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ARTICLE

A network paradigm predicts drug synergistic effects using downstream protein–protein interactions

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Abstract

In some cases, drug combinations affect adverse outcome phenotypes by binding the same protein; however, drug-binding proteins are associated through protein–protein interaction (PPI) networks within the cell, suggesting that drug phenotypes may result from long-range network effects. We first used PPI network analysis to classify drugs based on proteins downstream of their targets and next predicted drug combination effects where drugs shared network proteins but had distinct binding proteins (e.g., targets, enzymes, or transporters). By classifying drugs using their downstream proteins, we had an 80.7% sensitivity for predicting rare drug combination effects documented in gold-standard datasets. We further measured the effect of predicted drug combinations on adverse outcome phenotypes using novel observational studies in the electronic health record. We tested predictions for 60 network-drug classes on seven adverse outcomes and measured changes in clinical outcomes for predicted combinations. These results demonstrate a novel paradigm for anticipating drug synergistic effects using proteins downstream of drug targets.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Current knowledge of drug–drug interactions (DDIs) emphasize the drug target level by identifying shared transporters, enzymes, or pharmacodynamic targets, and do not prioritize proteins downstream of targets.

WHAT QUESTION DID THIS STUDY ADDRESS?

Here, we sought to address if proteins downstream of drug targets were sufficient to predict DDIs; we used protein interaction network analysis and real-world evidence to predict and detect rare DDIs mediated by downstream proteins.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

These results provide evidence that downstream proteins are sufficient for anticipating drug–drug effects.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

From these results, anticipating drug side effects during drug discovery and clinical development may consider drug interactions mediated through downstream proteins.

INTRODUCTION

Many drug–drug interactions (DDIs) associated with adverse effects occur from a shared binding protein, where drugs share similar targets, enzymes, carrier, or transporter proteins. For example, one drug can inhibit an enzyme that is responsible for metabolism of another drug substrate. However, not all DDIs are explained by this mechanism. Regulatory guidances recommend clinical[1] and in vitro experiments[2] to evaluate a drug's potential for drug interactions; these recommendations emphasize the study of drug metabolizing enzymes and transporters that are relevant to other marketed therapies. DrugBank's DDI database[3,4] curates DDIs based on shared protein mechanisms and PharmGKB[5] curates drug–gene interactions, but neither consider the effects of proteins downstream of drug targets. In contrast, evidence suggests that drugs synergize through pathway effects without shared binding proteins. For example, the combined use of the chemotherapeutic drugs paclitaxel and carboplatin reduced hematopoietic toxicity experienced with carboplatin alone yet the combination did not affect the pharmacokinetics of either single drug,[6] suggesting a non-shared-protein mechanism.

Using in silico methods, such as protein–protein interaction (PPI) network models, to anticipate drug effects is attractive because of the relative ease and scale of these methods for making predictions. These approaches have successfully predicted opportunities for drug repurposing,[7-10] for treating co-morbid conditions,[11] for identifying DDIs,[12,13] and for understanding disease mechanisms.[14] Already, there is mounting evidence that single and combination drug effects propagate through protein networks. Yet, downstream PPIs are not routinely used to anticipate drug effects in regulatory and industry settings because of the propensity of these models to overpredict drug phenotypes. We recently developed a per-phenotype PPI network approach that improved prediction performance 50% and increased average precision 76–95% when anticipating single drug adverse events, compared with global approaches.[15] Interestingly, downstream proteins, relative to drug targets, were highly weighted in predicting a drug's adverse outcome. Further, downstream proteins distinguished true from false positive predictions and were integral to preventing overprediction. Because drug effects propagate through networks and our previous discovery that downstream proteins were predictive of drug adverse outcomes, we hypothesized that downstream proteins could be predictive of DDIs when drugs did not share binding proteins.

We explored the extent to which PPIs downstream from the targets of two drugs were sufficient to predict DDIs in cases where the drugs had distinct binding proteins (motivated in Figure 1; we refer to drug targets, enzymes, carriers, or transporters as "targets" in the rest of this analysis). To complete this analysis, we generated a novel set of adverse drug reaction (ADR) pairs by extracting these relationships from the drugs' labels. Informed by the success of our per-phenotype PPI approach, we used meta-analysis to prioritize proteins downstream of targets of drugs labeled with the same ADR and re-classified drugs using these network proteins. We then predicted DDIs for drugs using their network class. We validated predicted combinations using novel observational studies in the electronic health record (EHR) and demonstrated an ability to detect rare DDIs using protein interactions downstream of their targets. Although we used ADRs as a case study, our network paradigm is broadly applicable to

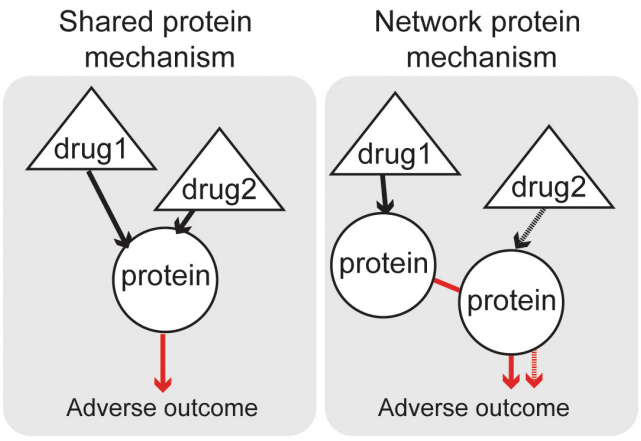

FIGURE 1 A downstream-protein paradigm for detecting drug–drug interactions (DDIs) is distinct from a shared-protein paradigm. In a shared protein mechanism, two drugs share a protein target (transporter, metabolizing enzyme, etc.), and this competition causes an adverse outcome. In a network protein mechanism, protein–protein interactions connect drugs' target proteins. A DDI is caused when a second drug targets a protein downstream of the original drug. Downstream proteins can be used to reclassify drugs.

all biological phenotypes, suggesting a relatively simple, and useful approach for anticipating drug synergistic effects generally.

