Predicting Response to Platin Chemotherapy Agents with Biochemically-inspired Machine Learning

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Running Title:
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Selection of effective genes that accurately predict chemotherapy response could improve cancer outcomes. We compare optimized gene signatures for cisplatin, carboplatin, and oxaliplatin response, and respectively validate each with cancer patient data. Supervised support vector machine learning was used to derive gene sets whose expression was related to cell line GI50 values by backwards feature selection with cross-validation. Signatures at different GI50 thresholds distinguishing sensitivity from resistance contrast the contributions of genes at extreme vs. median thresholds. Ensembles of gene signatures at different thresholds are combined to reduce dependence on specific GI50 values for predicting drug response. The most accurate models for each platin are: cisplatin: BARD1, BCL2, BCL2L1, CDKN2C, FAAP24, FEN1, MAP3K1, MAPK13, MAPK3, NFKB1, NFKB2, SLC22A5, SLC31A2, TLR4, TWIST1; carboplatin: AKT1, EIF3K, ERCC1, GNGT1, GSR, MTHFR, NEDD4L, NLRP1, NRAS, RAF1, SGK1, TIGD1, TP53, VEGFB, VEGFC; oxaliplatin: BRAF, FCGR2A, IGF1, MSH2, NAGK, NFE2L2, NQO1, PANK3, SLC47A1, SLCO1B1, UGT1A1. TCGA bladder, ovarian and colorectal cancer patients were used to test cisplatin, carboplatin and oxaliplatin signatures (respectively), resulting in 71.0%, 60.2% and 54.5% accuracy in predicting disease recurrence and 59%, 61% and 72% accuracy in predicting remission. One cisplatin signature predicted 100% of recurrence in non-smoking bladder cancer patients (57% disease-free; N=19), and 79% recurrence in smokers (62% disease-free; N=35). This approach should be adaptable to other studies of chemotherapy response, independent of drug or cancer types.
KEY WORDS. Chemotherapy response, support vector machine, gene signatures, cancer, cisplatin, oxaliplatin, carboplatin, machine learning, bladder cancer, breast cancer, ovarian cancer
INTRODUCTION

Chemotherapy regimens are selected based on overall outcomes for specific types and subtypes of cancer pathology, progression to metastasis, other high-risk indications, and prognosis¹,², and variability in tumor resistance has led to tiered sequential strategies for selection of agents based on their overall efficacy³. We and others have developed machine learning (ML)-based gene signatures aimed at predicting response to specific chemotherapeutic agents and minimizing chemoresistance based on inhibition of growth or drug targets (GI₅₀ or IC₅₀)⁴-⁶. In this study, we present integrated ML models of platin responses (cis-, carbo- and oxaliplatin). Previous studies have reviewed the genes⁷, gene products⁸ and specific individual pathways⁹, but not developed a comprehensive model of the global cell response to the drug. We use integrated models based on expression of multiple genes to predict responses to each of these platin agents, for the first time, at different resistance levels.

Cisplatin, carboplatin and oxaliplatin are each widely prescribed compounds for their antineoplastic effects. While each contains platinum to form adducts with tumour DNA, their effectiveness differs for specific types of cancers, such as bladder (cisplatin), ovarian (cisplatin and carboplatin) and colorectal cancer (oxaliplatin). Carboplatin differs in structure from cisplatin, exchanging the latter’s dichloride ligands with a CBDCA (cyclobutane dicarboxylic acid) group, while oxaliplatin is paired with both a DACH (diaminocyclohexane) ligand and a bidentate oxalate group. These chelating ligands have greater stability and solubility to aqueous solutions, which lead to differences in drug toxicity compared to cisplatin¹⁰. Oxaliplatin can be up to two times as cytotoxic as cisplatin, but it forms fewer DNA adducts¹¹. The large hydrophobic DACH ligand which overlaps the major groove is thought to prevent binding of certain DNA repair enzymes such as the POL polymerases, and may contribute to the low cross-resistance between
oxaliplatin and cisplatin and carboplatin. While all three drugs can enter the cell via copper transporters, organic cation transporters are oxaliplatin-specific and likely play a role in its efficacy in colorectal cancer (CRC) cells where these transporters are commonly overexpressed. Oxaliplatin specifically plays a role in interfering with both DNA and RNA synthesis, unlike cisplatin which only interferes with DNA. It is these intrinsic properties between the platinum drugs which lead to differences in their activity and resistance profiles, despite their similar mode of action.

We derived gene signatures to predict drug response at different sensitivity and resistance levels for each of these agents. We and others have used supervised learning algorithms, including random forest models; support vector machine (SVM) models; neural networks; and linear regression models to make these predictions. Pathway and network analysis of gene expression have been used to indicate hundreds of genes potentially up- and down-regulated upon cisplatin treatment. Cisplatin-specific gene signatures have been developed with integrative approaches such as elastic net regression using inferred pathway activity of bladder cancer cell line data. These methods have implicated genes that have not been described previously. Supervised ML with biochemically-relevant genes has also been useful for predicting drug response. A concern with each of these ML approaches is that an insufficient number of samples coupled to a large number of features, i.e. gene expression changes, in each sample can result in overfitting of the model affecting its generalizability with other sources of data. We therefore reduce the number of dimensions by selecting genes biologically relevant to the drugs under observation. Additional selection criteria are necessary when the number of genes implicated in peer-reviewed reports is still prohibitively large compared to sample size.
Biochemically-inspired gene signatures have shown good performance in predicting treatment response. A paclitaxel ML signature based on tumor gene expression had a higher success predicting the pathological complete response rate (pCR) for sensitive patients (84% of patients with no / minimal residual disease) than models based on differential gene expression (GE) analysis. For gemcitabine, a signature derived from both expression and copy number (CN) data from breast cancer cell lines was derived, and subsequently applied to analysis of nucleic acids from patient archival material. Multiple other outcome measures used to validate gene signatures include prognosis, Miller-Payne response, and disease recurrence. Binary SVM classifiers based on discrete time thresholds have been used to classify continuous outcome measures such as prognosis and recurrence. By contrast, pCR is simpler to interpret with binary SVM models. Nevertheless, differences in clinical recurrence have been noted between patients demonstrated with pCR and those who do not exhibit disease pathology. This source of variability in defining patient response can confound transferability of SVM models between different datasets.

