

INDI: a computational framework for inferring drug interactions and their associated recommendations

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Inferring drug–drug interactions (DDIs) is an essential step in drug development and drug administration. Most computational inference methods focus on modeling drug pharmacokinetics, aiming at interactions that result from a common metabolizing enzyme (CYP). Here, we introduce a novel prediction method, INDI (INferring Drug Interactions), allowing the inference of both pharmacokinetic, CYP-related DDIs (along with their associated CYPs) and pharmacodynamic, non-CYP associated ones. On cross validation, it obtains high specificity and sensitivity levels (AUC (area under the receiver-operating characteristic curve) ≥ 0.93). In application to the FDA adverse event reporting system, 53% of the drug events could potentially be connected to known (41%) or predicted (12%) DDIs. Additionally, INDI predicts the severity level of each DDI upon co-administration of the involved drugs, suggesting that severe interactions are abundant in the clinical practice. Examining regularly taken medications by hospitalized patients, 18% of the patients receive known or predicted severely interacting drugs and are hospitalized more frequently. Access to INDI and its predictions is provided via a web tool at <http://www.cs.tau.ac.il/~bnet/software/INDI>, facilitating the inference and exploration of drug interactions and providing important leads for physicians and pharmaceutical companies alike.

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Introduction

Simultaneous administration of two or more medications is by now a common practice, but may result in significant drug–drug interactions (DDIs), altering medication effectiveness or even harming the patient (Manzi and Shannon, 2005). A DDI is defined as any drug effect that is greater/less than expected in the presence of another drug (Crowther *et al*, 1997). As the number of approved drugs increases, the number of potential interactions between prescribed medications rapidly rises (Khouri *et al*, 2006). Moreover, certain patient groups (e.g., elderly patients (Bergendal *et al*, 1995; Juurlink *et al*, 2003; Doubova Dubova *et al*, 2007) or cancer patients (Riechelmann *et al*, 2007)) are typically administered numerous medications, exposing them to a high risk of multiple interactions. The increase in potential drug interactions renders the experimental discovery of those intractable, calling for large-scale computational prediction methods.

DDIs are conventionally categorized into *pharmacokinetic* interactions, whereby a drug is affecting the processes by which another drug is absorbed, distributed, metabolized or excreted (Zhang *et al*, 2009), and *pharmacodynamic*

interactions, where the effects of one drug are modified by the effect of another on its site of action or by affecting the same or cross-talking signaling pathways (Jonker *et al*, 2005; Imming *et al*, 2006). Most previous work concerns the prediction of pharmacokinetic DDIs. Due to the complex nature of the problem, those algorithms typically handle separately the absorption, distribution, metabolism and excretion of each compound, relying on different properties of the compound such as its chemical structure, permeability, solubility and polarity (Boobis *et al*, 2002). Subsequently, physiologically based pharmacokinetic modeling algorithms attempt to integrate these individual predictions into coherent and predictive models (Brown *et al*, 1997; Boobis *et al*, 2002).

Within the pharmacokinetic processes, the metabolism part covers the largest, yet poorly understood aspect and consequently the most difficult to evaluate and predict (Boobis *et al*, 2002). Most of the metabolism-related DDIs involve the Cytochrome P450 (CYP) enzyme superfamily (Wrighton and Stevens, 1992; Goshman *et al*, 1999; Ekins and Wrighton, 2001). Several methods focus on predicting *in-vivo* affinities of drug–CYP interactions from *in-vitro* experiments (Hutzler *et al*, 2005; Fowler and Zhang, 2008; Jamei *et al*, 2009;

Obach, 2009; Zhou and Zhou, 2009), while others attempt at *in-silico* modeling of drug–CYP interactions (Hudelson *et al*, 2008) (e.g., using the rate of metabolism of a drug in the presence of a CYP inhibitor (Kato *et al*, 2003)). The main shortcoming of these models is the need for tuning several pharmacokinetic parameters such as steric hindrance, lipophilicity, distribution volume, renal clearance and enzyme degradation rates (Boobis *et al*, 2002; Kato *et al*, 2003; Obach *et al*, 2007). A different approach is employed for predicting pharmacodynamic interactions, depending primarily on combining single drug *in-vitro* or *in-vivo* measurements of pharmacodynamic constants (Tallarida, 2001; Jonker *et al*, 2005; Li *et al*, 2007).

Additional prediction methods (that are not type specific) follow two distinct approaches. The first approach obtains *in-vitro* chemogenomic profile measurements of drug-perturbed cellular systems. These approaches infer interactions based on coupled perturbations (Nelander *et al*, 2008) or similarity between these profiles (Jansen *et al*, 2009). These methods were so far tested on limited cell types and validated only at small scale. The second approach mines potential DDIs from adverse drug reaction (ADR) reports (Tatonetti *et al*, 2012a,b). However, the latter methods suffer from several limitations, including various biases in the ADRs such as under-reporting, duplicate reports or change in reporting methodologies over time (Rawlins, 1988; van der Heijden *et al*, 2002; Bate and Evans, 2009), the necessity to pre-define drug classes, and the inability to handle novel and rarely used drugs for which no or limited reports exist (Tatonetti *et al*, 2012a).

Here, we present a large-scale *in-silico* DDI prediction method: INferring Drug Interactions (INDI), handling both pharmacokinetic and pharmacodynamic DDIs and overcoming the caveats of previous methods. The algorithmic framework follows the pairwise inference scheme previously employed to drug-indication prediction (Gottlieb *et al*, 2011): Given a query drug pair, INDI computes its similarity to drug pairs that are known to interact, exploiting seven different drug–drug similarity measures. The similarity scores of each drug pair according to each similarity measure pairs allows INDI to determine the likelihood that the query drug pair interacts. We further extended this framework to predict interaction-specific traits. Specifically, INDI allows (i) recommending the type of action to take upon administration of the two drugs (contraindicate, generally avoid, adjust dosage or monitor) and (ii) inferring the CYP isoforms involved when the interaction is CYP-related. The latter may enable physicians to seek alternative medications involving different drug–CYP interactions, as well as considering patient-specific genetic polymorphisms related to certain CYP enzymes (Bertilsson, 1995; Goshman *et al*, 1999) (e.g., patients who are CYP2D6-deficient (Ingelman-Sundberg, 2004)).

Drug interactions are a major cause of concern in the clinical practice. To investigate the prevalence of interactions in regularly taken medications, we applied INDI to three sources associated with clinical usage of drugs. We find that known and predicted DDIs cover the majority of drug events reported in the FDA adverse event reporting system. We further demonstrate the prevalence of known DDIs as well as the frequency of our predictions in regularly taken (chronic) medications reported by hospitalized patients in Israel and in

commonly administered drug combinations, demonstrating their association with frequency of admissions.

To conclude, the novelty in this paper is threefold: (i) the application of our algorithmic framework allows, to the best of our knowledge, the first unbiased *in-silico* DDI prediction algorithm; (ii) the extension of the algorithm to predict interaction-associated traits; and (iii) the exploration of known and predicted DDIs in clinical usage.