MATERIALS AND METHODS

Data and code availability

The data and code used in this paper and referenced in this section are available at https://github.com/jenwilson521/Designated-Medical-Event-Pathways. Note: In the computational analysis, we used "DME" as shorthand for "ADR." For transparency, we noted each script used for each analysis in the Results section. Patient data from the EHR analysis is not made available to respect patient privacy and data use agreements.

Extracting adverse reaction phenotypes from drug labels

An algorithm was built using Linguamatics, a natural language processing software, to extract designated medical events (DMEs; ADRs) as MedDRA Preferred Terms from the black box warning, warnings and precautions, and adverse reactions sections of the US Food and Drug Administration (FDA) product labels. All available FDA product labels (as of December 2017) were obtained from DailyMed and indexed in Linguamatics. For each ADR, the related MedDRA Preferred Term, Lower Level Term, and colloquial terms were searched (i.e., "SJS" was an additional term searched for "Stevens–Johnson syndrome"). Drugs with one or more ADRs in their product label were exported for analysis in PathFX. The data from this analysis are included in File S1 (supp1_Drugs_labeled_for_AEs.pdf, additionally, supp1_Drugs_labeled_for_AEs.txt is available in the Github to facilitate reproducibility).

PathFX modeling of marketed drugs and identification of pathway associations to ADRs

To find pathway associations to ADRs, we used the PathFX algorithm[8] to identify network relationships between drug targets and ADR-associated proteins. Compared with other methods, PathFX used a data-driven approach to discover network associations, the algorithm generated "white-box" predictions of drug associations, and demonstrated high specificity in predicting drug-ADR effects.[8] We used drug targets from DrugBank[3] (version 5.1.0) as inputs to PathFX. This analysis yielded a dataset of drug-ADR associations and downstream proteins associated with ADRs from drug labels. A summary of the PathFX algorithm approach and detailed description of the analysis and results is included in the Supplemental Materials and Methods.

Network meta-analysis

We next used meta-analysis to identify downstream proteins shared between drugs with the same ADR. PathFX contained multiple phenotypes associated with ADRs from a drug label (e.g., "Hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency" and "Hemolytic anemia" were both considered as a prediction of "anemia"), and we collapsed these phenotypes when investigating each ADR. For a full description of the meta-analysis and pathways considered, please see the Supplemental Materials and Methods.

Considering hypotheses for clinical evaluation

We leveraged data in TWOSIDES[16-18] as a filter for predicted drug combinations. TWOSIDES used data from the FDA Adverse Event Reporting System (FAERS) for identifying adverse outcomes that were statistically associated with combinations of drugs. We searched TWOSIDES for our predicted combinations to assess whether drug combinations were observed clinically and to get an estimate of the potential effect size of a drug combination on an adverse outcome. We used the scripts */char_data/charac_novel_combinations.py* and */Code/charac_novel_combos_using_int.py* to investigate if TWOSIDES supported our predicted drug combinations for ADR-associated network proteins (ARPs) or any protein on a shortest-path between a drug target and ADR protein (SPs). We leveraged drug synonyms from DrugBank (*contained in /data/drugbank_vocabulary.csv*) to match drug combinations from TWOSIDES with our predicted DDIs. We later filtered drug combinations that overlapped from our predictions and TWOSIDES if the predicted adverse drug reactions (ADRs) were synonymous.

We next aggregated predicted DDIs by network class. We used drug–drug-network protein-ADR data from */data/cotherapy/potential_co_therapies.xlsx* and the filtered drug–drug combinations from TWOSIDES to generate predictions for our expanded observational studies. These predictions are contained in */char_data/network_mechanisms_for_ehr_ml.xlsx* and are summarized in File S5-6.

Novel observation studies using the Optum Clinformatics Dataset

We pursued 60 novel observational studies using the Optum Clinformatics dataset using best practices for propensity matching patients to control confounding. Importantly, we used a de-identified dataset that did not require institutional review board (IRB) approval. A full description of the dataset and methods are provided in the Supplemental Materials and Methods.

RESULTS

Network analysis of single drugs with the same ADR

We first discovered PPI associations between a drug's target(s) and ADRs listed in the drug product's FDA-approved drug labeling. Specifically, we focused our investigation on a list of designated medical events which are ADRs of high priority in regulatory review. We used a natural language processing method to extract ADRs from the warnings, boxed warnings, adverse reactions, and precautions sections of the drugs' labels. This analysis yielded associations between 1970 drugs and 34 ADRs. This provided a unique dataset for interrogating network proteins of drugs associated with ADRs.

