Drugs synergistically affect adverse outcomes through protein-protein interaction networks

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**Abstract:** In some cases, drug combinations may cause adverse outcomes by binding a shared protein target, however, drug-binding proteins associate with each other through protein-protein interaction networks within the cell, suggesting that adverse outcomes could result from long-range, network effects. Here we investigated whether combinations of drugs could affect severe adverse outcomes if the drug’s binding proteins were known to interact through protein-protein interaction networks. We first identified drug-drug-adverse-outcome relationships using an interaction network analysis and validated these predictions using an observational study in the electronic clinical record. Specifically, we predicted and measured that aspirin exacerbates pancreatitis for drugs whose targets are proximal to aspirin targets in the protein-protein interactome and that salbutamol increased risk for sepsis if a drug’s network contained beta-adrenergic receptor 2 (ADrB2), which is a salbutamol-binding protein. These results demonstrate a novel paradigm for considering adverse outcomes from drug combinations when outcomes do not arise from binding a shared protein target and the network approach hypothesizes potential mechanisms for these effects.

**One Sentence Summary:** Protein-protein interactions of drug targets can mediate severe adverse outcomes of drug combinations.

**Main Text:**

While dysregulated pathways are widely accepted for their role in disease, protein network methods for pathway discovery are not yet sufficiently validated to be routinely considered for explaining drug mechanistic effects. Indeed, especially in oncology, detailed biochemical analysis of signaling pathways has informed drug mechanisms and signaling networks have guided cancer therapeutic target selection (*1*) and predicted mechanisms for drug resistance (CITE). Many have considered network-based drug discovery, or “network pharmacology”, as a promising new frontier for mechanistic drug discovery(*2*). Yet, network pharmacology lacks robust and reliable methods for defining drug pathways with mechanistic value(*2*). Towards the goal of elucidating general engineering principles for network methods, we first investigated if the downstream proteins discovered from a network analysis for drug pathways had mechanistic value. We considered drug combination analysis as one way to test the mechanistic relevance of network-selected downstream proteins and in the process discovered a framework for considering synergistic drug combination effects on adverse outcomes.

Regulatory and industry scientists are greatly concerned with investigating drug combination effects; however, it is experimentally intractable to test all possible drug combinations used clinically. From a pharmacokinetic perspective, drug-drug interactions (DDI) often result from the binding of a shared “on” or “off” target protein such as a metabolizing enzyme or transporter. Binding of one drug can affect the metabolism or distribution of a second drug and ultimately affect drug efficacy (*3*). For instance, warfarin and diclofenac both bind the protein, albumin. Administering diclofenac after warfarin will displace warfarin, increase warfarin concentration in the plasma, and can cause serious bleeding (*3*). Because of these known effects, FDA review requires rigorous investigation of possible DDIs using proteins known to affect drug disposition.

However, not all drug combination effects can be attributed to shared protein targets, suggesting that downstream drug mechanisms may be involved. For instance, the combined use of the chemotherapeutic drugs, paclitaxel and carboplatin, reduced hematopoietic toxicity experienced with carboplatin alone and the combination did not affect the pharmacokinetics of either single drug (*4*). While many acknowledge that drug outcomes could arise from network effects, it is difficult to consider these effects if the drug pathway mechanisms are unknown. Protein-protein interaction (PPI) network methods propose novel pathway mechanisms by investigating binding partners of drug-binding proteins, but these mechanisms are largely unvalidated. We hypothesized that PPIs could explain drug combination effects where drug combinations did not share a protein target (motivated in **Fig. 1.).**

**PathFX and meta- analyses discover candidate proteins implicated in drug-DME mechanisms**

We first endeavored to discover network associations between a drug’s targets and severe adverse outcomes listed on their drug labels. Specifically, we investigated designated medical events (DMEs) which are severe adverse drug outcomes of highest priority in regulatory review. We used the PathFX algorithm (*5*) to draw networks for 1,136 drugs for which we extracted DME relationships from the drug labels (**Fig. 2.,** **Sup. File 1.**). PathFX used a drug’s “on” and “off” target binding proteins to discover protein-protein interactions based on the amount and quality of evidence supporting these molecular interactions. The network of drug targets and protein interactions are considered a “draft” of the drug’s pathway. PathFX also discovered phenotypes for which the drug’s pathway proteins are enriched as compared to the interaction network as a whole. We used these network pathways to discover DME phenotypes and used the protein-interaction paths to discover proteins that are candidates for being involved in the drug’s pathway (**Fig. 2B**, for a full explanation of the methods and network analysis, see Materials and Methods).