Results and discussion

Assembly of a DDI gold standard

We downloaded 74 104 DDIs involving 1227 drugs from DrugBank (Wishart *et al*, 2008) (10 702 interactions) and from www.drugs.com (Drugsite Trust, Drugs.com) (70 099 interactions tagged as major or moderate DDIs). We distinguished between three types of drug interactions (Materials and methods): (i) *CYP-related DDIs (CRDs)*, in which both drugs are known to be metabolized by the same cytochrome P450 (CYP) enzyme and there is supporting evidence that the interaction is CYP-related (10 106 interactions); (ii) *potential CYP-related DDIs (PCRDs)*, in which both drugs are metabolized by the same CYP but there is no evidence that the interaction is CYP-related (18 261 interactions); and (iii) *non-CYP-related DDIs*, where no CYP is shared between the drugs (NCRDs, 45 737 interactions). We disregarded other metabolizing proteins that may be involved in drug interactions due to the low number of such interactions (Materials and methods). An outline of the gold-standard assembly process is depicted in Figure 1A.

We report in the Supplementary Material (Section 1) the correlations between the numbers of drug interactions, indications and side effects as well as the observed fluctuations in these numbers over a period of 50 years (1961–2010). Importantly, we found that the number of NCRDs is positively correlated with the number of indications of a drug. The fact that promiscuous drugs, indicated for multiple diseases, tend to have higher number of pharmacodynamic interactions, underscores the need for predicting such interactions.

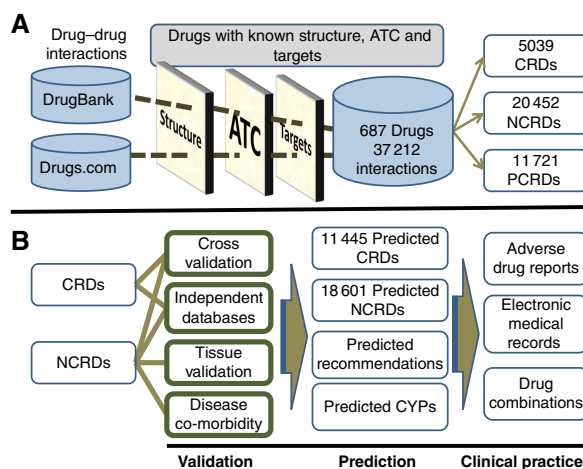


Figure 1 Illustration of the gold-standard assembly (A) and a schematic layout of the validation strategies, predictions and clinical implications (B).

INDI—an algorithm for DDI prediction

We designed a novel algorithm for INDI with two objectives in mind: (i) predicting both new CRDs and new NCRDs and (ii) developing a general strategy that allows predicting interactions for novel drugs for which no interaction information is currently available.

Given a gold-standard set of known DDIs, INDI ranks additional drug pairs based on their similarity to the known DDIs. This similarity-based approach was shown to be successful in predicting drug targets (Perlman *et al*, 2011)

and indications (Gottlieb *et al*, 2011). The algorithm works in three steps (Figure 2): (i) construction of drug–drug similarity measures; (ii) constructing classification features based on these similarity measures; and (iii) application of the classifier to these features to predict new DDIs. To address the first objective, we used two distinct parts of our DDI gold standard—one consisting of CRDs only and the other consisting of NCRDs only. Overall, we collected 5039 CRDs, spanning 352 drugs and 20 452 NCRDs, spanning 671 drugs for which all drug–drug similarity measures could be computed (Materials and methods).

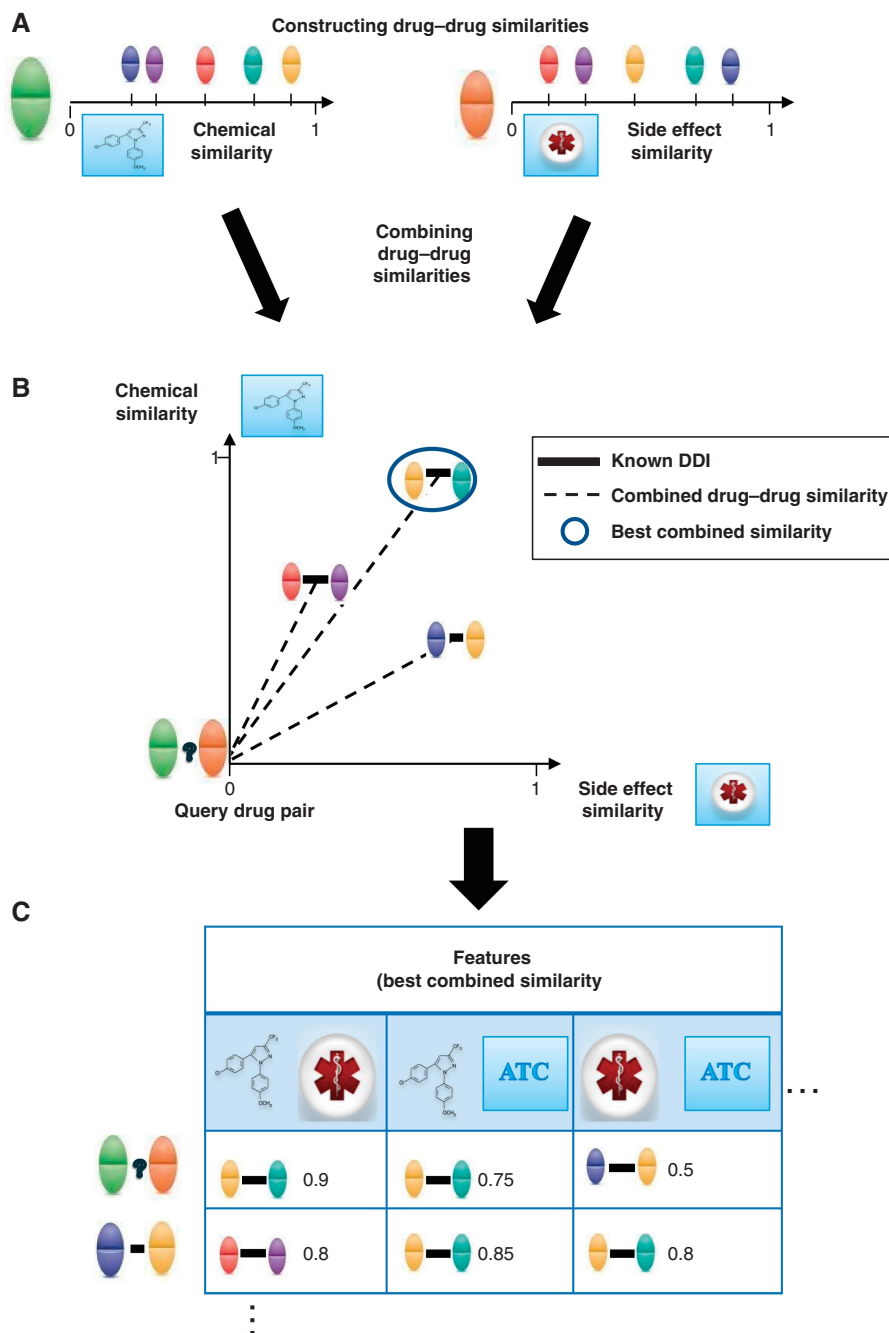


Figure 2 Illustration of INDI algorithm: computation of drug–drug similarity measures (e.g., (A)); scoring query drug pairs according to their similarity to known DDIs (B); and integration of the similarities to classification features and subsequent interaction prediction (C).