For network analysis, we restricted our analysis to 1136 drugs that had drug-binding proteins listed in DrugBank[3] and further restricted to 970 drugs whose targets were connected in our PPI network.[8] We used the PathFX algorithm[8] to create networks for these drugs (Figure 2, File S2). Compared with other PPI network models, PathFX used the amount and quality of evidence supporting PPIs around drug targets to prioritize downstream proteins and then used statistical enrichment to discover phenotypes enriched in the drug's network. Importantly, PathFX was naïve to a drug's true set of phenotypes (e.g., an ADR from the drug label or the drug's intent-to-treat disease) and instead used the corpus of evidence to anticipate drug network associations (further discussed in the Methods section). PathFX discovered network associations for 424 drugs to 24 ADRs.

This analysis discovered downstream proteins that were common to multiple drug-ADR pairs and distinct to ADRs (Figure 3a, File S3 and S4). For example, for drugs labeled with sepsis, their networks shared drug-binding and downstream proteins (Figure 3b). Because of these patterns, we reclassified drugs based on shared downstream proteins (Figure 2b). For instance, multiple drugs associated with sepsis contained the adrenoreceptor beta 2 (ADRB2) downstream of their drug targets; this yielded two new classes for sepsis-associated drugs: "ADBR2 network" drugs and "non-ADBR2 network" drugs. We repeated this reclassification for all shared downstream proteins across all 24 ADRs and tracked two types of network proteins for classification – ARPs or any SPs. We discovered 172 network classes (each corresponding to 172 "non-Gene-net" classes) across 12 ADRs or 1623 classes across 24 ADRs using ARPs, or SPs, respectively.

Next, using non-ADR drugs, we predicted novel DDIs for each network class where non-ADR drugs had target proteins downstream in the network class. For instance, ADBR2 is a target for the drug albuterol and albuterol is not associated with sepsis on its label. We predicted that "ADBR2 network" drugs would interact with albuterol to affect sepsis outcomes. In total, we predicted 18,988 drug–drug-ADR combinations using ARPs (51,605 combinations using SPs) from network classification. We further removed predicted DDIs if the drugs shared any target proteins because we were motivated to understand DDI effects due to downstream proteins. This yielded 6098 drug–drug-ADR triplets using ARPs (19,741 triplets using SPs) representing 5246 unique drug–drug pairs using ARPs (11,904 unique pairs using SPs) for further consideration (some drug–drug pairs were associated with multiple ADRs).

Literature, TWOSIDES evidence supports combination effects and suggests directionality

We estimated the sensitivity of our method by using TWOSIDES,[17,18] a well-regarded dataset for drug combination effects. TWOSIDES uses the FDA Adverse Event Reporting System (FAERS) to detect DDIs based on the relative reporting rates of combination drugs as compared with single drugs while controlling for confounding variables.[17,18] Predicted DDIs in TWOSIDES indicated combinations prescribed in the real world. For reference, TWOSIDES contained 42,920,391 drug–drug-ADR sets reported for 211,990 unique drug–drug pairs. Of note, TWOSIDES contained DDIs for 12,726 unique ADRs and included many more and milder side effects than our analysis (e.g., diarrhea and headache). We next counted our total drug–drug predictions and drug–drug-ADR triplets tracking both ARPs and SPs (Table 1). We first filtered our predictions by drug–drug combinations documented in TWOSIDES, reasoning that if a drug combination was reported in TWOSIDES, the combination was likely prescribed in the real world. To estimate the sensitivity of our method, we counted predicted drug–drug-ADR triplets documented in TWOSIDES (Table 1). From these results, using ARPs relative to SPs generated a higher sensitivity for detecting DDIs (80.7% vs. 50.2%).

