Drugs synergistically affect clinical outcomes through downstream protein-protein interactions

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**Abstract:** In some cases, drug combinations cause adverse outcomes by binding the same protein; however, drug-binding proteins are associated through protein-protein interaction networks within the cell, suggesting that adverse outcomes could result from long-range, network effects. We first used protein-protein interaction network analysis to classify drugs based on proteins downstream of their targets and predicted drug combination effects where drugs shared network proteins but had distinct binding proteins (e.g. targets, enzymes, or transporters). While the majority of predicted drug combinations are rarely prescribed, 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 outcomes using novel observational studies in the electronic health record and tested predictions for 10 network-drug classes on 5 adverse outcomes. These results demonstrate a novel paradigm for anticipating drug synergistic effects using proteins downstream of drug tagets.

**One Sentence Summary:** We predicted adverse outcomes for drug combinations using protein-protein interactions downstream of drug targets.

**Main Text:**

Using *in silico* methods, such 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 (*1*-*4*), for treating co-morbid conditions (*5*), for identifying drug-drug interactions (DDIs) (*6*, *7*) and for understanding disease mechanisms(*8*). Already, there is mounting evidence that drug single and combination 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 over-predict 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 to global approaches. (submitted, doi: <https://doi.org/10.1101/2020.12.15.422844>). Interestingly, performance relied on downstream proteins to accurately predict a drug’s adverse outcome.

Many drug interactions occur from a shared binding protein, where drugs share similar targets, enzymes, carrier or transporter proteins, yet not all DDIs are explained by this mechanism. Regulatory guidance for clinical{download:e\_4nco01} and in vitro experiments{Administration:1ZXXB0ZZ}, the Drugbank’s drug-drug interaction database (*9*, *10*)} and the PharmGKB(*11*) curates DDIs based on shared protein mechanisms. In contrast, there’s evidence that drug synergize through pathway effects without shared binding proteins. 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 (*12*). Because drug effects propagate through networks and our 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 downstream proteins – or protein-protein interactions between drugs’ targets – were sufficient to predict DDIs where the drugs had distinct binding proteins (motivated in **Fig. 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 drug-adverse-reaction (AR) pairs by extracting these relationships from the drugs’ labels. Informed by the success of our per-phneotype PPI approach, we used meta-analysis to prioritize proteins downstream of drugs labeled with the same AR and re-classified drugs using these network signatures. We then predicted DDIs for drugs using their network class. We validated predicted combinations using novel observational studies in the electronic health record and demonstrated an ability to detect rare DDIs using protein interactions downstream of their targets, suggesting a novel network-based mechanism for anticipating DDIs.

**PathFX, meta-analyses discover network signatures for drugs with the same AR**

We first discovered PPI associations between a drug’s target(s) and adverse drug reactions listed in the drug product’s FDA-approved drug labeling. Specifically, we focused our investigation on a list of designated medical events which are adverse drug reactions (ARs) of high priority in regulatory review. We used a natural language processing method to extract ARs from the warnings, boxed warnings, and precautions sections of the drugs’ labels. This analysis yielded associations between 1,970 drugs and 34 ARs. This provided a unique dataset for interrogating network signatures of drugs associated with ARs.

For network analysis, we restricted our analyis to 1,136 drugs that had drug-binding proteins listed in DrugBank(*9*) and further restricted to 970 drugs whose targets were connected in our PPI network(*2*). We used the PathFX algorithm (*2*) to create networks for these drugs (**Fig. 2.,** **Sup. File 1.**). Compared to 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. PathFX discovered network associations for 424 drugs to 24 ARs.

This analysis discovered downstream proteins that were common to multiple drug-AR pairs and distinct to ARs (**Fig 3A.**, **Sup. File 2.**, **Sup. File 3.**). For example, for drugs labled with sepsis, their networks shared drug-binding and downstream proteins (**Fig. 3B**). Beause of these patterns, we reclassified drugs based on shared downstream proteins (**Fig. 2B**). For instance, multiple drugs associated with sepsis contained 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 ARs, and tracked two types of network proteins for classification – AR-associated network proteins (ARPs) or any protein on a shortest-path between a drug target and AR protein (SPs). We discovered 172 network classes across 12 ARs or 1,623 classes across 24 ARs using ARPs, or SPs, respectively.