We additionally used this analysis to discover downstream proteins that were associated with multiple drug-DME pairs and produced a heatmap to summarize gene patterns across DMEs (**Fig 3.**, **Sup. File 2.**, **Sup. File 3.**). By merging networks associated to the same DMEs and counting proteins that were represented in multiple networks, we hypothesized these as candidate proteins involved in the drugs’ downstream pathway mechanism for affecting the DME. For instance, in the case of sepsis (**Fig. 3B**) we discovered network proteins that were drug binding in some drug networks and downstream (“intermediate”) in other networks. Initially we were interested in understanding if the proteins discovered in a network approach were of mechanistic value and hypothesized that drug combinations could be a means to test whether or not the discovered network proteins were related to drug mechanisms. To understand the mechanistic value of downstream proteins, we generated a list of possible drug combinations using proteins that were intermediate in DME networks and were bound by compounds not associated with the DME from the original analysis (examples provided below).

**Literature, TWOSIDES evidence supports combination effects and suggests directionality**

We used a natural-language approach to prioritize predicted combination drug effects using co-mentions from PubMed abstracts. For instance, the inhaled, beta-2 agonist, albuterol (also known as salbutamol), binds the adrenoreceptor beta 2 (ADRB2) protein, and ADRB2 is implicated in the interaction pathway of 3 drugs that are associated with sepsis on their drug labels (paroxetine, atropine, and cocaine). Salbutamol is not associated with sepsis on its drug label, yet in our search of published abstracts, we discovered that salbutamol is associated with sepsis in a rat model. Specifically, we discovered and manually validated the following sentence to support further consideration for observational study: *“This study showed for the first time that oral administration of salbutamol exerted protective effects on CLP-induced sepsis and related lung injury in rats”* (*6*). The full list of predicted combinations, their relevant network proteins, and literature evidence are provided in **Sup. File 4.**

To consider feasibility of detecting drug combination effects and measure potential outcomes, we referenced TWOSIDES(*7*). TWOSIDES uses the FDA Adverse Event Reporting System (FAERs) to detect drug combination effects based on the relative reporting rates of combo drugs as compared to single drugs while controlling for confounding variables. Drug combination effects that are reported in TWOSIDES would indicate drug combinations that are prescribed at sufficient rates for later detection in the clinical health record. Additionally, the proportional reporting ratios (PRRs) reported in TWOSIDES could further highlight the magnitude of effect on adverse outcomes expected from drug combinations. Indeed, some of our predicted combination effects are reported in TWOSIDES (**Table 1**, full results in  **Sup. File 5.**). Further, as PathFX does not contain directional pathway information, measuring a drug-combination effect in TWOSIDES suggests whether the combination synergizes clinically to affect the severe outcome of interest.

**Aspirin increased the probability of pancreatitis for drugs with network associations to aspirin-binding proteins**

We investigated the effect of aspirin prescribed in combination with drugs where PathFX identified aspirin-binding proteins in the drug networks. PathFX identified network associations for 80 drugs with pancreatitis listed on the drugs’ labels (**Sup. File 1.**). From this 80 drug set, 8 drugs contained at least one of the following aspirin-binding proteins – tumor protein p53 (TP53), endothelin receptor type A (EDRNA), or nuclear factor of kappa light chain gene enhancer of B-cells, inhibitor alpha (NFKBIA) – in their networks and did not share other drug-binding proteins with aspirin as documented in DrugBank(*8*). Of the remaining 72 drugs, 28 drugs did not contain aspirin-binding proteins in their networks nor share drug-binding proteins with aspirin. We used the 8-drug set to define a “target” cohort and the 28-drug set to define a “comparator” cohort (full drug set included in **Sup. File 1.**). We first measured the relative risk of pancreatitis between these two drug sets without a combination therapy. Patients were included in the target/comparator cohorts if they had an exposure to one of the drugs in 8-drug/28-drug sets. For the second analysis we considered the effect of the combination drug on either drug set. We defined a combined exposure as having an aspirin “DRUG ERA” that started between the start and end of an exposure to either the 8- or 28- drug set and further measured hazard ratios for patients in these groups (**Fig. 4**., **Tab. 2**). The probability of pancreatitis occurring in the 8-drug set is increased relative to the 28-drug set when aspirin is used concurrently: HR = 1.01 with the combination compared to HR=0.580 without the combination. In both comparisons we used a matched patient set which uses large-scale propensity matching to select patient cohorts where confounding variables are matched for any two target or comparator patients(*9*).