We apply biochemically-inspired ML to predict and compare the cellular and patient responses to cisplatin, carboplatin and oxaliplatin. We train models for classification of platin resistance with cancer cell line data and validate with patient GE and outcome data. Our previous gene signatures were based on median GI50 for each drug. This has been a necessary compromise, however in this study we consider signatures that differ at the highest vs. the lowest levels of drug resistance. A series of gene signatures are derived by shifting the GI50 thresholds that distinguish sensitivity from resistance. The frequency of genes selected at median vs. extreme thresholds highlights pathways that define these responses among different patient subsets.
RESULTS

Selection of Platin Drug Related Genes

We documented genes in the peer-reviewed literature associated with drug effectiveness or response (Supplemental References). For cisplatin, carboplatin and oxaliplatin, this implicated 178, 90, and 288 genes, respectively (Suppl. Table S1). Multiple factor analysis (MFA) was used to determine which genes were correlated to GI$_{50}$ in breast cancer cell lines through either GE and/or CN$^{13}$, significantly reducing the sizes of the gene sets for cisplatin (N=39), carboplatin (N=28), and oxaliplatin (N=55). Genes with significant relationships to GI$_{50}$ and direction of correlation (positive or inverse) are indicated in Figure 1. The diverse functions of these genes included apoptosis, DNA repair, transcription, cell growth, metabolism, immune system, signal transduction and membrane transport. Analysis of IC$_{50}$ and gene expression levels for cisplatin-treated bladder cancer cell lines confirmed these relationships evident from GI$_{50}$ values of different breast cancer lines. IC$_{50}$ values were related to GE for CFLAR, FEN1, MAPK3, MSH2, NFKB1, PNKP, PRKAA2, and PRKCA$^{20}$. Similarly, separate bladder cell line IC$_{50}$ values from the Genomics of Drug Sensitivity in Cancer project (http://www.cancerrxgene.org; N=17) were correlated with GE for CFLAR, FEN1, and NFKB1, in addition to ATP7B, BARD1, MAP3K1, NFKB2, SLC31A2 and SNAI1.

We performed MFA on the GI$_{50}$ values for cisplatin, carboplatin and oxaliplatin, without consideration of either GE or CN. Responses to cis- and carboplatin were directly correlated (a 6.2º separation between vectors), but neither was related to the oxaliplatin response (Figure 2). Previous studies have shown that cisplatin-resistant cell lines are generally sensitive to oxaliplatin$^{21-23}$. 

SVM-based signatures were initially derived for each platin drug from breast cancer cell line GE data. A 13-gene signature for cisplatin at the median $\text{GI}_{50}$ threshold (5.2% misclassification rate) consisted of $\text{BARD1}$, $\text{BCL2L1}$, $\text{FAAP24}$, $\text{CFLAR}$, $\text{MAP3K1}$, $\text{MAPK3}$, $\text{NFKB1}$, $\text{POLQ}$, $\text{PRKAA2}$, $\text{SLC22A5}$, $\text{SLC31A2}$, $\text{TLR4}$, and $\text{TWIST1}$. A similarly derived carboplatin signature included $\text{AKT1}$, $\text{ATP7B}$, $\text{EGF}$, $\text{EIF3I}$, $\text{ERCC1}$, $\text{GNGT1}$, $\text{HRAS}$, $\text{MTR}$, $\text{NRAS}$, $\text{OPRM1}$, $\text{RAD50}$, $\text{RAF1}$, $\text{SCN10A}$, $\text{SGK1}$, $\text{TIGD1}$, $\text{TP53}$, and $\text{VEGFB}$ (10.4% misclassification). For oxaliplatin, the final SVM model consisted of $\text{AGXT}$, $\text{APOBEC2}$, $\text{BRAF}$, $\text{CLCN6}$, $\text{FCGR2A}$, $\text{IGF1}$, $\text{MPO}$, $\text{MSH2}$, $\text{NAGK}$, $\text{NAT2}$, $\text{NFE2L2}$, $\text{NOTCH1}$, $\text{PANK3}$, $\text{PRSS1}$, and $\text{UGT1A1}$ (2.1% misclassification). A cisplatin SVM generated from 17 bladder cancer cell lines in cancerRxgeneresulted in 2 equally accurate signatures (with 11.8% misclassification) consisting of either $\text{PNKP}$ and $\text{PRKCA}$ or $\text{ATP7B}$, $\text{CFLAR}$, $\text{FEN1}$, $\text{MAPK3}$, $\text{NFKB1}$ and $\text{SLC22A11}$. These models were not useful for predicting patient outcomes due to the limited size of the training set.