We predicted novel DDIs for each class where non-AR 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 (51,605) drug-drug-AR combinations from network classification using ARPs, or SPs, respectively. 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 6,098 (19,741) drug-drug-AR triplets representing 5,246 (11,904) unique drug-drug pairs for further consideration (some drug-drug pairs were associated with multiple ARs).

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

We estimated the sensitivity of our method by using TWOSIDES(*13*, *14*), a gold standard dataset for drug combination effects. TWOSIDES used the FDA Adverse Event Reporting System (FAERS) to detect DDIs based on the relative reporting rates of combination drugs as compared to single drugs while controlling for confounding variables(*13*, *14*). Predicted DDIs in TWOSIDES indicated combinations prescribed in the real world. For reference, TWOSIDES contained 42,920,391 drug-drug-AR sets reported for 211,990 unique drug-drug pairs. Of note, TWOSIDES contained DDIs for 12,726 unique ARs and included many more and milder side effects than our analysis (e.g., diarrhoea, headache). Of our 5,246 (11,904) unique unique drug-drug pairs, 405 (964) combinations (7.7%) (8.1%) were reported in TWOSIDES, an indication that the 405 (964) combinations were likely prescribed in the real world. These 405 (964) drug-drug pairs encompassed 456 (1,565) drug-drug-AR pairs (some combinations were predicted to affect multiple ARs), of which 368 (786) predicted drug-drug-ARs were reported in TWOSIDES. This corresponded to a sensitivity of 80.7% (50.2%) for predicting DDIs using network classification and excluding drugs with shared target proteins. From these results, using ARPs generated a higher sensitivity for detecting novel DDIs.

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, and a non-sepsis-labeled drug, albuterol, and the AR, sepsis, but our analysis didn’t indicate whether coadministering 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 AR. We searched for combo-drug-AR 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 ARs to understand how the combo drug may affect the AR. For instance, the beta-2 agonist 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 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”* (*15*). The full list of predicted drug-drug-AR combinations, their relevant network proteins, and manually curated literature evidence are provided in **Sup. Files 4** (predictions for SPs)and **5** (predictions for ARPs)**.**

We further investigated predicted drug-drug-AR 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 (*16*), and investigated the proportional reporting ratios (PRRs) reported for our prioritized combimbinations. PRRs were a sufficient proxy for the possible magnitude of effect of predicted DDIs. Indeed, predicted combination effects are reported in TWOSIDES (**Table 1**, full results in  **Sup. File 4**). 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 ARs 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 deidentified Optum Clinformatics dataset that included over 88 million US patients, both privately-insured and Medicare beneficiaries, largely under the age of 65. 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(*17*). We leveraged the Observational Health Data Sciences and Informatics (OHDSI) network tools, specifically CohortMethod to measure AR 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 (*18*) (*19*)). The LEGEND study is one relevant application where CohortMethod measured cardiovascular outcomes across first-line anti-hypternsive drugs(*18*). Our approach was conceptually similar, however, we aggregated drugs into network classes instead of by their chemical structures or therapeutic use classes. We used CohortMethod to test two network DDI predictions to validate our ability to detect DDIs in the real world. We later used *clinical language model based representations* (CLMBR) because this platform was sufficiently scable to test an additional 58 class predictions.

**Albuterol increased the probability of sepsis for drugs with network associations to the albuterol-binding protein, beta-2 adrenergic receptor 2 (ADRB2) in an observational study**

We first investigated the effect of albuterol on ADBR2 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 S2**). We hypothesized that concomitant use of albuterol would alter 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 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 *Materials and Methods.* We further used large-scale propensity matching to estimate counfounding and then matched patients based on their propensity score to estimate relativel risk. Propensity matching aggregates all available patient data in the health record including commonly-considered confounders such age, diagnoses, demographics as well as other data such as number of visits, time to visits, that also reflect patient characteristics

(*16*, *20*, *21*). After matching, we discovered good covariate balance between the target and comparator cohorts and sufficient patient attrition for measuring outcomes (**Fig. S1., Table S3.**).

