**Results**

*Altered pancreatitis outcomes for network-classified drugs in a novel 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 like the analysis of albuterol, except that we classified pancreatitis-associated drugs by aspirin 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 S2**. 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., Sup. File 6.**).

After designing cohorts and looking for covariate balance, we measured hazard ratios for patients in these groups (**Fig. 4**., **Table 3**) using cox proportional hazards. The risk 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; this yielded an HR-ratio of 1.74. The T-E-N-net class had a lower risk of pancreatitis compared to the non-T-E-N-net class, and the addition of aspirin increased the risk of the T-E-N-net class compared to the non-T-E-N-net class. The change in 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 (*27*), suggesting that aspirin could exacerbate pancreatitis when used in combination with other drugs associated with this ADR. While insufficient to make conclusions, at least one study referenced the concomitant use of aspirin and aripiprazole (a T-E-N-net class drug) and documented an occurrence of pancreatitis; the report documented a 50-year old woman experiencing pancreatitis when aspirin and aripiprazole were used concomitantly, though the case report concluded that the pancreatitis may have been due to a third medication (*28*). 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.

**Materials and Methods**

*Summary of PathFX analysis*

PathFX used drug-binding proteins as inputs to first identify a relevant protein-protein interaction network around these targets, and next used the full list of network genes/proteins to identify phenotypes associated with these genes/proteins relative to the entire interactome. The original interaction network published with PathFX contained an edge score for all protein interactions. The edge score reflected the amount and quality of evidence (e.g., the number of publications, and the type of experimental analysis used to discover the interaction) and all scores are normalized from 0-1. A higher score reflects more and greater quality of evidence that the proteins interact. This scoring was based on the MIScore(*38*) method and is fully elaborated in(*8*). PathFX used a depth-first search to discover protein-protein interactions around a drugs’ target(s). The depth first search stops when a path score falls below the empirically derived threshold. This path score threshold was derived by measuring path uniqueness per network gene across a wide range of thresholds. At each threshold, and for each gene, the uniqueness of a path was measured as the difference between the path’s score and the average of all path scores for a gene. Path scores greater than the average were considered unique and path scores below the average were considered not unique. The empirical threshold was selected by counting the proportion of total unique paths in the network. At high score thresholds (e.g., 0.99) very few path scores exceeded this threshold and very few paths were unique. As we measured lower values (e.g., 0.7) many more paths were discovered, but the proportion of paths above the average path score for a gene peaked and then diminished. We formulated the scoring this way because highly connected, and highly studied genes (e.g., ubiquitin or tumor protein P53 (TP53)) could be compared to their own averages. This would generate a stricter threshold for including highly studied genes without penalizing network gene with fewer interacting partners. In the originally PathFX publication, this score was set to 0.77. Unique to our approach, this path score was not optimized for capturing drug-disease associations but was set to minimize biases such as hub bias when including protein interactions in a drug pathway. Conceptually, this path score represented an interaction distance where we had the strongest support from the corpus of underlying data to support that a downstream protein was likely relevant to a drug-induced effect.

After prioritizing downstream proteins, PathFX used a multiple hypothesis corrected Fisher’s exact test to identify biological phenotypes enriched in the drug’s network. This analysis created a table of phenotype associations, a p-value for the strength of those associations, and network genes associated with the phenotype. Importantly, PathFX was naive to the drug’s labeled phenotypes when discovering associations and only uncovered phenotype connections based on the supporting data.

*Application of PathFX to understand drug-ADR relationships mediated by downstream proteins*

We used PathFX(*8*) to discover networks for all drugs in DrugBank *(* analysis contained */ PathFX/ scripts/ run\_PathFX\_all\_drugBank.py*). We uploaded the association tables created by PathFX into the GitHub folder: */data/ all\_drugbank\_network\_association\_files/.* We next investigated whether downstream proteins connected drug targets to ADR-associated proteins. We calculated the sensitivity and specificity for each ADR. A drug-ADR association was counted as a true positive or false negative if the drug’s network contained or did not contain an ADR-related phenotype from the drug’s label. Further, for this ADR set, we considered all drugs in DrugBank that did not have the ADR listed on their drug label as negatives. We investigated the pathways for these drugs and considered the drugs as false positives or true negatives if the pathway contained or did not contain a phenotype relevant to the ADR. Our analysis of PathFX results is contained in */Code/ read\_drug\_to\_DME\_data.ipynb*. We manually combined the raw data, the outputs from sensitivity and specificity analysis, and the results from the pathway analysis into */Code/supp2\_true\_positives\_summary.xlsx I*(**Sup. File 2**).