**Salbutamol increases the probability of sepsis for drugs with network associations to the salbutamol-binding protein, beta-2 adrenergic receptor, 2 (ADRB2)**

We next investigated the effect of salbutamol prescribed in combination with drugs where PathFX identified salbutamol-binding proteins in the drug networks. PathFX identified network associations for 29 drugs with sepsis listed on the drugs’ labels (**Sup. File 1.**). From this 29 drug set 2 drugs contained ADRB2 in their networks and did not share other drug-binding proteins with salbutamol as documented in DrugBank(*8*). Of the remaining 27 drugs, 18 drugs did not contain salbutamol-binding proteins in their networks nor share drug binging proteins with salbutamol. We used the 2-drug set to define a “target” cohort and the 18-drug set to define a comparator cohort (full drug set included in **Sup. File 1.**). We first measured the relative risk of pancreatitis between these two drug sets without a combination therapy. Patients were included in the target/comparator cohorts if they had an exposure to one of the drugs in 2-drug/18-drug sets. For the second analysis we considered the effect of the combination drug on either drug set. We defined a combined exposure as having a salbutamol “DRUG ERA” that started between the start and end of an exposure to either the 2- or 18- drug set and further measured hazard ratios for patients in these groups (**Fig. 4**., **Tab. 2**). The probability of sepsis occurring in the 2-drug set is increased relative to the 18-drug set when salbutamol is used concurrently: HR=0.792 with the combination compared to HR=0.525 without the combination. Again, in both comparisons we used a matched patient set which uses large-scale propensity matching to select patient cohorts where confounding variables are matched for any two target or comparator patients(*9*).

**Discussion**

We endeavored to understand the mechanistic value of protein-protein interactions for explaining drug mechanisms and tested the validity of these interactions using drug combination studies in the electronic health record. We were motivated to understand the mechanistic value of network-identified proteins for explaining drug mechanisms to provide further advancing these methods to have value in decision-making settings. The altered effect on adverse outcomes in the presence of drug combinations affirm the utility of these identified proteins. Unfortunately, we were unable to test all predictions, largely due to lack of sufficient patient exposures. However, in terms of deriving engineering principles to advance to network methods, our results further promote the value of discovering drug effects beyond the drug’s targets and potentiate the use of network methods for discovering protein interactions that are relevant to drug pathways.

Network methods are increasing in their popularity for relatively rapid assessment of drug effects and have been applied in multiple therapeutic projects including drug repurposing(*10*), and predicting drug combinations(*11*-*13*). The success in these approaches suggested that networks contained proteins and interactions that were relevant to explaining drug mechanisms. Indeed, these discoveries motivated our investigation into further validating the mechanistic value of these pathways through observational studies. In contrast to these approaches, we took a phenotype-specific approach. Instead of identifying a single mathematical relationship to discover drug combinations, we instead discovered and tested genes specific to each adverse outcome phenotype. Thus, another limitation of our approach is that it is not rapidly applied to test drug combination effects across a range of phenotypes. However, we were encouraged by another recent result where we discovered that phenotype-specific network proteins could better distinguish true from false positive drug-DME associations (submitted). From an engineering perspective, these results suggest that effective network models will curate and learn pathways for each phenotype of interest rather than discovering a single, unifying graph principle that can be applied to all phenotypes of interest.

Our results generated a series of further questions both for understanding drug combination effects as well as techniques for further advancing network methods. For instance, further experimental validation is required to affirm our hypotheses that the identified network proteins are involved in drug mechanisms. In this analysis we were unable to consider drug dosing and it’s likely that dosing could modulate drug combination effects. We used PubMed abstracts to prioritize predicted combination therapies as an orthogonal data set to data used in PathFX, but we did have my other predicted drug combinations that could have clinical impact even if they didn’t have literature evidence. We additionally predicted that some of our combination drugs could have a mitigating effect on an adverse outcome. However, in these scenarios, the predicted combination drug often had an established role in minimizing an adverse effect, and thus, it was not possible to discern if a clinical effect was due to protein-protein interactions discovered in our analysis. Our analysis emphasized drug combination effects on adverse outcomes, but it is also possible to probe these interaction networks for hypothesis to identify drugs with synergistic effects on disease outcomes as well.

We discovered and validated network-level drug combinations for their effects on DMEs of interest. We identified aspirin as a drug that increased the probability of pancreatitis when used with drugs that bind proteins proximal to aspirin drug-binding proteins. Similarly, we discovered that salbutamol increased the probability of sepsis in drugs that bind proteins proximal to salbutamol-binding proteins. In therapeutic development, “drug interactions” are usually considered and studied for the ability of drugs to bind the same protein – a drug target, a transporter or metabolizing protein. Our study further expands a growing body of knowledge that drugs can exert synergistic effects without sharing drug-binding proteins. We were eager to explore this hypothesis not just for better understanding and anticipating drug adverse outcomes, but also to lay the groundwork for rational design of drug combinations for synergistic effects on single- or multiple- diseases based on known protein-protein interactions between the drugs’ targets.