**GI$_{50}$-Threshold Independent Modeling**

In our previous studies, we set median $\text{GI}_{50}$ value as the threshold to distinguished drug resistance and sensitivity.$^{5,6}$ An important question is whether the genes contributing to drug response are consistent among different cell lines, each with their own unique $\text{GI}_{50}$ values. Different ML models were obtained by shifting the $\text{GI}_{50}$ threshold, which changed the labels of resistant vs. sensitive cell lines. After feature selection, the compositions of the corresponding gene signatures for each threshold were compared. Finally, ensemble averaging of all of these optimized Gaussian SVM models derived for different $\text{GI}_{50}$ thresholds was used to create a threshold-independent ML-based signature.
Kinase (*MAPK3, MAP3K1*) genes and apoptotic family members (*BCL2, BCL2L1*) were most common in the cisplatin signatures at different GI50 thresholds, with consistent representation of error-prone and base-excision DNA repair genes as well (Figure 3A; Supplementary Table S2A). The kinases are more concentrated in signatures with lower drug sensitivity thresholds, whereas *BCL2* and *BCL2L1* are more ubiquitous at all levels. The error prone polymerases, *POLD1* and *POLQ*, are more frequent in models with lower sensitivity thresholds, while the flap endonuclease *FEN1* tends to be present at high levels of resistance. Thresholded models for carboplatin-related genes commonly contained the apoptotic family member *AKT1*, transcription regulation genes *ETS2* and *TP53*, as well as cell growth factors *VEGFB* and *VEGFC*, although the latter was less common at lower sensitivity thresholds (Figure 3B). Common oxaliplatin-related genes included transporters *SLCO1B1* and *GRTP1* (but not *SLC47A1*), transcription genes *NFE2L2, PARP15* and *CLCN6*, as well as multiple metabolism-related genes (Figure 3C).

SVM models were also derived using the cisplatin and/or carboplatin-treated TCGA (The Cancer Genome Atlas) bladder urothelial carcinoma patients, using post-treatment time to recurrence as a surrogate for different resistance thresholds (similarly performed in Mucaki *et al.* [2017]24; Supplementary Table S3). Similar trends to cell line SVMs are apparent; *POLQ* is frequently selected when the resistance threshold is set at a longer time period, while *FEN1* appears when it is shorter. However *BCL2*, which is present in a majority of breast cancer cell line SVMs, is present in only one model derived from TCGA data. Similarly, *MSH2* was rarely selected using cell lines, yet appears in nearly all patient derived SVMs with > 1 year recurrence.

GI50-thresholded models for each platin drug, generated with the breast cancer cell line data, produced 70 cisplatin, 83 carboplatin, and 83 oxaliplatin SVM models,
respectively. Each model was validated using available platin-treated patient datasets\textsuperscript{25–29}. The chemotherapy response metadata differed between studies. Als \textit{et al.}\textsuperscript{28} reported survival post-treatment, whereas Tsuji \textit{et al.}\textsuperscript{29} categorized patients as responders and non-responders. TCGA provided two different measures which were used to assess predictive accuracy in our models – chemotherapy response and disease-free survival. Accuracy is similar using either measure (Supplementary Table S4A); however recurrence and disease-free survival was used as the primary measure of response as it was more often recorded in the TCGA data sets tested. Patients from Als \textit{et al.} with a ≥ 5 year survival post-treatment were labeled as sensitive to treatment. The differences between these metadata may, in part, account for the differences in the prediction accuracy of the thresholded SVM models.

At higher resistance thresholds for any platin drug (low GI\textsubscript{50}), where more cell lines are labeled sensitive, the positive class (disease-free survival) is correctly classified, while the negative class (recurrence) is highly misclassified (Suppl. Figures 1 and 2). The reverse is true for models built using lower resistance thresholds (high GI\textsubscript{50}). We therefore state SVMs generated at these extreme thresholds are not very useful at predicting patient data. When used to predict recurrence in the TCGA datasets, sensitivity and specificity appears to be maximized in models where the GI\textsubscript{50} threshold for resistance was set near (but not necessarily at) the median (Suppl. Figure 1; Suppl. Tables S4A to 4C). While this pattern holds true for Tsuji \textit{et al.}\textsuperscript{29}, oxaliplatin models where GI\textsubscript{50} thresholds were set above the median could better separate primary and metastatic CRC patients (best model predicting 92.6% metastatic and 60.7% primary cancers; Suppl. Table S4C). While less consistent, cisplatin models generated with thresholds above median GI\textsubscript{50} performed better when evaluating the Als \textit{et al.}\textsuperscript{28} patient dataset (Suppl. Figure 2).
Models were further evaluated for their accuracy in TCGA patients using various recurrence times post-treatment to classify resistant and sensitive patients (0.5 - 5 years; Supplemental Table S5A-C). The best performing cisplatin model (BARD1, BCL2, BCL2L1, CDKN2C, FAAP24, FEN1, MAP3K1, MAPK13, MAPK3, NFKB1, NFKB2, SLC22A5, SLC31A2, TLR4, TWIST1; GI$_{50}$ of 5.11; hereby identified as Cis1) was able to accurately predict 71.0% of bladder cancer patients who recurred after 18 mo. (N=31; 58.5% accurate for disease-free patients [N=41]). Response of TCGA bladder patients treated with carboplatin (without cisplatin; N=19) were best predicted by Cis12 two years post-treatment (80% accurate for responding patients [N=5]; 93% for recurrent patients [N=14]). The best performing carboplatin model (AKT1, EIF3K, ERCC1, GNGT1, GSR, MTHFR, NEDD4L, NLRP1, NRAS, RAF1, SGK1, TIGD1, TP53, VEGFB, VEGFC; designated Car1 [Suppl. Table S5B]) was developed using a GI$_{50}$ threshold of 4.42, predicting recurrence of ovarian cancer after 4 years at an accuracy of 60.2% (N=302; 61.0% accurate for disease-free patients [N=108]). These models were also used to test TCGA bladder cancer patients treated carboplatin but not cisplatin (N=19), of which the best performing model (Car73) was 84% accurate for patients after 1 year of treatment (100% for responding patients [N=11]; 62.5% accuracy for recurrent [N=8]). Two additional carboplatin models are tied for overall accuracy (84%; Car9 and Car51), but more successfully predict non-responsive patients (87.5%; 82% accuracy for responding patients). These three models share four genes: AKT1, ETS2, GNGT1, and VEGFB. For oxaliplatin, the best performing model (BRAF, FCGR2A, IGF1, MSH2, NAGK, NFE2L2, NQO1, PANK3, SLC47A1, SLC10B1, UGT1A1; GI$_{50}$ of 5.10; designated Oxa1 [Suppl. Table S5C]) accurately predicted 71.6% of the disease-free TCGA CRC patients after one year (N=88; 54.5% accuracy predicting recurrence [N=11]). These models, as well as the previously mentioned SVMs based on bladder cell line data, were added to the online web-based SVM calculator (http://chemotherapy.cytognomix.com; introduced in
Dorman et al. [2016] which can be used to predict platin response using normalized
gene expression values.