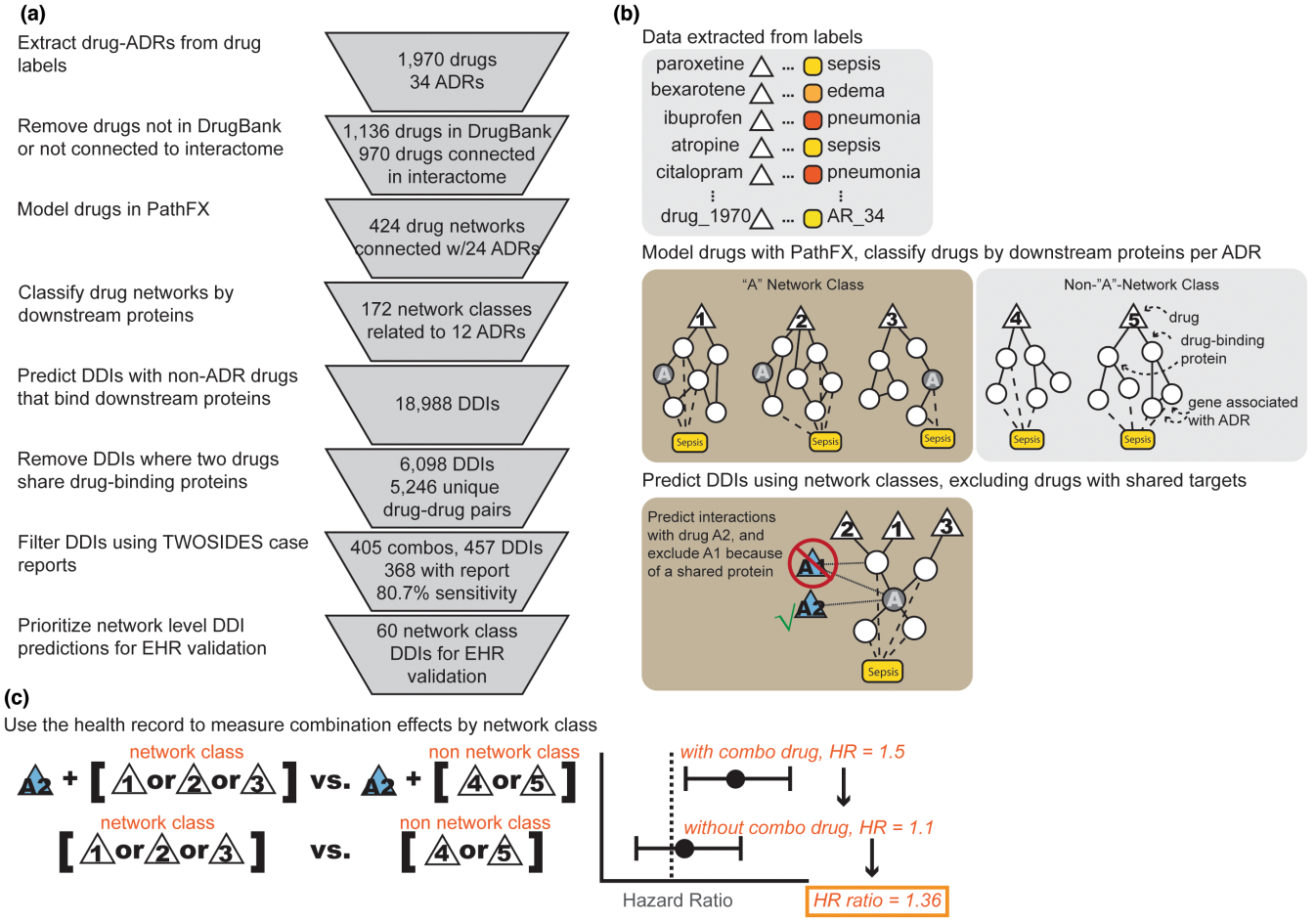

FIGURE 2 Project workflow used pathway modeling and electronic health record (EHR) analysis to assess drug–drug interactions (DDIs) predicted by network class. (a) Starting with drug-adverse drug reaction (ADR) relationships from drug labels, we used protein–protein interaction (PPI) modeling and network classification to predict DDIs (workflow demonstrated for ADR-associated network proteins [ARPs]). We filtered predicted DDIs using TWOSIDES for further validation. (b) The starting drug-ADR dataset comprised 1970 drugs associated with 34 different ADRs. For the 970 drugs with targets connected to our interactome, we constructed networks and looked for downstream associations to ADRs. We used downstream ARPs to define network classes and predicted DDIs where non-ADR drugs targeted downstream proteins. The figure depicts a hypothetical "GENE A" class based on the downstream protein, "A." (c) We validated predicted DDIs by measuring hazard ratios between "network" and "non-network" classes with (top row) and without (bottom row) the predicted combination drug and took the hazard ratios (HRs) to estimate the DDI effect. Hypothetical example shown to depict experimental set-up.

Our PathFX analysis identified non-directional associations and motivated us to pursue complementary data sources. For instance, our network analysis discovered an association between the drug paroxetine that had sepsis on its label, a non-sepsis-labeled drug, albuterol, and the ADR, sepsis, but our analysis did not indicate whether co-administering albuterol with paroxetine would reduce or worsen sepsis. We used the literature to infer the directionality of drug effects. Specifically, we used sentences to identify if a combination drug would worsen or mitigate the drug-induced ADR. We searched for combo-drug-ADR relationships within PubMed abstracts using natural language processing. Using emerging results from the literature was important because it did not replicate data used in the network analysis. We manually curated sentences containing mention of combo drugs and ADRs to understand how the combo drug may affect the ADR. For instance, the agonist compound albuterol, binds the ADRB2 protein, which is downstream in the interaction network of three drugs that are associated with sepsis on their drug labels (paroxetine, atropine, and cocaine). Albuterol is not associated with sepsis on its drug label, yet in our search of published abstracts, we discovered that albuterol is associated with sepsis in a rat model. Specifically, we discovered and manually validated the following sentence to support further consideration of these combinations in our study: "This study showed for the first time that oral administration of albuterol exerted protective effects on CLP-induced sepsis and related lung injury in rats."[19] The full list of predicted drug–drug-ADR combinations, their