Table I Performance of predicted interactions in cross validation

Training set	CRDs		NCRDs	
	# Drugs	AUC	# Drugs	AUC
All	352	0.93 ± 0.003	671	0.96 ± 6e ⁻⁴
Chemically dissimilar drugs ^a	186 ± 2	0.9 ± 0.005	301 ± 3	0.95 ± 0.004
Drugs sharing no targets ^a	–	–	164 ± 3	0.93 ± 0.01
Drugs with no ATC available	394	0.92 ± 0.002	815	0.94 ± 6e ⁻⁴

^aThe number of drugs slightly varies due to the removal process.

For the first algorithmic step, we assembled seven drug–drug similarity measures including chemical similarity, ligand-based chemical similarity based on Keiser *et al* (2009), similarities based on registered and predicted side effects (Atias and Sharan, 2010; Kuhn *et al*, 2010), the Anatomical, Therapeutic and Chemical (ATC) classification system and three similarity measures constructed between drug targets, including sequence similarity, distance on a protein–protein interaction (PPI) network and Gene Ontology (GO) (Ashburner *et al*, 2000) semantic similarity (Materials and methods).

The second algorithmic step integrates the drug–drug similarities to construct classification features and subsequently learns a classification rule, distinguishing between true and false DDIs (Materials and methods). For each query drug pair, we constructed features expressing its similarity to the closest gold-standard DDI, by adapting the scoring scheme of Gottlieb *et al* (2011) (Materials and methods). Each feature is based on a combination of two drug–drug similarity measures, resulting in 49 features overall. We then learned a logistic regression classifier that automatically weights the different features to yield a classification score.

We further extended the basic prediction algorithm to exploit the 49 features for inferring additional information related to the predicted DDIs: (i) recommendations of the type of action to take upon simultaneous administration of the interacting drugs (i.e., contraindicate, generally avoid, type of dosage adjustment or monitor); and (ii) for CRDs, the CYP isoforms affecting them.

We provide a web interface for querying predicted DDIs, their recommendations and their related CYPs, available at <http://www.cs.tau.ac.il/~bnet/software/INDI>. In order to extend our method to drugs for which not all seven similarity measures could be computed, we provide predictions also for drugs for which only the chemical structure and some drug targets are known (Materials and methods). Notably, the prediction performance remains similar also for this extended drug set (Table I).

Performance evaluation

We evaluated the performance of INDI in predicting DDIs using a total of 37 212 true DDIs (omitting the type of interaction, that is, pharmacodynamic or pharmacokinetic and including PCRDs). A schematic layout of the performed validations, summary of the predictions and the clinical practice implications is displayed in Figure 1B.

The application of INDI in a 10-fold cross validation setting (Materials and methods) yielded an area under the receiver-operating characteristic curve (AUC) of 0.97 ± 4e⁻⁴. Encouraged by these results, we then retained the information regarding the interaction type, applying INDI to predict CRDs and NCRDs using known DDIs from each type separately. Evaluating these predictions using a 10-fold cross validation setting, we obtained similarly high AUC scores of 0.93 ± 0.003 for CRDs and 0.96 ± 6e⁻⁴ for NCRDs (Table I). Using the best F1-measure (harmonic mean of precision and recall) over the different classifier ranks, these AUCs correspond to a recall and precision values of 0.89 ± 0.01 and 0.84 ± 0.01, respectively, for the CRD predictions and 0.93 ± 0.004 and 0.88 ± 0.004, respectively, for the NCRD predictions. We note that no single similarity measure obtains these high AUCs: the highest AUC was obtained using the ligand chemical similarity (0.86 for CRDs and 0.88 for NCRDs; Supplementary Table S1), and removing each single similarity measure had a marginal effect on the overall AUC (<0.02).

To confirm that INDI's performance is not biased by redundancy stemming from chemically similar drugs, we created a non-redundant drug set by filtering for similar drugs (Tanimoto coefficient ≥ 0.5; Materials and methods). The application of INDI to this set resulted in only a minor performance reduction (Table I; Supplementary Figure S1). In order to verify that this result is not biased by the type of chemical similarity computation, we further excluded the ligand similarity measure to obtain similar, minor, performance degradation for both CRDs and NCRDs (AUC difference < 0.01; Supplementary Figure S1). For NCRDs, we further verified that removing drugs sharing the same targets had a minor effect on performance (Table I).

As a second validation strategy, we predicted DDIs based on the smaller set of DrugBank DDIs and tested their intersection with the larger set of Drugsite Trust, Drugs.com interactions (excluding interactions appearing in DrugBank). Reassuringly, 10% of the predicted CRDs and 25% of the predicted NCRDs significantly overlapped the corresponding Drugsite Trust, Drugs.com DDIs ($P=0$ for both). For completeness, we carried out the same validation when training on the Drugsite Trust, Drugs.com set, constraining ourselves to the same set of drugs used when training on the DrugBank set. We obtained significant overlap of 32% of the predicted CRDs ($P=2e^{-7}$) and 18% of the predicted NCRDs ($P=4e^{-4}$) with the corresponding DrugBank DDIs (Supplementary Table S4). Additionally, we observed a significant overlap with PCRDs (12% of predicted CRDs, $P=5e^{-313}$ and 28% of predicted NCRDs, $P=0$, see Supplementary Material, Section 2).

Finally, we wished to compare INDI to a layman approach. To this end, we devised a naive method that predicts an interaction between a pair of drugs based solely on the similarity between them (rather than using known DDIs as in INDI). This naive approach proved to be a poor predictor for all similarity measures, resulting in AUCs < 0.6 for both the CRD and NCRD sets (Supplementary Figure S5; Supplementary Material, Section 3).

Prediction of novel DDIs

Next, we applied INDI to predict new interactions between 807 drugs (Materials and methods). Our full set of predictions includes 14 698 predicted CRDs and 28 108 predicted NCRDs. This set includes known PCRDs, which were further exploited for validation purposes. Excluding those from the prediction set, we acquired 11 445 and 18 601 novel CRDs and NCRDs, respectively (Supplementary Table S2). In all, 1781 of the predicted CRDs and 3208 of the predicted NCRDs involve drugs with no DDI information (*novel drugs* in our context). Notably, we observed a high correlation between the numbers of predicted and known CRDs of a drug, and similarly for the NCRD predictions. The detailed analysis, as well as analyses of the trends in the number of predicted interactors per drug over a period of 50 years appears in the Supplementary Material (Section 1).

To evaluate our predictions, we compared them to a recent collection of DDIs mined from FDA Adverse Event Reporting System (Tatonetti *et al*, 2012b). Even upon exclusion of known DDIs from our prediction set, we observed a significant overlap between the two lists, where 39 and 33% of the CRD and NCRD predictions, respectively, overlapped the list of mined DDIs ($P = 4e^{-95}$ and $P = 4e^{-23}$, respectively).