To evaluate the consistency in the prediction of TCGA bladder cancer patients treated with cisplatin, the predicted distance from the hyperplane for all SVMs generated were plotted for each patient with a short recurrence time (<6 mo., N=10; Supplementary Figure 3). Despite showing similar levels of resistance to treatment, patterns differed between patients. While these patients would be expected to be indicated as highly cisplatin resistant (hyperplane distance < 0), two patients (TCGA-XF-A9SU and TCGA-FJ-A871) were predicted sensitive across nearly all SVM models. Similar variation was also seen in patients with either a long recurrence time (>4 years) or no recurrence at all after 6 years (Suppl. Figure 4).

Threshold independent models were generated for each individual platin drug at different GI₅₀ thresholds through ensemble ML, which involves the averaging of hyperplane distances for each model to generate a composite score for each TCGA patient tested. Hyperplane distances across all 70 cisplatin models were similar, with a mean score of -0.22 and a standard deviation of 3.5 hyperplane units (hu) across the set of patient data. The ensemble model classified disease-free bladder cancer patients with 59% accuracy and those with recurrent disease with 47% accuracy. Limiting ensemble averaging to only cisplatin models generated at a moderate GI₅₀ threshold (ranging from 5.10 to 5.50) did not significantly improve accuracy (44% for disease-free and 66% for recurrent patients; Suppl. Table S6A). For carboplatin, ensemble ML did not produce significantly better predictions than random, regardless of the GI₅₀ threshold interval selected (Suppl. Table S6B) or the similar mean hyperplane distances (-0.11 +/- 3.9 hu). For oxaliplatin, the ensemble ML model (mean = -0.12 +/- 2.7 hu) was most accurate after 1 year (60% accuracy for disease-free and 73% for recurrent patients;
As in cisplatin, limiting this analysis to oxaliplatin SVM models with moderate GI$_{50}$ thresholds did not significantly increase accuracy.

To determine the impact of individual genes on overall model accuracy, each gene within every SVM model was excluded, and model accuracy was reassessed (Supplementary Table S2A; S2B and S2C contain carbo- and oxaliplatin models, respectively). Genes which consistently significantly increase misclassification (averaging > 16% increase) in moderate threshold SVMs (GI$_{50}$ thresholds set from 5.1 to 5.5) include ERCC2, POLD1, BARD1, BCL2, PRKCA and PRKCB. ERCC2 and POLD1 perform critical functions in nucleotide and base excision repair, respectively. PRKCA and PRKCB are paralogs with significant roles in signal transduction. BARD1 has been shown to reduce apoptotic BCL2 in the mitochondria$^{30}$, and has a key role in genomic stability through its association with BRCA1. Genes with a high variance in increased misclassification between different models include NFKB1, NFKB2, TWIST1, TP63, PRKAA2, and MSH2. The variance of these genes may be due to epistatic interactions with other biological components, including the other genes in the SVM. For example, NFKB1 and NFKB2 are jointly included in 7 SVMs generated at a moderate GI$_{50}$ threshold. There is evidence of possible epistasis in that the removal of either of these genes, but not necessary both, will have a large impact in model misclassification rates (≥ 18.0% increase). The misclassification variance of NFKB1 with NFKB2, is significantly lower than in SVM models lacking NFKB2.

**Predicting cisplatin response in patients based on smoking history**

Tobacco smoking is known as the highest risk factor for the development of bladder cancer$^{31}$. We therefore subdivided the patients based on their smoking history and tested the thresholded models (Supplementary Tables S7 and S8). When testing
patients who were lifelong non-smokers, the prediction accuracy of Cis1 predicted all non-smoking patients who were recurrent after 18 months as cisplatin-resistant (N=5). Prediction accuracy for disease-free patients was 57.1% (N=14). Another model (Cis18; Suppl. Table S7) had performed equally as well for non-smokers, and these two models share 7 genes: BCL2, BCL2L1, FAAP24, MAP3K1, MAPK13, MAPK3, and SLC31A2. Threshold independent analysis predicted disease-free equally well, but recurrence was less accurate (66.7%). Note that non-smokers make up a small subset of the patients tested (N=19). Threshold-independent prediction of recurrence in patients with a smoking history was 46% accurate (N=13), while disease-free patients were correctly predicted at a rate of 58% (N=19). Recurrence in these patients was best predicted by a model built at the median GI50 threshold (Cis2). Accuracy improved for both disease-free (57.7% -> 61.9%) and recurrent patients (76.0% -> 78.6%) when excluding patients who quit smoking more than 15 years before diagnosis. Genes in this SVM which are not present in the two models which performed well for non-smokers include CFLAR and PRKAA2.

Tobacco smoking has a significant impact on cytosine methylation levels in the genome32. CpG island methylation has been associated with smoking pack years in a subset of the TCGA bladder urothelial carcinoma patients25. We suspected that the level of methylation measured in t SVMs which performed best for smoking and non-smoking patients might differ, and with possible concomitant effects on GE. When ranking each gene from Cis1 by highest methylation and GE, 88 of 1080 patient: gene combinations showed the expected inverse correlation between methylation levels and GE (i.e. high methylation and low GE). Inverse correlation of methylation and GE was more common than direct correlation (i.e. high methylation and high GE; N=17). However, direct correlation was more common in patients with a recent smoking history (70.5%). This
pattern was also observed for Cis2, which best predicted recurrence in smokers. In cases where methylation and GE are directly correlated, we propose that smoking may alter expression by other effects, eg. mutagenic, rather solely than by epigenetic inactivation through methylation.