References and Notes:

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Supplementary Materials:

Materials and Methods

Figures S1-S#

Tables S1-S5

Code, data, supplemental methods: <https://github.com/jenwilson521/Designated-Medical-Event-Pathways>

References (*##-##*)

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**Fig. 1.** Network-level combination effects arise when drug-binding proteins share protein-protein interactions. On the left, adverse combination effects may occur if two drugs bind the same – “on” of “off” – target protein. In contrast, on the right, adverse combination effects may occur if two drugs bind proteins that share molecular, protein-protein interactions. The diagram shows two drug-binding proteins that directly interact, but the drug targets may also interact through an intermediate protein as well.

**A screenshot of a cell phone

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**Fig. 2.** **Project workflow uses pathway modeling and electronic health records to assess network-level combination effects**. (**A**) We extract drug-DME associations from the warnings, boxed warnings, and precautions sections of the drug labels for 1,136 drugs using a natural-language processing approach. (**B**) We create networks using PathFX and identify DME network phenotypes and the network genes and proteins associated with the phenotype. In the example, drugs 1,2, and 3 all have gene “A” in their networks. Gene “A” may not directly be associated with the DME but may exist along a pathway associating a drug’s targets to the DME. Drugs 4 and 5 are associated with the same DME but do not have gene “A” in their networks. (**C**) We identify candidate drug combinations using knowledge that combination drugs bind network genes but do not share other targets with the DME-associated drugs and are not associated with the DME on their drug labels. Using a natural-language processing approach, we identify sentences that co-mention the predicted combination drugs (e.g. “X” or “Y”) and the DME. Literature evidence prioritizes drug combinations for testing the electronic health record. (**D**) We conducted multiple drug combination observational studies. To test the effects of predicted combination therapy, “X”, we first measure hazard ratios for the DME of interest using exposure to drugs 1, 2, or 3 to define the target cohort and exposure to drugs 4 or 5 as the comparator cohort (bottom). We next measure the hazard ratios for the DME of interest using overlapping drug exposure to drugs 1, 2, or 3 and drug “X” to define the target cohort and overlapping exposure to drugs 4 or 5 and drug “X” to define the comparator cohort.

A close up of a map

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**Fig. 3. Network meta-analysis reveals patterns among network drug-DME associations.** (**A**) A heatmap highlights the network proteins that were associated with the most drug-DME network associations. Columns represent 24 DMEs where networks discovered an interaction pathway between the drug’s targets and DME-associated genes. Rows are network proteins – both drug-binding (red) and downstream, intermediate proteins (grey). Heatmap coloring represents the number of drug-DME pathways in which the protein was implicated. For some drugs, their networks were associated with multiple DME-relevant phenotypes and proteins were counted based on each phenotype association. For instance, (**B**) The heatmap displays proteins (columns) discovered in networks for drugs associated with sepsis (rows). Coloring indicates whether the protein was drug-binding (red) or an intermediate network protein (grey). Network proteins may be drug-binding in one network but intermediate in another network.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Combo drug** | **DME-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 (salbutamol ) | atropine | sepsis | sepsis | 5 |
| urosepsis | 10 |

**Table 1. TWOSIDES supports predicted drug combination effects**. PRR = proportional reporting ratio as published in(*7*).

**Graphical user interface, application

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**Fig. 4. Adverse event hazard ratios are altered in drugs predicted to have combination network effects.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Comparison** | **HR** | **lower 0.95** | **upper 0.95** |
| *Pancreatitis* | | | |
| TP53,EDNRA,NFKBIAnetworkDrugs\_vs\_nonNetworkDrugs | 0.580 | 0.519 | 0.648 |
| Aspirin+TP53,EDNRA,NFKBIANetworkDrugs\_vs\_Aspirin+NonNetworkDrugs | 1.001 | 0.514 | 1.959 |
| *Sepsis* | | | |
| ADRB2networkDrugs\_vs\_nonNetworkDrugs | 0.525 | 0.499 | 0.552 |
| Salbutamol+ADRB2NetworkDrugs\_vs\_Salbutamol+NonNetworkDrugs | 0.792 | 0.739 | 0.848 |

**Table 2. Adverse event hazard ratios are altered in drugs predicted to have combination network effects.**