We further assessed the predictions in two ways. The first is based on the assumption that NCRDs tend to occur among drugs affecting the same tissues. We associated drugs with tissues via their indicated diseases or via tissue-specific expression of their targets (see Materials and methods for details of the tissue association scheme and the validation of the above assumption). Reassuringly, our predicted interactions involved drugs that were also significantly associated with the same tissues ($P = 2e^{-34}$ for disease-based and $P = 7e^{-41}$ for gene-expression based drug-tissue associations). A similar trend, albeit with a lower magnitude, was observed for the CRD predictions (Supplementary Material, Section 2). The second validation, assumes that NCRD drug pairs affect similar or cross-talking mechanisms of actions and, thus, may also treat disease pairs with shared molecular mechanisms, contributing to co-morbidity of those pairs. Indeed, predicted NCRDs were found to be enriched with the corresponding co-morbidity pairs (see Supplementary Material, Section 2 for details).

Analysis of interactions between drug classes

We used our predictions to study which drug classes should not be administered concomitantly in general. We utilized the third level of the ATC drug classification system to compute

interactions between ATC classes, using only interactions involving severe recommendations (i.e., generally avoid or contraindicate, encompassing 8% of the DDIs). Figure 3A and B display the interaction networks formed by CRDs and NCRDs, respectively (for clarity, we display only interactions between classes involving a significant fraction of each class' drugs, see Materials and methods). Most of the inter-class interactions in these figures are supported by both known and predicted interactions (solid lines). We identified two predicted novel CRD-based and four NCRD-based inter-class interactions. We found that these novel inter-class interactions extend known interactions from other classes (see Supplementary Material, Section 4).

Predicting DDI recommendations and related CYPs

In addition to predicting DDIs, we aimed to provide additional information characterizing them: (i) recommendations, describing the type of action to take upon administration of the drugs predicted to interact and (ii) CYPs influencing the predicted CRDs. In order to predict recommendations, we used the four major recommendations available for all Drugsite Trust, Drugs.com and some of the DrugBank interactions: (i) contraindicated (3% of the CRD and NCRD training set); (ii) generally avoid (9%); (iii) adjust dosage (2%); and (vi) monitor (84%). The remaining 2% could not be mapped to any of these recommendations (Materials and methods). For the 'adjust dosage' recommendation, we provided finer-grained categorization by further partitioning the recommendation into four subcategories: (i) decrease dosage (53% of the CRD and NCRD training set with adjust dosage recommendation); (ii) increase dosage (9%); (iii) limit dosage (15%); or (iv) adjust dosage interval (17%). Additional 5% DDIs had an unspecified adjust dosage recommendation. Recall that each predicted interaction (CRD or NCRD) exploits a set of 49 most similar known DDIs, computed by the DDI prediction algorithm. We formed four novel features describing each DDI prediction by computing the frequency of each of the four recommendations in these 49 known DDIs, and trained a logistic regression classifier for each recommendation separately using these four features (Materials and methods).

On a 10-fold cross validation test, we obtained high AUC scores for each of the recommendation types (Table II). Predicting each recommendation type independently, we kept the most severe recommendation whenever more than one type was predicted for a drug pair. Overall, we predicted recommendations for over 97% of the predicted DDIs (Table II). In order to validate the predicted recommendations, we exploited the fact that 21% of the predicted CRDs are known NCRDs or PCRDs (with associated recommendations) and 33% of the predicted NCRDs are known CRDs or PCRDs (with associated recommendations). We obtained significant agreements between the predicted and known recommendations (see Supplementary Table S5 and Supplementary Material, Section 5). Restricting ourselves to those predicted DDIs with predicted adjust dosage recommendation, we used the same methodology to further predict one of the four

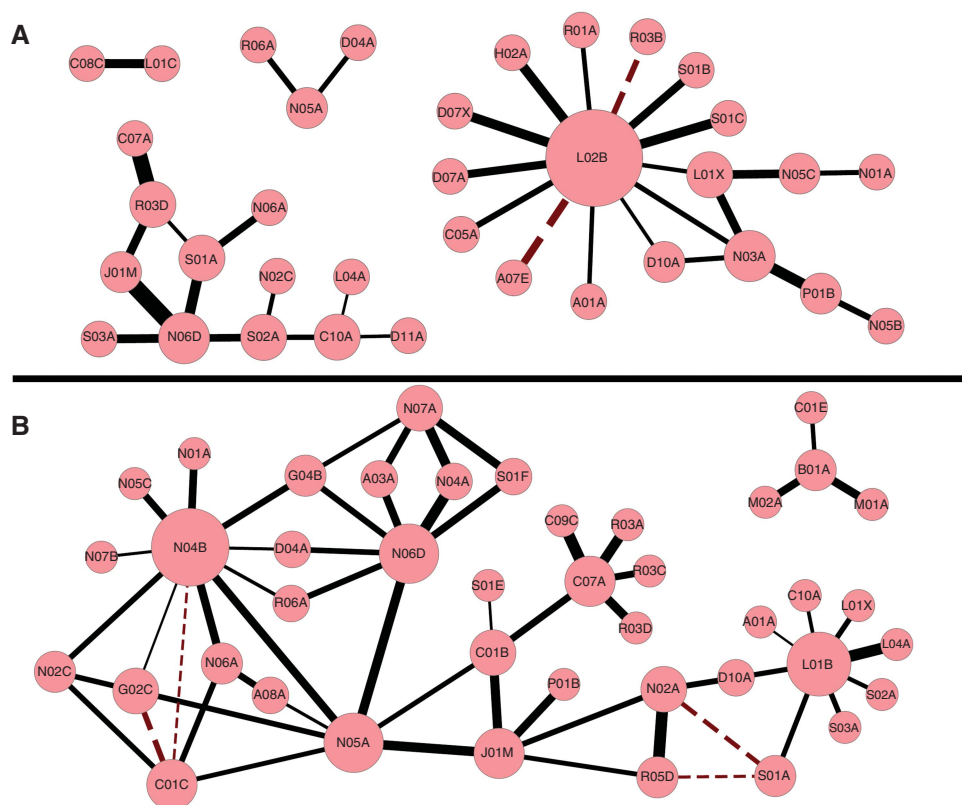


Figure 3 Networks of CRDs (A) and NCRDs (B) with severe recommendations between third level ATC classes. Black solid lines denote interactions supported by both known and predicted interactions, while red dashed lines denote interactions supported by predicted interactions only. Edge width corresponds to the average percentage of interacting drugs from each class and node size corresponds to the number of interactors (degree) of the class. The first letter of each ATC category denotes the top level, anatomical, class, including Alimentary tract and metabolism (A), Blood and blood forming organs (B), Cardiovascular system (C), Dermatologicals (D), Genito-urinary system and sex hormones (G), Systemic hormonal preparations, excluding sex hormones and insulins (H), Antiinfectives for systemic use (J), Antineoplastic and immunomodulating agents (L), Musculo-skeletal system (M), Nervous system (N), Antiparasitic products, insecticides and repellents (P), Respiratory system (R) and Sensory organs (S).