To determine which genes in these models led to discordant predictions of patient outcome, we conducted a bioinformatic experiment to gradually alter the expression of each signature gene until the misclassification was corrected. If the GE value required to cross this threshold exceeded ≥ 3-fold the highest/lowest expression of that gene, it was interpreted as a minor contributor to the prediction. Genes which could not correct a discordant prediction included PRKAA2, NFKB1, NFKB2 and TWIST1. Significant genes which, when altered, corrected discordant predictions included MAP3K1, MAPK3, SLC22A5 and SLC31A2. Altering BCL2L1 expression was more likely to correct the discordant predictions of Cis1 (4 out of 5) than with Cis2 (2 out of 4).

DISCUSSION

Using gene expression signatures, we derived both GI50 threshold-dependent and -independent ML models which predict the chemotherapy responses for cisplatin, carboplatin and oxaliplatin, respectively. The cisplatin model Cis1 (Supplementary Table S5A) most accurately predicted response in bladder cancer patients after 18 months, and Car1 (Suppl. Table S5B) best predicted response in ovarian cancer patients after 4 years. Oxa1 (Suppl. Table S5C) more accurately predicted disease-free patients than recurrent disease at the one year treatment threshold. The thresholds which best represented time-to-recurrence differed between the platin drugs in each cancer type. Cisplatin gene signatures had noticeably improved performance when smoking history was taken into account.
The three platin drugs produce distinctly different gene signature models. Initial gene sets exhibited some overlap between platin drugs (N=67 between any two platins), but very few of these were correlated by MFA of GI$\text{}_{50}$ with multiple platin drugs ($\text{ATP7B}$, $\text{BCL2}$ and $\text{MSH2}$). Despite the close similarity between cisplatin and carboplatin GI$\text{}_{50}$ response (see Figure 2), only one gene ($\text{ATP7B}$) was related by MFA to GI$\text{}_{50}$ levels of both drugs. $\text{BCL2}$ and $\text{MSH2}$ correlated with both cisplatin and oxaliplatin GI$\text{}_{50}$. The increase in misclassification caused by the elimination of $\text{MSH2}$ from any SVM model in which it was present was significant; for example, in oxaliplatin model Oxa21 (Suppl. Table S5C) and cisplatin model Cis14 (Suppl. Table S5A) misclassification was increased by 19.1% and 28.2%, respectively. These differences may reflect the spectrum of activity, sensitivity, and toxicity of these signature genes$^{21-23,33,34}$.

Gene signature models derived from cell lines and tested on patients differ in their outcome measures. The exact GI$\text{}_{50}$ cell line threshold that is most predictive of patient outcome is not known, and different groups use different methods to discretize GI$\text{}_{50}$ values$^{35,36}$. Therefore, we developed ML models for platin drugs which predict drug response without relying on arbitrary GI$\text{}_{50}$ thresholds. For cisplatin, SVM ensemble averaging generated on different resistance thresholds shows a small increase in accuracy over most models, better representing the sensitive, disease-free class (59% accuracy). Interestingly, ensemble averaging of only the models built using a moderate GI$\text{}_{50}$ thresholds yielded results which better represented the resistance class. This result closer matches the accuracy of Cis1, and may be due to Cis1 having a greater overall impact on the ensemble prediction. When limiting ensemble averaging to only those models with the highest area under the curve (AUC) at each resistance threshold, differences in predictions were negligible. Ensemble ML can potentially avoid problems...
with poor performance and overfitting by combining models that individually perform slightly better than chance\textsuperscript{37}. It is difficult to reconcile gene signatures without features known to be related to chemoresistance with tumor biology. Our thresholding approach may reveal potentially important genes and pathways associated with platin resistance. It would be preferable to explore pathways related to signature genes to improve accuracy, identify potential targets for further study of chemoresistance, and expand the model parameters to take into account alternate states besides those captured in the original signature\textsuperscript{38}. Signatures for resistance may be useful for developing targeted intervention to re-sensitize tumours. For example, the mismatch repair (MMR) gene \textit{MSH2} is commonly present in gene signatures at high resistance levels for oxaliplatin, which is of interest, as MMR deficiency has been shown to be predictive for oxaliplatin resistance\textsuperscript{34}. Indeed, \textit{MLH1}, \textit{MSH2} and \textit{MSH6}-deficient cells are more susceptible to oxaliplatin, despite MMR-deficiency being associated with cisplatin resistance\textsuperscript{33}. The autoimmune disease-associated gene \textit{SIAE}, which has been previously shown to have a strong negative correlation to oxaliplatin response in advanced CRC patients\textsuperscript{39}, was selected in the majority of thresholded oxaliplatin models (Supplementary Table S2C). The gene \textit{BCL2}, which was commonly selected for cisplatin (Figure 3A), was rarely selected for oxaliplatin (Figure 3C). At the highest levels of resistance to cisplatin, models were enriched for genes belonging to DNA repair, anti-oxidative response, apoptotic pathways and drug transporters (Figure 3A). These gene pathways are known to be involved in cisplatin resistance\textsuperscript{40,41} and these specific genes may be explored in subsequent work to identify the contribution to chemotherapy response in a biochemical context.

It may be feasible to predict responses to combination chemotherapy with the models described here. Not included in the present analysis were signatures for
methotrexate, vinblastine, and doxorubicin, which comprise the MVAC cocktail used to
treat bladder cancer. This was due primarily to a lack of patients treated with this drug
combination in the TCGA bladder dataset (N=11). Individual signatures for several of
these drugs have been derived and analyzed using the patient data from METABRIC
(Molecular Taxonomy of Breast Cancer International Consortium)\textsuperscript{24}. A reasonable
approach to predicting combination chemotherapy would first determine the probability
of sensitivity or resistance to individual drugs, accounting for the misclassification rate by
each (defined as $d_1$, ..., $d_n$). The ML classifiers output these probabilities, analogous to
their misclassification rates in a set of patients treated identically. If the model predicts
that the patient is sensitive to drug $d_1$ with 90% probability, and sensitive to drug $d_2$ with
5% probability, the probability of sensitivity to the combination is $1 - (1 - 0.9)*(1 - 0.05) =$
90.5%, and the probability of resistance is 9.5%. The correlated responses could be
estimated for drug pairs, $d_1$ and $d_2$, and then adjusted for the combined probability of the
pair to $d_{12}$, based on the features that are shared by the signatures of both drugs. The
probability of sensitivity would then be given by $1 - (1 - d_{12})*(1 - d_3)*...*(1 - d_n)$.