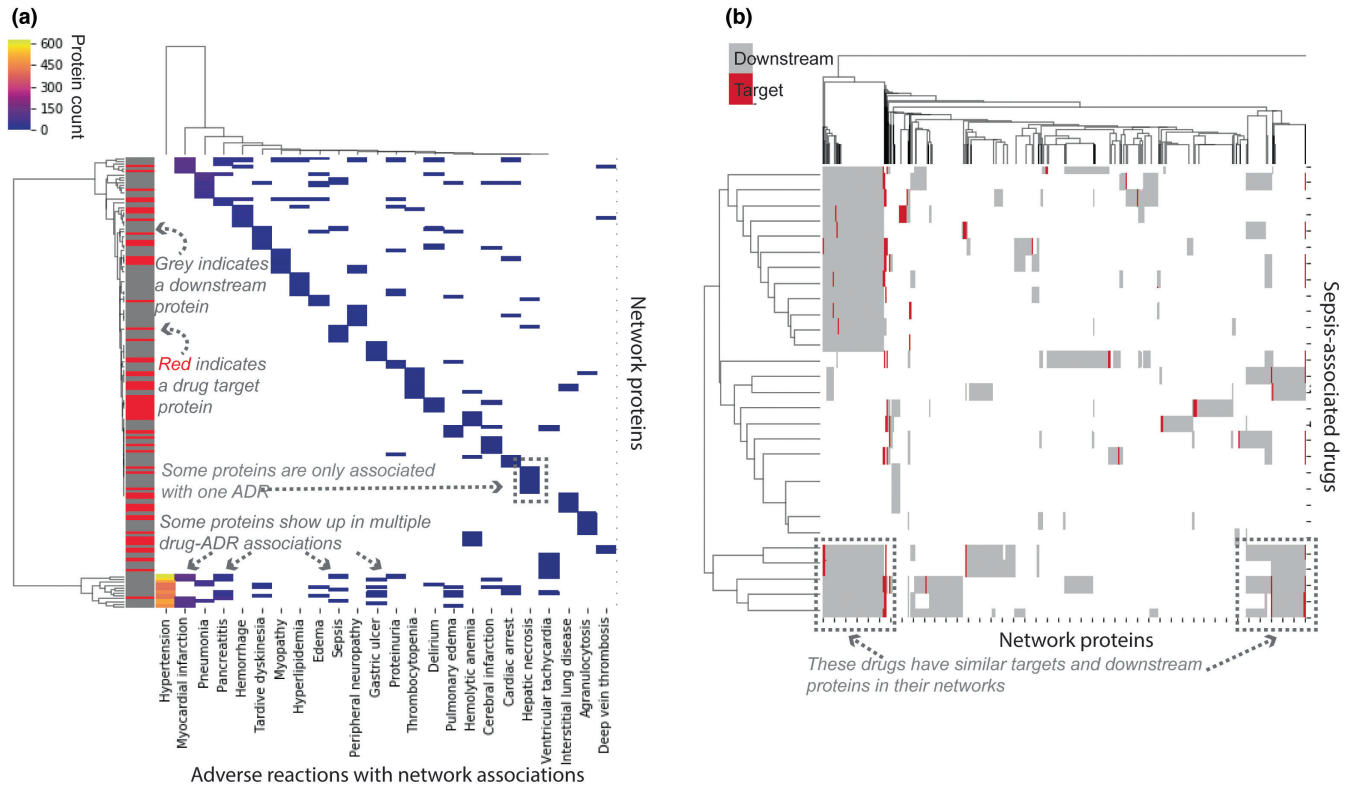

FIGURE 3 Drug networks have common target and downstream proteins across and within adverse drug reactions (ADRs). (a) The most common target (red) and downstream (gray) proteins (rows) for all drug networks associated with 24 ADRs (columns). Protein count indicates in how many drug networks the protein appears (b) Target (red) and downstream (gray) proteins (columns) for all sepsis-associated drugs networks (rows).

TABLE 1 Sensitivity of DDI prediction using network classification

	ADR-associated proteins (ARPs)	Proteins on a shortest path between drug target and ADR-gene (SPs)
Drug pairs documented in TWOSIDES/ Total predicted drug pairs	405/5246 (7.7%)	964/11,904 (8.1%)
Drug–drug-ADR triplet in TWOSIDES/ Predicted drug–drug-ADR triplets	368/456 (80.7%)	786/1565 (50.2%)

Abbreviations: ADR, adverse drug reaction; DDI, drug–drug interaction.

relevant network proteins, and manually curated literature evidence are provided in File S7.

We further investigated predicted drug–drug-ADR combinations with clinical data using published databases and novel observational studies. To consider feasibility of detecting drug combination effects in patient data, we again referenced TWOSIDES,[17,18] and investigated the proportional reporting ratios (PRRs) documented for the 368 combinations discovered using ARPs. We used PRRs documented in the TWOSIDES database; importantly, TWOSIDES corrected for hidden and unmeasured covariates when calculating predicted drug combination effects. PRRs were a sufficient proxy for ADR severity of predicted DDIs. Indeed, predicted combination effects were discovered in TWOSIDES (Table 2, full results in File S6). Further, because PathFX networks do not contain directional pathway information, measuring a drug-combination effect in TWOSIDES suggested that drug combinations may increase risk for ADRs in the real world.