Table II Performance of predicted recommendations in cross validation

Recommendation	CRDs		NCRDs	
	% of predictions	AUC	% of predictions	AUC
Contraindicate	1%	0.9 ± 0.01	1%	0.96 ± 0.008
Generally avoid	5%	0.91 ± 0.007	7%	0.97 ± 0.002
Adjust dosage	2%	0.88 ± 0.01	1%	0.97 ± 0.005
Decrease dosage	27% ^a	0.94 ± 0.01	43% ^a	0.98 ± 0.01
Increase dosage	24% ^a	0.84 ± 0.08	2% ^a	0.91 ± 0.04
Limit dosage	49% ^a	0.96 ± 0.005	– ^b	–
Adjust dosage interval	– ^b	–	51% ^a	0.99 ± 0.009
Monitor	89%	0.93 ± 0.003	88%	0.98 ± 7e ⁻⁴

^aPercentage out of the adjust dosage recommendation.

^bNo predictions due to insufficient training data.

subtypes, providing such a subcategorization for 85 and 96% of the adjust dosage predicted CRDs and NCRDs, respectively (see Supplementary Material Section 5 and Supplementary Table S5 for further details and validation).

Using a similar methodology, we predicted which CYP enzymes may be the cause of the predicted CRDs—essential information in drug design (de Groot, 2006). Extracting the

CYP enzymes known to cause the interaction from each CRD description, resulted in seven CYP enzymes which appear prevalently in known CRDs (3A4, 2D6, 2C9, 1A2, 2C8, 2C1 and 2B6; Materials and methods). Overall, we predicted related CYPs for 99% of the CRD predictions, 18% of which have more than one CYP (versus 14% of the known CRDs). The predictions were validated in a cross validation setting

obtaining high AUCs (see Supplementary Material, Section 5 for details and an additional validation).

Analysis of the prevalence of known and predicted DDIs in clinical data

We sought to analyze the prevalence of DDIs in three independent clinically related sources: (i) ADRs; (ii) chronic medications taken by hospitalized patients in Israel; and (iii) common drug combinations.

To analyze ADRs, we used a comprehensive set of over 270 000 of them from the FDA Adverse Event Reporting System (Materials and methods). We considered drug pairs for which the number of ADRs is significantly higher than would be expected by chance, resulting in 1988 drug pairs involving drugs included in our CRD and NCRD sets (Materials and methods). We found that 6% ($P=4e^{-27}$) of these drug pairs are known CRDs, 15% ($P=e^{-25}$) are known NCRDs and 20% ($P=e^{-131}$) are known PCRDs. Additionally, 4% ($P=0.004$) of the drug pairs are novel predicted CRDs and 8% ($P=3e^{-19}$) are novel predicted NCRDs. Moreover, a significant portion of those drug pairs (4%, $P=6e^{-20}$) exhibits severe recommendations (see Supplementary Material, Section 6 for further details).

Next, we investigated the frequency of DDIs in drug pairs taken on a regular basis (*chronic* drugs). We retrieved electronic medical records of patients hospitalized in internal medicine departments at the Rabin Medical Center, Israel over a period of 1 year (Materials and methods). We investigated the frequency of severe DDIs in chronic drugs reported to the medical staff by 9413 of these patients, each taking at least two medications at admission time. We found that 3, 8 and 7% of the patients were taking at least two chronic medications with known severe CRDs, NCRDs or PCRDs, respectively. Additional 1% of the patients took chronic medications involving predicted severe CRDs and 3% of the patients took chronic medications with predicted severe NCRDs. Expectedly, these percentages follow the random expectation. Overall, 19% of the patients took at least two chronic medications with known or predicted severe DDIs, with 12% taking more than one such combination. Next, we compared the admission frequency of three patient groups over the course of 1 year (removing patients who passed away during that period for an unbiased comparison): (i) patients taking medications with at least one known severe interaction among them (group A); (ii) patients taking medications with predicted severe interactions, but with no known severe interactions (group B); and (iii) patients without any severe interactions (known or predicted) (group C). Expectedly, patients from groups A and B take more drugs than those in group C (11.6 ± 10 and 10.3 ± 7.3 for groups A and B, respectively, versus only 6.3 ± 5.9 for group C, $P=e^{-165}$ and $P=3e^{-24}$, respectively). Interestingly, we found that the patients from groups A and B were hospitalized 2 ± 1.6 and 2 ± 1.4 times on average during the year, while patients from group C were hospitalized only 1.8 ± 1.3 times on average (Wilcoxon ranked sum test $P=2e^{-27}$ and $P=e^{-10}$, respectively). We note that no significant age or gender differences were observed between both groups A or B and group C.

Additionally, no enrichment of the primary reason for hospitalization was observed (using the primary discharge ICD-9-CM code, two-sided Fisher exact test with false discovery rate of 0.01). We found that patients from group A tended to have richer medical history than group C patients, as expressed by the number of associated diagnosis and procedural ICD-9-CM codes prior to their hospitalization (number of past ICD codes = 3.5 ± 28 versus 2.7 ± 2.4 , $P=6e^{-16}$). However, no significant difference in medical history was observed between groups B (number of past ICD codes = 3 ± 2.4) and C. In order to compensate for the effect of medical history richness on the number of hospitalizations for group A patients, we further stratified these patients into those with: (i) scarce history (0–4 associated codes); (ii) moderate history (5–10 codes); and rich history (>10 codes). We found the same significant difference in number of hospitalizations between patients in group A and those in group C. Precisely, patients with both scarce history ($P=e^{-12}$) and moderate history ($P=0.003$) displayed significant differences, while rich history patients showed no significant difference.

One of the drug combination reported by these patients is a predicted CRDs (a predicted CYP3A4-related CRD), predicted to be contraindicated. This interaction involves Roxithromycin, a semi-synthetic macrolide antibiotic, very similar in composition, chemical structure and mechanism of action to Erythromycin (according to DrugBank) and Alfuzosin, an α -adrenergic blocker used to treat benign prostatic hyperplasia. This prediction is not surprising, since the similar antibiotic, Erythromycin—a potent inhibitor of CYP3A4, is known to contraindicate Alfuzosin by significantly increasing the plasma concentrations and pharmacologic effects of Alfuzosin, primarily metabolized by this isoenzyme (according to Drugsite Trust, Drugs.com). Several relatively frequent interactions (38 patients) were predicted to induce a severe NCRD. One example of such a predicted interaction involves Atenolol, a cardioselective β -adrenergic blocker and Methyldopa, an α -2 adrenergic agonist primarily used as antihypertensive agent. According to a third, independent, DDI database, ePocrates (www.epocrates.com), this combination has indeed an increased risk of severe rebound hypertension on α -2-agonist withdrawal (antagonistic effects, unopposed α -adrenergic stimulation).