The predictive accuracy for the same model could differentiate highly between
the two datasets. \textbf{Cis3} (Supplemental Table S5A) had a high predictive accuracy and
AUC for TCGA bladder cancer patients (AUC=0.64). However, the AUC was lower when
applied to the Als et al.\textsuperscript{28} dataset (AUC=0.18). Patient metadata in the latter study only
indicated patient survival times, while we base the expected TCGA patient outcome on
time to disease recurrence. As the basis of our expected outcome differs between
datasets, these differences may be acting as a confounding factor to determine accuracy
of gene signatures. The datasets also differ in how expression was measured
(microarray vs. RNA-seq). The relevance of models based on training and testing data
from different platforms can affect the accuracy of validation, which might not be
improved by data normalization. In this study, datasets were subjected to z-score normalization. In subsequent studies, other techniques to correct for some of these effects have been described and could be applied\(^42\).

In summary, we describe GI\(_{50}\)- or IC\(_{50}\)-threshold-independent ML models to predict chemotherapy response to platin agents in cancer patients. Ensemble machine learning produced combined signatures that were more accurate than most individual models generated with different thresholds. Genes associated cisplatin response included those which exacerbate resistance in patients with a history of smoking. The methodology described here should be adaptable to other drugs and cancer types. With a range of models for multiple drugs, it may be possible to improve the efficacy of treatment by tailoring treatment to a patient's specific tumour biology, and reduce treatment duration by limiting the number of different therapeutic regimens prescribed before achieving a successful response\(^43\).

**MATERIALS AND METHODS**

**Data and preprocessing**

Microarray GE and data were from breast cancer cell lines were used to train ML-based gene signatures of drug response based on respective growth or target inhibition data (GI\(_{50}\) or IC\(_{50}\)). Cell lines were treated with either cisplatin (N=39), carboplatin (N=46), or oxaliplatin (N=47)\(^13\). Bladder cancer cell line GE and IC\(_{50}\) measurements for cisplatin were obtained from cancerRxgene (N=17). However, all testing was performed on breast cancer cell line data as the number of bladder cancer cell lines was insufficient to produce accurate signatures. RNA-seq GE and survival measurements were downloaded from TCGA for bladder urothelial carcinoma (N=72 patients treated with cisplatin)\(^25\), ovarian epithelial tumor (N=410 treated with
carboplatin)\textsuperscript{26} and colorectal adenocarcinoma (N=99 treated with oxaliplatin)\textsuperscript{27}. GE of cisplatin-treated patients of cell carcinoma of the urothelium (N=30)\textsuperscript{28} and for oxaliplatin-treated CRC patients (N=83)\textsuperscript{29} were obtained from the Gene Expression Omnibus. Clinical metadata and GE for TCGA patients were obtained from Genomic Data Commons (https://gdc.cancer.gov/), while methylation HM450 (Illumina) data for these patients was downloaded from cBioPortal\textsuperscript{44}.

Initial gene sets for developing signatures for each drug were identified from previously published literature (see Supplemental References) and databases, such as PharmGKB and DrugBank\textsuperscript{45,46}. Genes were then eliminated that showed no or little expression in TCGA bladder cancer patients (where RNA-seq by Expectation Maximization [RSEM] is < 5.0 for majority of individuals). The final gene sets were chosen using MFA to analyze interactions between GE, CN, and GI\textsubscript{50} data for the drug of interest\textsuperscript{47}. Genes whose GE and/or CN showed a direct or inverse correlation with GI\textsubscript{50} were selected for SVM training. This reduces the overall number of genes for SVM analysis, and thus helps to avoid a data to size sample imbalance. For cisplatin, MFA was repeated using IC\textsubscript{50} values for 17 bladder cancer cell lines; however, the available CN data generally showed a lack of variation in the cell lines for these genes. Instead, the available IC\textsubscript{50} values for three other cancer drugs (doxorubicin, methotrexate and vinblastine) were compared with the IC\textsubscript{50} of cisplatin by MFA.

Applying an SVM model directly to patient data without a normalization approach is imprecise when training and testing data are not obtained using similar methodology (i.e. different microarray platforms). To compare the cell line GE microarray data and the patient RNA-seq GE datasets, expression values were normalized by conversion to z-scores using MATLAB\textsuperscript{48}. Although Log2 intensity values from microarray data were not
available for TCGA samples, RNA-seq based GE and log₂ intensities from microarray data are highly correlated⁴⁹.

**Machine Learning**

SVM were trained with breast cancer cell line GE datasets¹³ with the Statistics Toolbox in MATLAB⁴⁸ using Gaussian kernel functions (fitcsvm), and then tested with a leave-one-out cross-validation (using ‘crossval’ and ‘leaveout’ options). A greedy backwards feature selection algorithm was used to improve classification accuracy⁵⁰. The gene subset with the lowest misclassification with cross-validation is selected as the model for subsequent testing with patient GE and clinical data.

*Derivation of gene signatures for different drug resistance thresholds*

We have previously set a conventional GI₅₀ threshold distinguishing sensitivity from resistance at the median of the range of drug concentrations that inhibited cell growth by 50%⁶. We hypothesized that different gene signatures could be derived for different levels of drug resistance by varying this threshold. ML experiments for classifying resistance or sensitivity at GI₅₀ values generated a series of optimized Gaussian SVM models whose performance were assessed with patient expression data for each signature. A heat map which illustrates the frequencies of genes appearing in these models was created with the R language `hist2d` function.