*Extended explanation of network meta-analysis*

For each ADR, we took the union of all shortest pathways between a drug target and ADR-associated genes *(/Code/merge\_networks\_for\_DMEs.ipynb*). For instance, the drug, alemtuzumab, is associated with hemolytic anemia on its label. Alemtuzumab’s pathway was associated with the phenotypes, ‘autoimmune hemolytic anemia’ because the pathway contained the genes, FCGR3B, CD3G, IGHV2-5, and FCGR3A, and ‘hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency’ because the pathway contained the genes FCGR2B, FCGR2A, FCGR2C, CD3G, and IGHV2-5 (**Sup. File 2**). We found all shortest pathways between alemtuzumab’s drug targets and these genes. We repeated this process for all true positive drug pathways for hemolytic anemia and took the union of these shortest paths to create the merged pathway for hemolytic anemia. We repeated this process for all ADRs *(/Code/merge\_networks\_for\_DMEs.ipynb*).

We further ranked all network nodes (drug binding and intermediate proteins) by the number of drug-ADR pathways in which they occurred. For the alemtuzumab example, the gene CD3G is counted twice because it is involved in the pathway between alemtuzumab and two ADR phenotypes (‘autoimmune hemolytic anemia’ and ‘hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency’). The total count for CD3G was 7 because it also occurred in the pathways for the drugs, natalizumab, rituximab, and pegademase. The full list of drug-ADR pathways for CD3G includes: Natalizumab:Hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency; Rituximab:Hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency; Alemtuzumab:Autoimmune hemolytic anemia; Rituximab:Autoimmune hemolytic anemia; Natalizumab:Autoimmune hemolytic anemia; Pegademase bovine:Hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency; and Alemtuzumab:Hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency (**Sup. File 3***, supp3\_DME\_merged\_node\_counts.xlsx*). For each ADR, we took the top 12 genes and plotted these counts across ADRs to look for patterns across genes using the seaborn and pandas modules in python for creating heatmaps (*Code/merge\_networks\_for\_DMEs.ipynb,* **Sup. File 4***, supp4\_DME\_heatmap\_top\_node\_counts.xlsx, plotted in* ***Fig 3***).

For example, in tardive dyskinesia, PathFX identified 35 cases where drug pathways contained an association to the tardive dyskinesia phenotype. For all other ADRs, the number of merged pathways is contained in **Sup. File 2***, supp2\_true\_positives\_summary.xlsx*. For some ADRs, pathways analysis did not uncover an association between the drug’s target(s) and the ADR (i.e., the sensitivity = 0, **Sup. File 2**). All the genes in the merged pathway constitute an “ADR pathway”.

*Network images and heatmaps*

To create network images, we wrote a custom python script for creating images with drugs oriented above drug-binding proteins and phenotypes. The script used the merged networks created previously and created a layered array where drugs were plotted in the topmost layer, drug targets in the second layer, intermediate and downstream proteins in the third layer, and ADR phenotypes in the fourth, and bottom layer. All scripts are included in the ‘code’ folder of the directory. *(/Code/ merge\_networks\_for\_DMEs.ipynb* and */Code/ find\_co\_therapy\_networks.ipynb*).

*Identifying novel co-therapies and determining directionality of effect*

We again used drug-protein binding data from DrugBank to identify drugs that bound an ADR pathway gene (discovered previously) and were not associated with an ADR on their drug label *(/Code/find\_predicted\_cotherapies.ipynb, /char\_data/* *charac\_novel\_combinations.py,* and */Code/* *charac\_novel\_combos\_using\_int.py*). We tracked two types of ADR pathway genes: ADR-associated genes (ARPs) and proteins along shortest paths between drug targets and ADR-associated genes (SPs). We predicted drug combinations for both types of network genes and these results are stored in *supp5\_all\_SP\_drug\_class\_predictions.xlsx* (SPs) and *supp6\_ARPs\_PRRs\_clin\_results.xlsx* (ARPs). The ARPs were a subset of the SP set.

We then sought separate data that could validate the predicted combination drug’s association to the ADR. We conducted a search of co-mentions of combination drugs and the ADRs. The rule-based natural language processing tool Linguamatics was used to identify MEDLINE abstracts that contained sentences with co-mentions of drugs and ADRs in December 2018, yielding a set of PubMed IDs for relevant abstracts. This search yielded a set of PubMed IDs for abstracts that contained sentences that contained co-mentions of the combination drugs and ADR phenotypes in the same sentence. Importantly, this set did not contain drugs associated with ADRs on their labels and the co-mentions of combination drugs and ADRs were not used in the PathFX predictions. Co-mentions in PubMed could represent emerging effects or exceedingly rare relationships to ADRs that would not have required the drug to have the ADR on its label. We manually read the abstracts to confirm relevance of the abstract and infer directionality of the drug’s effect on the ADR (e.g., aggravates the ADR or mitigates) (*/data/ Drug-DME\_Eval\_final.xlsx*). We summarized our predictions of potential drug interactions to create tables linking drugs with labeled ADRs to potential aggravating or preventative drug interacting partners *(/Code/summarize\_predictions.ipynb***, Sup. File 7:** *supp7\_summary\_drug\_interactions.xlsx*).