Last, we explored combinations of drugs that are frequently administered concomitantly, listed in the Drug Combinations Database (DCDB) (Liu *et al*, 2010). DCDB holds approved or investigational drug combinations, specifying potential interactions between their components. We found that 12% of these drug combinations are known CRDs (including one contraindicated interaction) and 19% are known NCRDs (including three severe interactions). The use of such combinations implies that their benefit may, in some cases, outweigh the adverse effects caused by the induced interactions. Similarly, we found that 6% (9 pairs) of the drug combinations intersecting our drug sets are predicted CRDs and 13% (20 pairs) are predicted NCRDs. Three and 12 drug combinations, respectively, are novel predictions (i.e., not known PCRDs; Supplementary Table S3).

Importantly, we predicted a recommendation of ‘generally avoid’ for two predicted NCRDs, requiring special attention.

The first prediction involves Fluorouracil and Methotrexate, both antineoplastic antimetabolites, a combination used in adjuvant treatment for early breast cancer (Bonadonna *et al*, 1995). This combination was indeed found to be mutually antagonistic under some circumstances (Tattersall *et al*, 1973; Maugh, 1976) and maintaining a 24-h period between administrations of the two drugs was found to be preferable (Marsh *et al*, 1991). The second, related potential severe NCRD drug combination, involves Methotrexate and Leucovorin, the last being an active metabolite of folic acid. According to the Drugsite Trust, Drugs.com website, co-administration of Methotrexate with other agents known to induce hepatotoxicity may potentiate the risk of liver injury. Indeed, it is reported that the addition of Leucovorin to Fluorodeoxyuridine metabolite produces greater hepatotoxicity than when Fluorodeoxyuridine used alone (King and Perry, 2001). We highlight an additional example of a predicted CRD between Tolterodine, an antimuscarinic drug used to treat urinary incontinence, and Ergonovine, an ergot alkaloid with uterine and vascular smooth muscle contractile properties in the Supplementary Material (Section 7).

Conclusions

Our interaction prediction framework, INDI, leverages on the comprehensive set of known DDIs, as well as a set of basic drug properties such as chemical structure, drug targets and side effects. Unlike many DDI prediction methods, INDI does not require the collection of kinetic and dynamic parameters (Boobis *et al*, 2002; Obach *et al*, 2007). INDI attains very high rates of specificity and sensitivity in cross validation (AUC = 0.93 and 0.96 for CRDs and NCRDs, respectively). Furthermore, our predictions attain significant coverage of independent drug interaction sources for both interaction types ($P = 0$), as well as significant fraction of an independent drug interaction types such as PCRDs (12% of predicted CRDs, $P = 5e^{-313}$ and 28% of predicted NCRDs, $P = 0$). As more experimental evidence accumulates and PCRDs are easily classified to pharmacodynamic and pharmacokinetic types, INDI may further improve its prediction power.

A limitation of our method is that it does not take into consideration the method of administration of the drug (e.g., taken orally, injected or applied externally such as ointment or ophthalmic drops). As a specific example, a corticosteroid may produce an interaction when taken orally while having no effect when applied to the skin or taken as an ophthalmic solution. As this information is crucial for determining whether an interaction will take place in clinical reality, we addressed this limitation by manually removing predicted interactions involving topically applied drugs, as the probability of such interactions remains low. Nevertheless, this limitation should be taken into account by the physician in each case individually.

We extended our prediction scheme to two additional traits: (i) predicting the relevant recommendation for a predicted DDI; and (ii) predicting the CYP enzymes associated with a CRD. While the former is essential for selecting the correct type of action to take upon co-administration of the drugs, the latter highlights suspected CYPs, allowing the consideration of

patient-specific genetic polymorphisms related to certain CYP enzymes (Bertilsson, 1995; Goshman *et al*, 1999) (e.g., patients who are CYP2D6-deficient (Ingelman-Sundberg, 2004)). These extensions demonstrate the potential applicability of our approach for improving patient treatment and, ultimately, tailoring it to the patient's needs. We note that while we were able to predict the recommendation of the required type of dosage adjustment, we are limited in predicting the magnitude of the adjustment.

Using known and predicted DDIs, we explored their implications in clinical data. Using adverse drug reports, we were able to show that known and predicted DDIs significantly cover the majority of the adverse pairs of drugs reported. Additionally, we found an abundance of known and predicted severe DDIs in chronically administered medications, available in medical record data from hospitalized patients in Israel. These patients were hospitalized significantly more frequently than others. Finally, we detected two potentially severe DDIs in frequently used drug combinations. Our web tool, available in <http://www.cs.tau.ac.il/~bnet/software/INDI>, may aid physicians and researchers to exploit INDI's predictions in the clinical practice.

We suggest that our predictions may be beneficial in three areas: (i) drug development, especially in post-marketing surveillance, aiding in verification of hazardous interactions; (ii) large-scale clinical trial design, addressing and assessing potentially hazardous drug combinations; and (iii) driving and directing *in-vitro* validation of potentially hazardous interactions for efficiency and cost reduction of large-scale biological experiments. As a final word of caution, we note that while our predictions were validated *in-silico*, they should be further tested experimentally in order to establish their clinical implications. A special consideration should be given for drug combination whose benefit may outweigh the predicted adverse effects between them.

Materials and methods

Data sets

DDIs were extracted from DrugBank version 3 (Wishart *et al*, 2008) (freely available at www.drugbank.ca/downloads) and the <http://drugs.com> website (excluding DDIs tagged as minor), updated by Cerner Multum™ 21 June 2011. The DDIs from Drugsite Trust, Drugs.com were downloaded by using the generic drug names appearing in DrugBank to reach their corresponding Drugsite Trust, Drugs.com DDI index pages. From each such index page, we selected only the interacting drugs tagged as a major or moderate interaction. We provide a blinded drug-ID set and the set of similarity measures in order to allow readers to repeat the cross validation analysis (Supplementary File S1). Associations between drugs and Cytochrome P450 (CYP) were downloaded from DrugBank (Wishart *et al*, 2008), the Flockharts Interaction Table (Flockhart, 2007) and SuperCYP database (Preissner *et al*, 2010). DDIs between drugs associated with the same CYP and the interaction description specifically reports a CYP cause for the interaction were considered CRDs, while those lacking specific evidence in the interaction description were considered PCRDs.

Drug targets were obtained from DrugBank (Wishart *et al*, 2008), the DCDB (Liu *et al*, 2010), Matador (Gunther *et al*, 2008) and KEGG DRUG (Kanehisa *et al*, 2010) databases. Canonical simplified molecular input line entry specification (SMILES) (Weininger, 1988) of the drugs were extracted from DrugBank (Wishart *et al*, 2008). Drug side effects were obtained from SIDER (Kuhn *et al*, 2010). Drug-disease associations

were assembled from multiple sources including DrugBank (Wishart *et al.*, 2008), FDA drug labels in the DailyMed website (<http://dailymed.nlm.nih.gov>) and from <http://drugs.com> exploiting the MetaMap tool (Aronson, 2001) to parse textual indications in the same manner described in Gottlieb *et al.* (2011). Human PPIs were compiled from experimental and literature curated data (Xenarios *et al.*, 2002; Rual *et al.*, 2005; Stelzl *et al.*, 2005; Ewing *et al.*, 2007; Stark *et al.*, 2011). Protein sequences and GO annotations (Ashburner *et al.*, 2000) were downloaded from UniProt (Jain *et al.*, 2009). Drug combinations were downloaded from the DCDB database (Liu *et al.*, 2010). FDA Adverse Event Drugs were downloaded from the FDA Adverse Event Reporting System (AERS), reports available for the years 2004 through 2011 (first quarter). Drug approval years were retrieved from Drugs@FDA database (<http://www.accessdata.fda.gov/scripts/cder/drugsatfda/>) as of 19 October 2011.