A composite gene signature was created by ensemble averaging of all models generated at each resistance threshold. Ensemble averaging combines signatures through averaging the weighted accuracy of a set of related models³⁷. The decision function for the ensemble classifier is the mean of the decision function scores of the component classifiers, weighted by the AUC.
Randomization

The potential for models to overfit data during training and/or feature selection was assessed by permutation analysis with randomized cell line labels and with random sets of genes, as described previously\textsuperscript{6}. Using the median cisplatin GI\textsubscript{50} as the resistance threshold, 10,000 models based on random gene selection (15 genes) had higher rates of misclassification than the best median SVM models (2 signatures with 7.7% misclassification). Cisplatin, carboplatin and oxaliplatin GE data for random cell line label combinations (n=10,000) generated only 8, 1 and 1 signatures, respectively, with lower error rates than the best biochemically-inspired signatures.

ACKNOWLEDGEMENTS

Katherina Baranova contributed to the cisplatin gene signatures and Dimo Angelov developed automated feature selection. We thank Murray Junop for commenting on the manuscript. Compute Canada and Shared Hierarchical Academic Research Computing Network (SHARCNET) provided high performance computing and storage facilities.

CONFLICTS OF INTEREST

PKR cofounded CytoGnomix Inc., which hosts the interactive resource described in this study for prediction of responses to chemotherapy agents. The other authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

PKR and DL designed the methodology. EJM and JZ performed analyses. EJM and PKR wrote the manuscript.

FUNDING
PKR is supported by NSERC (RGPIN-2015-06290), Canadian Foundation for Innovation, Canada Research Chairs, and CytoGnomix.
REFERENCES

1. Cardoso, F. et al. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **23**, vii11–vii19 (2012).

2. Oostendorp, L. J., Stalmeier, P. F., Donders, A. R. T., van der Graaf, W. T. & Ottevanger, P. B. Efficacy and safety of palliative chemotherapy for patients with advanced breast cancer pretreated with anthracyclines and taxanes: a systematic review. *Lancet Oncol.* **12**, 1053–1061 (2011).

3. Alfarouk, K. O. et al. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Cancer Cell Int.* **15**, 71 (2015).

4. Gąsowska-Bodnar, A. et al. Survivin Expression as a Prognostic Factor in Patients With Epithelial Ovarian Cancer or Primary Peritoneal Cancer Treated With Neoadjuvant Chemotherapy: *Int. J. Gynecol. Cancer* **24**, 687–696 (2014).

5. Hatzis, C. et al. A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. *JAMA* **305**, 1873–1881 (2011).

6. Dorman, S. N. et al. Genomic signatures for paclitaxel and gemcitabine resistance in breast cancer derived by machine learning. *Mol. Oncol.* **10**, 85–100 (2016).

7. Zhang, S. et al. Organic Cation Transporters Are Determinants of Oxaliplatin Cytotoxicity. *Cancer Res.* **66**, 8847–8857 (2006).

8. Poisson, L. M. et al. A metabolomic approach to identifying platinum resistance in ovarian cancer. *J. Ovarian Res.* **8**, (2015).

9. Cadoná, F. C. et al. Guarana a Caffeine-Rich Food Increases Oxaliplatin Sensitivity of Colorectal HT-29 Cells by Apoptosis Pathway Modulation. *Anticancer Agents Med. Chem.* **16**, 1055–1065 (2016).
10. Kasparkova, J., Vojtiskova, M., Natile, G. & Brabec, V. Unique Properties of DNA Interstrand Cross-Links of Antitumor Oxaliplatin and the Effect of Chirality of the Carrier Ligand. *Chem. – Eur. J.* **14**, 1330–1341 (2008).

11. Woynarowski, J. M. *et al.* Oxaliplatin-Induced Damage of Cellular DNA. *Mol. Pharmacol.* **58**, 920–927 (2000).

12. Tashiro, T., Kawada, Y., Sakurai, Y. & Kidani, Y. Antitumor activity of a new platinum complex, oxalato (trans-l,2-diaminocyclohexane)platinum (II): new experimental data. *Biomed. Pharmacother.* **43**, 251–260 (1989).

13. Daemen, A. *et al.* Modeling precision treatment of breast cancer. *Genome Biol.* **14**, R110 (2013).

14. Yuan, Y. *et al.* Identification of the biomarkers for the prediction of efficacy in first-line chemotherapy of metastatic colorectal cancer patients using SELDI-TOF-MS and artificial neural networks. *Hepatogastroenterology.* **59**, 2461–2465 (2012).

15. L’Espérance, S., Bachvarova, M., Tetu, B., Mes-Masson, A.-M. & Bachvarov, D. Global gene expression analysis of early response to chemotherapy treatment in ovarian cancer spheroids. *BMC Genomics* **9**, 99 (2008).

16. Nickerson, M. L. *et al.* Molecular analysis of urothelial cancer cell lines for modeling tumor biology and drug response. *Oncogene* (2016). doi:10.1038/onc.2016.172

17. Yuryev, A. Gene expression profiling for targeted cancer treatment. *Expert Opin. Drug Discov.* **10**, 91–99 (2015).

18. Sataloff, D. M. *et al.* Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of outcome. *J. Am. Coll. Surg.* **180**, 297–306 (1995).

19. Ogston, K. N. *et al.* A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast Edinb. Scotl.* **12**, 320–327 (2003).
20. Earl, J. et al. The UBC-40 Urothelial Bladder Cancer cell line index: a genomic resource for functional studies. *BMC Genomics* **16**, 403 (2015).

21. Rixe, O. et al. Oxaliplatin, tetraplatin, cisplatin, and carboplatin: Spectrum of activity in drug-resistant cell lines and in the cell lines of the national cancer institute's anticancer drug screen panel. *Biochem. Pharmacol.* **52**, 1855–1865 (1996).