*Electronic Health Record Dataset: Optum Clinformatics Data Mart 7.0*

The Optum Clinformatics™ Data Mart Database (OptumInsight, Eden Prairie, MN) is a de-identified database from a large national insurance provider. The dataset contains over 88 million patients largely under the age of 65 and is frequently used for observational studies (*21*) We used a version of Optum standardized to OHDSI’s Observational Medical Outcomes Partnership (OMOP) common data model version 5 (<https://github.com/OHDSI/CommonDataModel>). The OMOP CDM used standard vocabulary concepts to map to international coding systems into a consolidated data resource.

*Novel observational study for assessing aspirin and albuterol combinations using CohortMethod*

Accessing data in the CDM format enabled us to produce anonymized code that is sufficiently standardized to enable deployment on other health record datasets in the CDM format. Anonymized code does not contain any server access information or any patient data that would jeopardize data security and facilitates reproducibility. All the anonymized SQL and R code used for the first two observational studies is contained in */Code/CohortMethod\_and\_SQL.* We used the following code to execute the following searches in the electronic health record:

* *count\_dmes\_outcomes.sql*: identified patients with ADR diagnoses from the CONDITION\_OCCURENCE table. For pancreatitis and sepsis, we used the concept ids 4192640 and 132797 which mapped to the SNOMED terms, “pancreatitis”, and “sepsis”, respectively. In both cases, we included descendent concepts of either primary term.
* *count\_drug\_eras\_singleDrug.sql*: identified patients exposed to a predicted combination drug from the DRUG\_ERA table (full list in **Sup. File 6**).
* *count\_drug\_eras\_from\_list.sql*: identified patients exposed to network or non-network class drugs from the DRUG\_ERA table (full list in **Sup. File 6**).
* *look\_for\_overlaps.sql*: identified patients that had overlapping drug exposures of classified drugs and predicted combination drugs.
* look\_for\_subsequent\_outcomes.sql: identified patients with an adverse outcome CONDITION\_OCCURENCE following exposure to a classified drug or combination of drugs. This search was largely for feasibility of estimating Hazard Ratios.

The DRUG\_ERA is a derived data table used in OMOP CDM databases. The eras are derived from drug exposure data using standardized algorithms. They reflect a continuous exposure to a single compound and can be derived from multiple drug exposure data types: for pharmacy prescriptions, a drug era begins at the start of the prescription and ends at the time of the last dispensed dose, for procedure drugs they reflect the date of administration, and drug eras may be combined if the gap between subsequent exposures is less than or equal to 30 days (https://www.ohdsi.org/web/wiki/doku.php?id=documentation:cdm:drug\_era).

To test these hypotheses, we used CohortMethod (*39*) tools and the Optum dataset (described previously) to conduct propensity score matching and estimate effect sizes. For transparency, the analyses for the sepsis and pancreatitis studies are contained in *count\_drug\_combo\_exposures\_sepsis.R* and *count\_drug\_combo\_exposures\_pancreatitis.R*, respectively.

For the sepsis study, we started with 29 drugs where their networks were associated with sepsis. Of these 29, 2 drug networks contained ADRB2, which is a target of the predicted combo drug, albuterol. These 2 drugs also did not share any of the albuterol drug targets. Of the remaining 27 drug networks, 18 drugs did not share drug-binding targets with albuterol and did not contain any albuterol-binding genes in their networks. All drugs are listed in **Table S1** and **Sup. File 6**, *supp6\_ARPs\_PRRs\_clin\_results.xlsx*.

Instead of manually defining patient covariates, we used the built-in function to create a propensity score that leveraged the totality of data for a given patient to reduce confounding. For this analysis the area under the curve (AUC) for the propensity model was 0.99. We used two methods to assess treatment vs. comparator effects: inverse propensity weighting (IPW) and matching. In IPW, we used the entire patient population and weighted patient subsets based on their propensity score to balance the representation of patient subsets in the overall estimation. In the matching approach, we used a subset of the patient population, and estimated the drug effects only on patients who are matched between the treatment and comparator groups based on their propensity scores (the propensity score is a sufficient proxy for shared confounding variables). We ultimately used matching to define patient cohorts as this was the best comparison of patients with similar clinical features. The patient attrition diagram and covariate balance table after matching are contained in **Fig S1** and **Sup. File 6**, respectively.