Drug–protein considerations

There are three known types of interactions between drugs and CYPs (some drugs interact with a CYP in more than one way): the drug is (i) metabolized by the CYP enzyme (substrates); (ii) inhibits the CYP activity (inhibitor); or (iii) increases the CYP activity (inducer) (Zhou, 2008). Using information regarding each drug–CYP interaction type, we observed that CRDs spanned all possible drug–CYP combinations (e.g., a drug that is a substrate of a certain CYP interacts with a drug that is an inhibitor of the same CYP, two drugs that are substrates of the same CYP, etc.). We observed that ‘simple’ interactions, where each drug involves just one type of drug–CYP interaction comprised only 11% of the CRDs. We thus ignored this type of information.

While CYPs are the major family related to drug metabolism, other proteins (e.g., uridine diphosphate glucuronyl transferase and P-glycoprotein) may be involved in drug metabolism. These proteins had a small number of exclusively related DDIs (23 DDIs overall, unrelated to CYPs) and thus were excluded from the analysis.

Similarity measures

We defined and computed seven drug–drug similarity measures. Three of the drug–drug similarities (5–7) are gene-related, based on drug targets downloaded from the DrugBank (Wishart *et al.*, 2008), DCDB (Liu *et al.*, 2010), Matador (Gunther *et al.*, 2008) and KEGG DRUG (Kanehisa *et al.*, 2010) databases. For drugs associated with more than one gene, maximal similarities between the associated genes were averaged (averaging over the contribution of each member in a drug pair for symmetry). In order to extend our method to drugs for which not all seven similarity measures could be computed, the web tool provides predictions for drugs lacking the ligand and annotation-based similarity measures (#2 and #4). All similarity measures were normalized to be in the range [0, 1].

We used the following drug–drug similarity measures:

- (1) *Chemical-based*: Canonical SMILES (Weininger, 1988) of the drug molecules were downloaded from DrugBank (Wishart *et al.*, 2008). Hashed fingerprints were computed using the Chemical Development Kit (CDK) with default parameters (Steinbeck *et al.*, 2006). The similarity score between two drugs is computed based on their fingerprints according to the two-dimensional Tanimoto score (Tanimoto, 1957), which is equivalent to the Jaccard score (Jaccard, 1908) of their fingerprints, that is, the size of the intersection over the union when viewing each fingerprint as specifying a set of elements.
- (2) *Ligand-based*: The Similarity Ensemble Approach (SEA) (Keiser *et al.*, 2007) relates protein receptors based on the chemical 2D similarity of the ligand sets modulating their function. Given a drug’s canonical SMILES, the SEA search tool compares it against a compendium of ligand sets and computes *E*-values for those ligand sets. To compute a drug–drug similarity we queried drugs using their canonical SMILES on the SEA tool. To obtain robust results, we queried the drug against the two ligand databases provided in the tool (MDL Drug data report and WOMBAT (Olah *et al.*, 2005)) and used two different methods to compute the drug fingerprint (Scitegic ECFP4 and Daylight), resulting in four lists of

similar ligand sets. Unifying the four lists and filtering drug–ligand set pairs with *E*-values $> 10^{-5}$, we obtained a list of relevant protein–receptor families for each drug. Finally, the similarity between a pair of drugs was computed as the Jaccard score between the corresponding sets of receptor families.

- (3) *Side-effect based*: Drug side effects were obtained from SIDER (Kuhn *et al.*, 2010), an online database containing drug side effects associations extracted from package inserts using text mining methods. We augmented this list by side effect predictions for drugs that are not included in SIDER based on their chemical properties (Atias and Sharan, 2010). Following this latter work, we defined the similarity between drugs according to the Jaccard score between either their known side effects or top 13 predicted side effects in case they are unknown (following the number suggested by Atias and Sharan (2010)). In order to avoid bias from side effects related to kidney failure, which is directly related to drug interactions (Rowland Yeo *et al.*, 2011), we removed such side effects from consideration including renal failure or insufficiency, tubular acidosis and papillary necrosis.
- (4) *Annotation-based*: We used the World Health Organization (WHO) ATC classification system (Skrbo *et al.*, 2004). This hierarchical classification system categorizes drugs according to the organ or system on which they act, their therapeutic effect, and their chemical characteristics. ATC codes were obtained from DrugBank. To define a similarity between ATC terms we used the semantic similarity algorithm of (Resnik, 1999). This algorithm associates probabilities $p(x)$ with all the nodes (i.e., ATC levels) x in the ATC hierarchy by computing the number of levels below x ; it then calculates the similarity of two drugs as the maximum over all their common ancestors ATC level c of $-\log(p(c))$.
- (5) *Sequence-based*: Based on a Smith–Waterman sequence alignment score (Smith *et al.*, 1985) between the corresponding drug targets (proteins). Following the normalization suggested in Bleakley and Yamanishi (2009), we divided the Smith–Waterman score by the geometric mean of the scores obtained from aligning each sequence against itself.
- (6) *Closeness in a PPI network*: The distances between each pair of drug targets were calculated using an all-pairs shortest paths algorithm on the human PPI network. Distances were transformed to similarity values using the formula described in Perlman *et al.* (2011):

$$S(p, p') = Ae^{-D(p, p')} \quad (1)$$

where $S(p, p')$ is the computed similarity value between two proteins, $D(p, p')$ is the shortest path between these proteins in the PPI network and A was chosen according to Perlman *et al.* (2011) to be $0.9 \cdot e$. Self-similarity was assigned a value of 1.

- (7) *GO based*: Semantic similarity scores between drug targets were calculated according to Resnik (1999), using the `csbl.go R` package (Ovaska *et al.*, 2008) selecting the option to use all three ontologies.

Combining measures to classification features

The classification features that we used were constructed from association scores calculated on all possible pairs of drug–drug similarity measures, resulting in 49 features. For a given similarity measure pair (i.e., feature), the score of a given drug pair (d_1, d_2) is calculated by considering the similarity of all known drug interactions to this pair. The computation is done as follows: First, for each known interaction (d_1', d_2') we compute the drug–drug similarities $S(d_1, d_1')$ and $S(d_2, d_2')$ (and symmetrically $S(d_1, d_2')$ and $S(d_2, d_1')$). Next, we follow the method of Gottlieb *et al.* (2011) to combine the two similarities to a single score by computing their geometric mean. Finally, the overall score is

$$\text{Score}(d_1, d_2) = \max_{d_1', d_2' \neq d_1, d_2} \sqrt{S(d_1, d_1') \cdot S(d_2, d_2')} \quad (2)$$

For purposes of validation, self-similarities (in case (d_1, d_2) itself is a known interaction) are excluded.