We measured the relative risk of sepsis between these two drug classes without a combination therapy and with co-adminstration of albuterol (**Fig. 4**., **Table 2**). The relative risk of sepsis occurring in the ADBR2-net class is increased relative to the non-ADBR2-net class when albuterol is used concurrently: HR=0.792 with the combination compared to HR=0.525 without the combination and 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 relative to non-ADBR2-network classs. We did not discover literature evidence supporting sepsis outcome 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 (*22*), 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 (*23*), further supporting that albuterol doesn’t affect sepsis when used alone.

**Aspirin increased the probability of pancreatitis for drugs with network associations to aspirin target proteins in an observational study**

We next investigated the effect of aspirin (also known as acetylsalicylic acid) prescribed in combination with drug network classes associated with aspirin target proteins. This analysis was similar to the analysis of albuterol, except that we classified pancreatitis-associated drugs by asprin target proteins. We discovered downstream network proteins for 80 drugs with pancreatitis listed on their labels. From this 80 drug set, eight drug networks contained at least one of the following aspirin target 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) – and did not share other aspirin target proteins. We classified these eight drugs as the “T-E-N-net” class. Of the remaining 72 drugs, 28 drugs did not share aspirin target proteins or have downstream connections to aspirin target proteins. We classified these 28 drugs as the “non-T-E-N-net” class. All drugs in both classes are listed in **Table S4**. We hypothesized that aspirin would increase the risk of pancreatitis when co-administered with the T-E-N-net class but not with the non-T-E-N-net class.

As above, we extracted patients from the Optum dataset to be used in the target/comparator cohorts if they had an exposure to the T-E-N-net/non-T-E-N-net classes, respectively. We defined combined exposure cohorts as mentioned previously, except that patients were required to have a drug exposure to aspirin that overlapped with an exposure to the T-E-N-net or non-T-E-N-net classes. We also used propensity score analysis and patient-matching to calculate the relative risk of pancreatitis with the aspirin combination. Again, we had sufficient patient attrition and covariate balance to pursue further analysis (**Fig. S2., Table S5.**).

After designing cohorts and looking for covariate balance, we measured hazard ratios for patients in these groups (**Fig. 4**., **Tab. 2**) using cox proportional hazards. The probability of pancreatitis occurring in the T-E-N-net class increased relative to the non-T-E-N-net class when aspirin is used concurrently: HR = 1.01 with the combination compared to HR=0.580 without the combination and yielded an HR-ratio of 1.74. The T-E-N-net class had a relatively lower risk of pancreatitis relative to the non-T-E-N-net class, and the addition of aspirin increased the relative risk of the T-E-N-net class compared to the non-T-E-N-net class. The change in relative risk of drugs with network associations to the combo drug in these two cases encouraged us to pursue further validation. Aspirin inhibits prostaglandins and inhibits pancreas ductal permeability (*24*), suggesting that aspirin could exacerbate pancreatitis when used in combination with other drugs associated with this AR. A case report of a 50-year old woman experiencing pancreatitis included that aspirin and aripiprazole, a T-E-N class drug, were both used concomittently, though the case report concluded that the pancreatitis may have been due to a third medication (*25*). However, our network classification predicted a DDI of pancreatitis for combined use of aspirin and aripiprazole, suggesting an alternative explanation for the patient’s pancreatitis outcome.

**Further discovery of drug interactions based on network associations.**

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 (**Table S6** and **Sup. File 5**). For these 58 predictions, we used the *clinical language model based representations* (CLMBR) method

(*26*), propensity score matching to adjust for confounding, and Cox regression models. CLMBR is a representation learning technique used to consolidate patient data prior to using additional machine learning to predict patient outcomes that outperformed other representations in prediction accuracy on five relevant clinical tasks

(*26*). Precomputed CLMBR representations enabled more rapid cohort definitions and HR-ratio estimation than CohortMethod. Similar to 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 measured HRs between network and non-network drug classes with and without drug combinations. This second pipeline also used propensity score matching to control for confounding variables.

Similar to 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 (**Table S6**). 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 HR between the net-GENE and non-net-GENE classes and for 8 of these cases, there were also sufficient patients to measure HRs between drug classes with the predicted combination drugs (**Fig. S3**). For the 8 classes we measured the change in Cox coefficient to estimate effects of the predicted DDIs (**Table 3, Fig. S4**). These DDI effects were moderate with HR-ratios that ranged 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, used to treat diarrhea. The lowest HR-ratio, 0.85, was measured for from drug-induced hypertension associated with prostaglandin E receptor 4 (PTGER4) network class drugs used in combination with misoprostol, used to treat stomach ulcers. This result suggests a protective effect of misoprostol for the PTGER4 network drug class.