We repeated this analysis procedure for the pancreatitis study. For this analysis, we started with 80 drugs where their networks were associated with pancreatitis. Of these 80, 8 drugs contained either TP53, EDRNA, or NFKBIA, which are targets of the predicted combo drug aspirin. These 8 drugs also did not bind any of aspirin’s drug targets. Of the remaining 72 drug networks, 28 drugs did not share drug-binding targets with aspirin and did not contain any aspirin-binding genes in their networks. All drugs are listed in **Table S2**. We used the same propensity score matching function and the AUC for the propensity model was 0.90. The attrition diagram after patient matching and the cohort covariate balance table are contained in **Fig S2** and **Sup. File 6,** respectively**.** In both studies, we observed patients for a 30-day risk window after the second, combination drug era was initiated.

*Novel observational studies for additional network predictions*

We pursued clinical validation of an additional 58 predicted DDIs from the ARP predictions because these had a greater sensitivity in the TWOSIDES dataset. To prioritize these combinations, we started with the 457 DDIs that were supported by a case report in TWOSIDES. We grouped these DDIs by network class (using downstream network proteins discovered by PathFX) and converted DrugBank identifiers to Anatomical Therapeutic Chemical (ATC) codes. We mapped DrugBank identifiers to all ATC codes but excluded combination products from the analysis. All network, non-network classes and drugs contained in these classes are included in **Sup. File 6**, *supp6\_ARPs\_PRRs\_clin\_results.xlsx***.** To conduct this analysis, we used a custom, more scalable pipeline using low dimension CLMBR patient representations

(*29*). These representations are a consolidated record of patient encounters with the health system – visits, diagnoses, drug exposures – and have been shown to outperform other patient representations on multiple clinical prediction tasks. Having precomputed patient representations allowed us to efficiently conduct multiple DDI studies because these representations could be reused across analyses.

We used these representations to conduct large-scale propensity matching of patients for each predicted DDI. Propensity score matching reduces confounding by limiting the analysis to similar patients. For all DDI studies, we conducted a baseline measurement of the ADR risk between the network and non-network class and a second measurement of the ADR risk between these classes when the combination drug was also used. To be included in a DDI study, each patient needs to have been exposed to drugs in either the network or non-network class and then be exposed to the predicted combination drug or a comparator. After identifying a matched cohort, we estimated hazard ratios using Cox regression model for the ADR outcome. This procedure is the same as used in CohortMethod (https://github.com/OHDSI/CohortMethod). For all measurements, the p-value represents the likelihood of the estimated hazard ratio relative to the null hypothesis that the hazard ratio is 1.

Fig. S1. Attrition diagram after performing patient matching in sepsis study.



Fig. S2. Attrition diagram after performing patient matching in pancreatitis study.

Chart, bar chart

Description automatically generatedFig S3. Cox coefficients measured for 21 of the 58 predicted class effects. We measured Cox coefficients for network class effects where there were sufficient patients exposed to the target and comparator drug classes (grey bars) and for the 8 classes where there were sufficient patients exposed to the combination drug and either the target or comparator drug classes (red bars). Experimental numbers correspond with network classifications in Sup. File 6.

Chart, waterfall chart

Description automatically generated

**Fig. S4.** Changes in the cox coefficient for 8 predicted classes with combination drugs compared to without the combination drug. Increased/decreased risk for the ADR is represented in red/blue bars respectively. Experimental numbers correspond with network classifications in **Sup. File 6**.

|  |  |
| --- | --- |
| **Drugs with network association to sepsis and their networks contain ADRB2,  “ADBR2-net”** | **Drugs with network association to sepsis and their networks do NOT contain ADRB2, “non-ADBR2-net”** |
| atropine, paroxetine | abatacept, canakinumab, certolizumab pegol, diphenoxylate, eculizumab, etanercept, gabapentin, glycine, golimumab, goserelin, infliximab, menthol, pramipexole, sulfasalazine, sumatriptan, ketoprofen, niacin, memantine |

**Table S1.** Drugs used in sepsis DDI study.

|  |  |
| --- | --- |
| **Drugs with network association to pancreatitis and their networks contain TP53, EDRNA, or NFKBIA, “T-E-N-net”** | **Drugs with network association to pancreatitis and their networks do NOT contain aspirin-binding proteins, “non-T-E-N-net”** |
| acamprosate, aripiprazole, atropine, droxidopa, pergolide, pilocarpine, pramipexole, ropinirole | aliskiren, amoxapine, benazepril, blinatumomab, busulfan, danazol, diphenoxylate, enalaprilat, fosinopril, gabapentin, hydroflumethiazide, isopropyl alcohol, lanreotide, levodopa, menthol, octreotide, olmesartan, oxaliplatin, pasireotide, pentazocine, prilocaine, quinapril, Ramipril, riluzole, tenecteplase, tramadol, trandolapril, vandetanib |

**Table S2**. Drugs used in pancreatitis DDI study.