We also pursued multiple novel observational studies to test our hypotheses and used two approaches to conduct this analysis. For these analyses, we used the de-identified Optum Clinformatics dataset version 7 that included over 88 million US patients, both privately insured and Medicare beneficiaries, largely under the age of 65 years. We accessed a version of the Optum dataset standardized to the Observational Medical Outcomes Partnership (OMOP) common data model (CDM); standardized data models have decreased heterogeneity between datasets and improved consistency in underlying data.[20] We leveraged

TABLE 2 TWOSIDES supports predicted drug combination effects

Combo drug	ADR-associated drug	Adverse event search term	TWOSIDES condition name	PRR
Aspirin	Aripiprazole	Pancreatitis	Pancreatitis chronic	20
			Pancreatitis relapsing	20
			Pancreatitis acute	12.1053
			Pancreatitis	7.56757
	Atropine		Pancreatitis	5
	Pramipexole		Pancreatitis	1.5
			Pancreatitis acute	0.5
	Ropinirole		Pancreatitis chronic	2.5
			Pancreatitis	1.09091
Albuterol	Atropine	Sepsis	Sepsis	5
			Urosepsis	10

Abbreviations: ADR, adverse drug reaction; PRR, proportional reporting ratio as published in ref.[18].

the Observational Health Data Sciences and Informatics (OHDSI) network tools, specifically CohortMethod to measure ADR outcomes for patients exposed to our predicted combinations. CohortMethod is a software package that facilitates extracting patient data from the EHR, conducting large-scale propensity matching for controlling confounding variables, and estimating outcome models, such as Cox regression (example applications refs.[21,22]). The LEGEND study is one relevant application where CohortMethod measured cardiovascular outcomes across first-line antihypertensive drugs.[21] Our approach was conceptually similar, however, we aggregated drugs into network classes instead of by their chemical structures or therapeutic use classes. We first used CohortMethod to test two network DDI predictions to validate our ability to detect DDIs in the real world. We used a customized pipeline to analyze the 58 network classes that encompassed the 368 drug–drug-ADR triplets documented in TWOSIDES.

Altered sepsis outcomes for network-classified drugs in a novel observational study

We first investigated the effect of albuterol on beta-2 adrenergic receptor 2 (ADRB2) network drugs. PathFX identified network associations for 29 drugs with sepsis listed on the drugs' labels. From this 29-drug set, two drugs, paroxetine and atropine, contained ADRB2, an albuterol (also known as salbutamol) drug target, downstream in their networks and did not share other target proteins with albuterol. Of the remaining 27 drugs, 18 drugs did not contain albuterol-binding proteins downstream in their networks nor share drug target proteins with albuterol. These 18 drugs were considered the "non-ADRB2-net" class (Table S1). We hypothesized that concomitant use of albuterol would alter the risk of sepsis for ADBR2-network drugs relative to non-ADBR2-network drugs.

For the first measurement, we measured the risk of sepsis for patients on ADBR2-network ("target" cohort) or non-ADBR2-network ("comparator" cohort) drugs. For the second measurement, we measured the risk of sepsis for patients with an overlapping exposure to albuterol + ADBR2-network drugs ("target" cohort) or albuterol + non-ADBR2-network ("comparator" cohort) drugs. To select patients with an overlapping exposure, we required patients have an albuterol "DRUG ERA" that started between the start and end of an exposure to either the ADBR2-network or non-ADBR2-network drugs and the risk for sepsis was observed for 30 days following the start of the second drug exposure. The drug era is considered a sufficient proxy to estimate an exposure to an active ingredient and the details of this data table are further explained in the Materials and Methods section. We further used large-scale propensity matching to estimate confounding and then matched patients based on their propensity score to estimate risk. Propensity matching aggregates all available patient data in the health record, including commonly considered confounders, such age, diagnoses, and demographics, as well as other data, such as number of visits and time to visits, that also reflect patient characteristics.[17,18,23,24] After matching, we discovered good covariate balance between the target and comparator cohorts and sufficient patient attrition for measuring outcomes (Figure S1, File S6).

We measured the risk of sepsis between these two drug classes without a combination therapy and with co-administration of albuterol (Figure 4, Table 3). The risk of sepsis occurring in the ADBR2-net class is increased

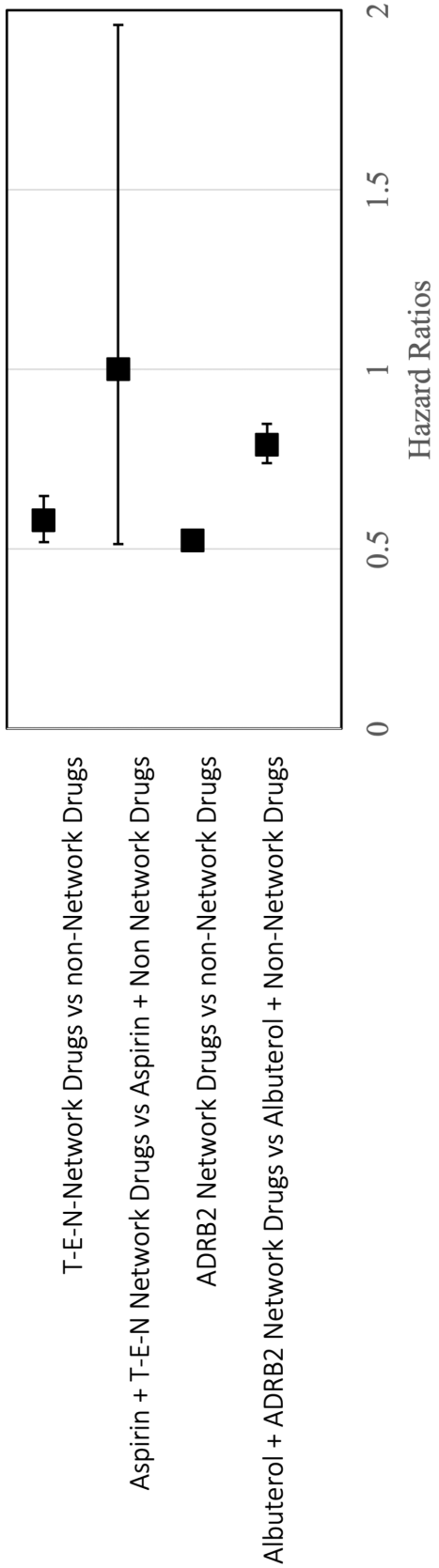

FIGURE 4 Hazard ratio estimates for ADBR2 and T-E-N network classes. We estimated the between class effects with and without a combination drug for two predicted drug–drug interaction (DDI) effects.