**Discussion**

We predicted drug-drug interactions (DDIs) using network classification and validated these predictions using DDI databases and novel observational studies. We first extracted a novel dataset of drug-averse reaction (AR) pairs using data extracted from drug labels. This dataset was crucial to our analysis and will be valuable to other investigations of drug ARs. We used network analysis to discover downstream proteins associated with ARs, 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. Compared to 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. We validated DDI predictions for albuterol and aspirin based on network classification and 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 DDI effects.

Fortunately, rigorous regulatory review and good clinical practices prevent the use of many harmful drug combinations. For these reasons, 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 realtively cheap and efficient and could aid in therapeutic development where anticipation of ARs is essential for therapeutic development. Our discovery of drug combinations that mitigated AR outcomes suggested a new paradigm for managing drug-induced ARs; specifically that mitigating therapies could be prescribed based on drug network class. Similarly, *in silico* network analysis could also inform safety analysis plans for clinical development.

Our results also have implications for advancing protein-protein interaction (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 AR risk in the presence of secondary drugs is evidence that drug effects could be attributed to downstream proteins. 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 similar to drug B’s network) but also for predicting mechanistic effects (e.g. drug A’s AR 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 can lead to a better understanding of adverse drug reactions and rational design of new therapeutic combinations. Drug synergy is a broad field where many frameworks are used to anticipate drug effects {Bansal:uy} {Menden:wk}. Some approaches leverage “supra-additive” effects of drugs used in combination (*27*) (*28*), yet these measurements often rely on complex and relatively costly high-throughput screens(*29*). While the performance of computational synergy prediction algorithms has increased, these effects have yielded little success in the clinic (*30*) (*31*). 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 (*30*). While we used drug-induced ARs as as the focus of this analysis, analysis of proteins downstream of drug targets could improve prediction of drug synergistic effects on disease outcomes.

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Supplementary Materials:

Materials and Methods

Figures S1-S3

Tables S1-S6

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

References (*1-7*)

**Diagram, shape

Description automatically generated**

**Fig. 1.** **Network vs. shared protein mechanism**. In a shared protein mechanism, two drugs share a protein target 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.

**Diagram

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**Fig. 2.** **Project workflow used pathway modeling and electronic health record analysis to assess DDIs predicted by network class**. (**A**) Starting with drug-AR relationships from drug labels, we used PPI modeling and network classification to predict DDIs (workflow demonstrated for ARPs). We filtered predicted DDIs using TWOSIDES for further validation. (**B**) The starting drug-AR dataset comprised 1970 drugs associed with 34 different adverse reactions (ARs). For the 970 drugs with targets connected to our interactome, we constructed networks and looked for downstream associations to ARs. We used downstream AR-associatied proteins (ARPs) to define network classes and predicted DDIs where non-AR 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 take the ratio of HRs to estimate the DDI effect. Hypothetical example shown to depict experimental set-up.

**Diagram

Description automatically generated Fig. 3. Drug networks have common target and downstream proteins across and within ARs.** (**A**) The most common target (red) and downstream (grey) proteins (rows) for all drug networks associated with 24 ARs (columns). Protein count indicates in how many drug networks the protein appears (**B**) Target (red) and downstream (grey) proteins (columns) for all sepsis-associated drugs networks (rows).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 (albuterol ) | atropine | sepsis | sepsis | 5 |
| urosepsis | 10 |

**Table 1. TWOSIDES supports predicted drug combination effects**. PRR = proportional reporting ratio as published in(*16*).

**Graphical user interface, text, application

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**Fig. 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 DDI 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 |

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

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *With Combo Drug* | | *Without Combo Drug* | |  |  |  |  |  |
| **HR** | **P-value** | **HR** | **P-value** | **HR Ratio** | **AR** | **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 |
| 0.97 | 1.69E-01 | 0.86 | 1.91E-14 | 1.14 | Hypertension | Hydrochlorothiazide | KCNMA1 | Exp12 |
| 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 |

**Table 3. Additional HR ratios estimated from EHR-ML analysis.** Network and non-network class drugs are listed in Table S6 and are refernced by the experimental number (“ExpNum”).