUSC. A recent study showed that *ARNTL* plays a potential tumor suppressor role in ovarian cancer.[73] *CTTN*, which encodes cortactin, was previously reported to mediate progression of NSCLC[74] and other cancer types.[75] Taken together, down-regulation of *CTTN* and *FOXP1* and up-regulation of *ARNTL* may be a potential MOA for the anti–lung cancer effects of resveratrol.[61,62] The aforementioned analysis lends itself to proper experimental validation in the future.

**Figure 7:** Potential mechanism-of-action (MOA) for resveratrol's predicted anticancer indications in breast and lung cancers. (**A** and **B**) The networks connecting resveratrol and significantly mutated genes in breast (**A**) and lung (**B**) cancer, respectively. In (**A**) and (**B**), the color of nodes represents the *z*-score of the association between gene expression and survival rate calculated by univariate Cox regression as described in a previous study.[44] The size of gene nodes denotes the amplitude (*a*) value of differential expression by the treatment of resveratrol using the Connectivity Map data.[40] The red edges denote up-regulation and blue edges denote down-regulation. (**C**) Kaplan-Meier survival curves show 6 overlapped genes (selected based on the most significant *z*-scores) between the significantly mutated genes in breast and lung cancer and the resveratrol down/up-regulated genes from the Connectivity Map. The curves were prepared by an online tool, PREdiction of Clinical Outcomes from Genomic profiles: https://precog.stanford.edu.




## DISCUSSION

Massive amounts of next-generation sequencing data generated from thousands of tumor genomes enabled the development of precision cancer medicine by the identification of new drugs or existing drugs via targeting cancer driver events or SMGs discovered in cancer genomes. In this study, we manually curated 693 SMGs from large-scale cancer genomics studies and found 121 druggable proteins encoded by SMGs. We found that 33.1% of patients could potentially benefit from current US FDA-approved targeted agents, and this number increased to 68.0% when utilizing drug repositioning strategies, suggesting that cancer genomic data forms a strong foundation for precision cancer medicine in the future. Furthermore, we proposed a network-based drug repositioning statistical framework with moderately high accuracy (66.7% success rate) to prioritize new potential indications for existing drugs by integrating SMGs, drug-gene signatures, and the PPI network. We also identified a potential MOA for the putative anticancer effects of resveratrol in breast and lung cancer. In summary, this study demonstrated the potential application of our network-based approach in identifying existing drugs for precision cancer medicine by integrating large-scale cancer genomics and drug pharmacological data.

### Limitations and future work

There are several possible limitations in the current network-based infrastructure. First, the data is still incomplete and biased. For example, we examined potential molecular mechanisms of resveratrol's anticancer effects in breast cancer and lung cancer by integrating drug-gene signatures and clinical analysis (Figure 7). However, CMap data, while innovative and powerful, suffer from the lack of control on the selection of the optimal drug dose, which should be subtoxic to produce informative expression profile data.[40] Second, the current CMap 2.0 version included drug-gene signatures identified in only 4 different cancer cell lines.[40] However, we studied more than 20 different cancer types in this work. We could not perform individual cancer type analyses using 4 different cancer cell lines versus over 20 different cancer types, but this would be possible when a future version of CMap is released to cover many cancer types. Third, compounds used at higher concentrations can lead to widespread effects due to off-target and secondary effects that are difficult to control in a high-throughput setting. Hence, experimental validations of the predicted MOA are warranted in the future. In addition, we could integrate high-quality, comprehensive drug-gene signatures from LINCSCLOUD (http://www.lincscloud.org) into our network-based approach for more precise anticancer drug repositioning in the future. Fourth, cancer is highly heterogeneous and each cancer may have its subtypes with unique molecular signatures. We will further explore these limitations in the future. Despite the above limitations, our work effectively utilizes the largest-ever cancer genomic dataset, thus providing a robust framework to identify potential existing drug candidates for drug repositioning in cancer.

As shown in Figure 1, we hypothesized that if SMGs or their coding protein neighbors in the PIN were overrepresented in the set of genes up- or down-regulated by a drug of interest for a particular cancer type, this drug would have a high potential of a new indication for this cancer type. This strategy is effective because it weights not only mutational effect but also related biological regulation in the molecular network.[76] However, accurately distinguishing oncogenes from TSGs in particular cancer types is a very difficult task. In the current study, we combined oncogenes and TSGs as a union of SMGs, which may cause some data bias or potential false positive discoveries. In the future, we will build more reliable network-based approaches by considering agonists for TSGs to restore gene function or inhibitors for oncogenes to inhibit their oncogenic potential. In addition, we will implement more robust statistical algorithms, such as Kullback-Leibler divergence, for prioritizing new drugs to inhibit the function of neighbors of TSGs in a transcriptional regulatory network by integrating comprehensive gene expression profiles perturbed by drug treatment[77] using the data identified from functional RNAi and CRISPR-Cas9 screens.[78,79] In addition to SMGs derived from cancer genomics data, we may integrate some cancer susceptibility genes generated from GWAS[2] or phenome-wide association studies,[80] like the data from GWAS Catalog[81] and phenome-wide association studies Catalog,[82] into our network-based framework to identify new potential druggable targets or new indications for existing drugs, which will aid in the timely development of precision medicine in broad phenotypes.

## CONCLUSION

In this study, we proposed an integrative network-based infrastructure that has the potential to advance the field of precision cancer medicine by prioritizing druggable targets and new indications for existing drugs through targeting cancer driver genes. Based on large-scale cancer genomics data across over 5000 cancer genomes from TCGA, we found that 33.1% of patients could benefit from currently targeted agents, and the benefit rate increased to 68.0% when utilizing a drug repositioning approach. Moreover, we developed a statistical framework for anticancer drug repositioning to integrate drug-gene signatures, manually curated cancer drivers, and the PPI network. We identified 284 potential indications connecting 28 cancer types and 48 existing drugs with a 66.7% success rate validated by literature data. Finally, we showcased a potential MOA for resveratrol anticancer effects through targeting several SMGs (e.g., *ARNTL*, *ASPM, CTTN, EIF4G1, FOXP1,* and *STIP1*) in breast and lung cancers via integrative network analysis and survival rate analysis. In summary, we demonstrated a promising network-based infrastructure to identify potential targets and anticancer drug repositioning candidates for precision cancer medicine by targeting cancer driver genes that can be discovered in massive amounts of cancer genomics data.

## CONTRIBUTORS

F.C. and Z.Z. conceived and designed the study. F.C. carried out experiments and most of the data analysis. J.Z. and M.F. participated in data analysis. F.C. and Z.Z. wrote the manuscript.

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## COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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## SUPPLEMENTARY MATERIAL

Supplementary material is available online at http://jamia.oxfordjournals.org/.

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