compared to the non-ADBR2-net class when albuterol is used concurrently: hazard ratio (HR) = 0.792 with the combination compared to HR = 0.525 without the combination; this yielded an HR ratio of 1.51. The risk of sepsis from paroxetine or atropine (ADBR2-network class) was less than non-ADBR2-network class drugs, however, the combined use of albuterol with paroxetine or atropine increased the risk of sepsis compared to non-ADBR2-network class. We did not discover literature evidence supporting sepsis outcomes in combined use of atropine or paroxetine with albuterol. A retrospective chart review supported that albuterol and atropine were both therapeutic options for systematic bradycardia,[25] suggesting that patients may have overlapping exposures to these drugs. A clinical trial in infants suffering from chronic lung disease observed that salbutamol (a synonym of albuterol) had no observable effect on patient sepsis,[26] further supporting that albuterol is not associated with sepsis when used alone.

Altered pancreatitis outcomes for network-classified drugs in a novel observational study

We additionally repeated this process, this time emphasizing the effect of aspirin (also known as acetylsalicylic acid) prescribed in combination with drug network classes associated with aspirin target proteins on the ADR, pancreatitis. We measured HRs for patients in these groups (Figure 4, Table 3) and observed a shift in HR for patients taking the predicted combination drug (full explanation included in the Supplemental Results).

Novel observational studies for 58 additional DDI classes discovered using ARPs

We sought validation for an additional 58 network-class DDIs predicted from using ARPs because these predictions had higher sensitivity for anticipating effects in TWOSIDES (File S6). For these 58 predictions, we estimated an HR using Cox regression on a 1–1 propensity score matched cohort with a caliper of 0.1. We used a logistic regression propensity score model trained on low dimensional CLMBR patient representations.[27] Precomputed CLMBR representations enabled more rapid cohort definitions and HR ratio estimation than CohortMethod. Like before, we included patients in our baseline/combo analysis if they had exposure to drugs in the network or non-network classes with/without the predicted combo drug, respectively. We

TABLE 3 Adverse event hazard ratios are altered in drugs predicted to have combination network effects

Comparison	HR	Lower 0.95	Upper 0.95
Pancreatitis			
T-E-N-Network Drugs vs. non-Network Drugs	0.580	0.519	0.648
Aspirin + T-E-N-Network Drugs_vs_Aspirin + non-Network Drugs	1.001	0.514	1.959
Sepsis			
ADRB2-network Drugs vs. non-Network Drugs	0.525	0.499	0.552
Albuterol + ADRB2-Network Drugs_vs_Albuterol + non-Network Drugs	0.792	0.739	0.848

Abbreviation: HR, hazard ratio.

measured HRs between network and non-network drug classes with and without drug combinations.

Like the two cases outlined above, we measured the relative risk between the net-GENE and non-net-GENE classes with and without the predicted combination drug for the 58 remaining class predictions (File S5). Not surprisingly because our drug interaction predictions are rare, we were unable to generate sufficient patient cohorts to measure HRs for all predicted classes. For 21 of the 58 total classes, we had sufficient patients to measure HRs between the net-GENE and non-net-GENE classes and for eight of these cases, there were also sufficient patients to measure HRs between drug classes with the predicted combination drugs (Figure S3). However, we removed one class prediction because the predicted combination drug's indication was too similar to the side effect. For the seven remaining classes, we measured the change in Cox coefficient to estimate effects of the predicted DDIs (Table 4, Figure S4). These DDI effects were moderate with HR ratios from 0.85–1.17. The highest HR ratio, 1.17, was measured for drug-induced hypertension associated with proopiomelanocortin (POMC) network class drugs used in combination with loperamide, an ingredient used to treat diarrhea. The lowest HR ratio, 0.85, was measured for drug-induced hypertension associated with prostaglandin E receptor 4 (PTGER4) network class drugs used in combination with misoprostol, an ingredient once used to treat stomach ulcers. This result suggests a protective effect of misoprostol for the PTGER4 network drug class.

DISCUSSION

We predicted DDIs using network classification and validated our predictions using DDI databases and novel observational studies. We first extracted a novel dataset of ADR pairs using data extracted from drug labels. This dataset was crucial to our analysis and will be valuable to other investigations of ADRs. We used network analysis to discover downstream proteins associated with ADRs, reclassified drugs by their downstream proteins, and predicted DDIs based on network classification. We demonstrated high sensitivity for detecting rare DDIs using ARPs for classification, further supporting that rare or emerging drug–drug effects may arise when drugs do not share protein targets. We validated DDI predictions for albuterol and aspirin based on network classification and for 58 additional DDI effects using novel observational studies. Overall, these results provide evidence for investigating downstream proteins for anticipating DDIs and that protein–protein interactions between drugs' targets are sufficient for identifying drug combination effects.