Performance evaluation

We used a 10-fold cross validation scheme to evaluate the accuracy of our prediction algorithm. The entire training set used for the cross validation included the 37 212 true interactions and a randomly generated set of drug pairs (not part of the positive set), the same size as the positive set in each training set. When training on each interaction type separately, the training set included 5039 (training on CRDs) or 20 452 (training on NCRDs) true drug interactions and a randomly generated set of drug pairs (not part of the positive set), the same size as the positive set in each training set. To obtain robust AUC score estimates, we performed 10 independent cross validation runs, selecting a different negative set and different random partition of the training set to 10 parts in each; we then averaged the resulting AUC scores. Expectedly, taking a negative set of twice the size as the positive set had a negligible effect on the resulting AUC score (less than e^{-4}). We used the MATLAB implementation of the logistic regression classifier (glmfit function with binomial distribution and logit linkage) for the prediction task.

In order to test the effect of redundant drugs on prediction accuracy, we created non-redundant sets of drugs filtered by two different criteria: (i) chemical similarity above a Tanimoto coefficient ranging from 0.8 to 0.5 and (ii) target sharing. To this end, we iteratively selected the most similar pair and randomly removed one of the pair's drugs. We repeated this procedure 10 times for each similarity threshold to construct different non-redundant sets and averaged over the resulting AUC score (reporting also the AUC standard deviation).

Novel predictions and prediction assessment

To predict novel interactions for drugs, we used a training set that included all the known DDIs and an equally sized, randomly generated set of drug pairs that are not known to interact. We applied the trained classifier to a set of all the remaining possible drug pairs, including drugs with no known DDIs. We repeated the analysis with another randomly picked negative set, distinct from the first one, to assign prediction scores also to the random negative set that we initially used for training. The negative set was comprised solely of drugs with known DDIs in order to ensure a more robust definition of negative interactions. Overall, we obtained classification scores for all 320 182 and 304 769 drug pairs in the CRD and NCRD training sets, respectively. Following Gottlieb *et al* (2011), we selected a cutoff for the ranked list of predictions according to the best F1-measure obtained from the cross validation.

In addition to the cross validation, we validated our predictions by training only on the DrugBank DDIs and comparing the resulting predictions to known interactions from Drugsite Trust, Drugs.com. Additional validations for NCRDs include testing their agreement with tissue-specific information on the drugs and with co-morbidity of their indicated diseases. For the tissue validation, we constructed a tissue-based drug-drug similarity measure. We initially verified that drugs interacting via known NCRDs are significantly more similar to each other based on shared tissues than non-interacting pairs of drugs, supporting our assumption (Wilcoxon ranked sum test, $P = 3e^{-81}$ for disease-based and $P = 7e^{-45}$ for expression based drug-tissue associations; see Supplementary Material (Section 2) for details).

All enrichment P -values were computed using a hypergeometric test and tissue specificity validation computed a Wilcoxon ranked sum test.

Prediction of DDI recommendations and DDI-causing CYPs

We extracted the set of four recommendations available in the descriptions of each DDI defined in the Drugsite Trust, Drugs.com source: Contraindicate, generally avoid, adjust dosage and monitor. We further searched for the following keywords, defined in decreasing rank of severity: contraindicate, avoid, adjust dose/dosage and monitor in the DrugBank descriptions, followed by manual curation. A DrugBank description containing more than one keyword was classified according to the most severe term. DDIs having no

recommendation were classified as 'unknown'. We further divided the predicted DDIs with an adjust dosage recommendation prediction into four subgroups: (i) decrease dosage; (ii) increase dosage; (iii) limit dosage; or (iv) adjust dosage interval.

Each predicted interaction (CRD or NCRD) exploits a set of 49 most similar known DDIs. We formed four novel features describing each drug pair by computing the frequency of each of the four recommendations in these 49 known DDIs. We further trained a logistic regression classifier for each recommendation separately using these four features (in fact, since the four features sum to one, we removed one of the redundant features—the 'monitor' frequency feature). Performing the cross validation for each recommendation type, we selected all the DDIs sharing the same recommendation as the positive set and another, equally sized, random set of negative examples, comprised of the DDIs from the remaining three recommendations (for the 'monitor' recommendation type, spanning 84% of the total DDIs, we used the 14% remaining DDIs with known recommendation as a negative set). Finally, we applied the same framework as in the DDI prediction task to predict the recommendation type for each of the CRD and NCRD predictions (including novel drugs), training separately on each recommendation, using the entire positive set and a negative set of equal size. We selected the best F1-measure from each recommendation cross validation for choosing each recommendation prediction cutoff. If a DDI prediction was classified to more than one recommendation, we kept only the most severe one. Predicting adjust dosage subcategories, we applied the same procedure, constraining ourselves only to these predicted DDIs receiving an adjust dosage recommendation. If a DDI was classified to more than one subcategory, we retained the more general top level adjust dosage recommendation without a finer specification (including 15 and 4% of the adjust dosage predicted CRDs and NCRDs, respectively).

For the prediction of the CYP enzymes which are a possible cause of a CRD prediction, we extracted the name of the CYP enzymes involved in the gold-standard CRDs from the interaction description. We used the top seven CYP enzymes appearing in our training set, appearing in > 10 DDIs each (we excluded CYP 3A3, 2E1 and 2A6, appearing in < 4 DDIs each in our set). Following the same methodology as in the recommendation task, we calculated the frequency of each of the seven CYP enzymes across the 49 features describing a CRD prediction. Unlike the recommendation algorithm, known CRDs may have more than one CYP causing them, thus we normalized the frequency by the maximal CYP occurring and retained all seven features (not necessarily summing to one).

Analysis of interactions between drug classes

In order to focus on prevalent interactions between drug classes, we required that each class contains at least five drugs in our set. Furthermore, for visualization purpose, we filtered inter-class interactions that involved < 25% or 40% of the drugs in each class for CRDs or NCRDs, respectively. Last, self-interactions were removed due to trivial additive interactions.

Analysis of ADRs

We downloaded the FDA AERS data files and extracted all events that include one or two drugs. We kept only drug pairs we could map both to DrugBank drugs, using the generic name, synonyms and brand names available in DrugBank, followed by a manual curation to identify additional inexact drug name matches, resulting in 31 841 unique drug pairs, reported 272 150 times overall. We next used a hypergeometric test to extract drug pairs that were reported more than would be expected based on the overall occurrences of each individual drug in these reports, and corrected for multiple hypothesis testing using a false discovery rate of 0.01. This resulted in 1988 drug pairs, 147 of which contained drugs with no known DDIs.

Analysis of electronic medical records

We retrieved electronic medical records describing the medications taken on a regular basis reported by patients administered in either

internal or acute geriatric units at the Rabin Medical center, Israel between August 2010 and August 2011. The patients were identified by a randomly generated patient id. The study was approved by the Helsinki Committee of the Rabin Medical Center.

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

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Author contributions: AG and RS conceived the paper. AG performed the experiments and analyzed the data. AG, GYS and YO performed manual verification of novel predictions. AG, ER and RS wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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