Compared with other network approaches, our analysis was, to our knowledge, unique in the requirement that we excluded drugs with shared protein targets. This allowed us to exclusively explore DDIs that resulted from downstream effects and not a shared protein mechanism. Other approaches to predicting DDIs are extensively reviewed in ref.[28] and our approach is most like the network propagation technique of Park et al.[29] PathFX, like their approach, begins with a diffusion-based approach to identify potential signaling cascades affected by binding a drug's target(s). Instead of requiring two drugs to have similar signaling networks, we only required that an ADR-associated drug "diffuse" to the target of a non-ADR drug target. We prioritized DDIs where multiple ADR drugs converged on similar non-ADR drug targets (e.g., ADRB2).

Fortunately, rigorous regulatory review and good clinical practices prevent the use of many harmful drug combinations, and this limited our ability to extensively validate every prediction. We could not measure DDI effect sizes for all network predictions, yet we found evidence for rare drug combinations. Nonetheless, in silico network analysis is relatively cheap and efficient and could aid in therapeutic development where anticipation of ADRs is essential for therapeutic development. Further, our predictions are not documented in routinely used DDI data sources and integration of these predictions could inform clinical care or further research efforts. Our discovery of

TABLE 4 Additional HR ratios estimated from EHR analysis

With combo drug		Without combo drug						
HR	*p* value	HR	*p* value	HR ratio	ADR	Combo drug	Downstream protein	ExpNum
1.09	4.61E-01	0.93	6.33E-04	1.17	Hypertension	Loperamide	POMC	Exp22
1.00	1.00E+00	0.88	6.65E-02	1.14	Pancreatitis	Sucralfate	EGF	Exp35
1.17	2.86E-03	1.15	9.72E-13	1.01	Hypertension	Sucralfate	EGF	Exp17
1.13	1.29E-02	1.12	1.48E-12	1.01	Edema	Aliskiren	REN	Exp2
1.00	1.00E+00	1.02	6.74E-01	0.98	Myopathy	Sucralfate	EGF	Exp29
1.08	6.94E-01	1.20	9.55E-21	0.90	Hypertension	Gentamicin	LRP2	Exp8
0.96	5.24E-01	1.12	3.48E-08	0.85	Hypertension	Misoprostol	PTGER4	Exp18

Note: Network and non-network class drugs are listed in File S6 and are referenced by the experimental number ("ExpNum").

Abbreviations: ADR, adverse drug reaction; EHR-ML, electronic health record; HR, hazard ratio.

drug combinations that mitigated ADR outcomes suggested a new paradigm for managing drug induced ADRs; specifically, that mitigating therapies could be prescribed based on drug network class. Similarly, in silico network analysis which predicts therapies to mitigate side effects could also inform safety analysis plans for clinical development.

Our results also have implications for advancing PPI networks for anticipating drug effects. There is sufficient evidence that PPI networks can anticipate drug effects and be used predictively for identifying repurposing opportunities. However, our analysis is distinct because of our emphasis on attribution; we aimed to ascribe drug effects to specific downstream proteins. Classifying drugs by their downstream proteins and measuring relative ADR risk in the presence of secondary drugs is evidence that drug effects could be attributed to downstream proteins discovered from PathFX network analysis. Further experimental validation would be required to investigate these hypotheses. However, it suggests that PPI methods are useful not just for pattern discovery (e.g., drug A's network is like drug B's network) but also for predicting mechanistic effects (e.g., drug A's ADR outcome is mediated by the downstream protein Y).

Our study expands a growing body of knowledge that drugs can exert synergistic effects without sharing drug-binding proteins, which may lead to a better understanding of ADRs and rational design of new therapeutic combinations. Drug synergy is a broad field where many frameworks are used to anticipate drug effects.[30,31] Some approaches leverage "supra-additive" effects of drugs used in combination,[32,33] yet these measurements often rely on complex and relatively costly high-throughput screens.[34] Although the performance of computational synergy prediction algorithms has increased, these effects have yielded little success in the clinic.[31,35] A community competition for synergy prediction noted that drugs with high experimental synergy contained drug targets in the same pathway and further, that well-predicted drug synergies occurred when combination drug targets were downstream of a shared protein.[31] Whereas we used drug-induced ADRs as the focus of this investigation, analysis of proteins downstream of drug targets could improve prediction of drug synergistic effects on disease outcomes.

AUTHOR CONTRIBUTIONS

J.L.W. wrote the manuscript. J.L.W. designed the research. J.L.W., E.S., and R.R. performed the research. J.L.W., E.S., and R.R. analyzed the data. E.S., N.S., R.B.A., and K.G. contributed new reagents/analytical tools.

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CONFLICT OF INTEREST

R.B.A. is a founder and stockholder in Personalis, and a stockholder in 23andMe; he declares no conflicts of interest. J.L.W. was a consultant for Sanofi from July to December 2021; she declares no conflicts of interest. All other authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

All data are available in the main text or the Supplementary Materials.

DISCLAIMER

The opinions expressed in this paper are those of the authors and should not be interpreted as the position of the US Food and Drug Administration.

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References 36–39 are cited in supporting information.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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