22. Mehmood, R. K. Review of Cisplatin and oxaliplatin in current immunogenic and monoclonal antibody treatments. *Oncol. Rev.* **8**, 256 (2014).

23. Kweekel, D. M., Gelderblom, H. & Guchelaar, H.-J. Pharmacology of oxaliplatin and the use of pharmacogenomics to individualize therapy. *Cancer Treat. Rev.* **31**, 90–105 (2005).

24. Mucaki, E. J. et al. Predicting Outcomes of Hormone and Chemotherapy in the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) Study by Biochemically-inspired Machine Learning. *F1000Research* **5**, 2124 (2017).

25. Robertson, A. G. et al. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. *Cell* **171**, 540-556.e25 (2017).

26. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* **474**, 609–615 (2011).

27. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**, 330–337 (2012).

28. Als, A. B. et al. Emmprin and survivin predict response and survival following cisplatin-containing chemotherapy in patients with advanced bladder cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **13**, 4407–4414 (2007).

29. Tsuji, S. et al. Potential responders to FOLFOX therapy for colorectal cancer by Random Forests analysis. *Br. J. Cancer* **106**, 126–132 (2012).
30. Tembe, V. et al. The BARD1 BRCT domain contributes to p53 binding, cytoplasmic and mitochondrial localization, and apoptotic function. *Cell. Signal.* **27**, 1763–1771 (2015).

31. Freedman, N. D., Silverman, D. T., Hollenbeck, A. R., Schatzkin, A. & Abnet, C. C. Association between smoking and risk of bladder cancer among men and women. *JAMA* **306**, 737–745 (2011).

32. Joehanes, R. et al. Epigenetic Signatures of Cigarette Smoking. *Circ. Cardiovasc. Genet.* (2016). doi:10.1161/CIRCGENETICS.116.001506

33. Raymond, E., Faivre, S., Chaney, S., Woynarowski, J. & Cvitkovic, E. Cellular and Molecular Pharmacology of Oxaliplatin. *Mol. Cancer Ther.* **1**, 227–235 (2002).

34. Alex, A. K. et al. Response to Chemotherapy and Prognosis in Metastatic Colorectal Cancer With DNA Deficient Mismatch Repair. *Clin. Colorectal Cancer* (2016). doi:10.1016/j.clcc.2016.11.001

35. Sos, M. L. et al. Predicting drug susceptibility of non-small cell lung cancers based on genetic lesions. *J. Clin. Invest.* **119**, 1727–1740 (2009).

36. Laderas, T. G., Heiser, L. M. & Sönmez, K. A Network-Based Model of Oncogenic Collaboration for Prediction of Drug Sensitivity. *Front. Genet.* **6**, (2015).

37. Clemen, R. T. Combining forecasts: A review and annotated bibliography. *Int. J. Forecast.* **5**, 559–583 (1989).

38. Airley, R. *Cancer chemotherapy*. (Wiley-Blackwell, 2009).

39. Li, X.-X. et al. RNA-seq identifies determinants of oxaliplatin sensitivity in colorectal cancer cell lines. *Int. J. Clin. Exp. Pathol.* **7**, 3763–3770 (2014).

40. Borst, P., Rottenberg, S. & Jonkers, J. How do real tumors become resistant to cisplatin? *Cell Cycle Georget. Tex* **7**, 1353–1359 (2008).

41. Wernyj, R. & Morin, P. Molecular mechanisms of platinum resistance: still searching for the Achilles heel. *Drug Resist. Updat.* **7**, 227–232 (2004).
42. Johnson, W. E., Li, C. & Rabinovic, A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostat. Oxf. Engl.* 8, 118–127 (2007).

43. Akamatsu, N., Nakajima, H., Ono, M. & Miura, Y. Increase in acetyl CoA synthetase activity after phenobarbital treatment. *Biochem. Pharmacol.* 24, 1725–1727 (1975).

44. Gao, J. *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* 6, pl1 (2013).

45. Whirl-Carrillo, M. *et al.* Pharmacogenomics Knowledge for Personalized Medicine. *Clin. Pharmacol. Ther.* 92, 414–417 (2012).

46. Law, V. *et al.* DrugBank 4.0: shedding new light on drug metabolism. *Nucleic Acids Res.* 42, D1091–D1097 (2014).

47. Abdi, H. & Williams, L. J. Principal component analysis. *Wiley Interdiscip. Rev. Comput. Stat.* 2, 433–459 (2010).

48. MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States.

49. Marioni, J. C., Mason, C. E., Mane, S. M., Stephens, M. & Gilad, Y. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.* 18, 1509–1517 (2008).

50. Bermingham, M. L. *et al.* Application of high-dimensional feature selection: evaluation for genomic prediction in man. *Sci. Rep.* 5, 10312 (2015).
FIGURE LEGENDS

**Figure 1.** Schematic of platinum drug sensitivity and resistance genes which showed MFA correlation for GI\textsubscript{50} of A) cisplatin, B) carboplatin, and C) oxaliplatin. The genes used to derive the SVM are shown in context of their effect in the cell and role in cisplatin mechanisms of action. GE and CN correlation with inhibitory drug concentration by MFA of breast (GI\textsubscript{50}) and bladder (IC\textsubscript{50}) cancer cell line data.

**Figure 2:** GI\textsubscript{50} values for cell lines treated with the three platin drugs were plotted in order of ascending oxaliplatin GI\textsubscript{50}. For most cell lines, there is a visible trend between the GI\textsubscript{50} for cisplatin and carboplatin, reflecting the correlation between the two drugs seen by MFA. Despite this correlation, carboplatin shows a much smaller variance (0.22) compared to cisplatin (0.37; oxaliplatin variance is 0.34).

**Figure 3.** The variation in gene composition of SVMs at different GI\textsubscript{50} thresholds for A) cisplatin, B) carboplatin, and C) oxaliplatin. GI\textsubscript{50} intervals are indicated on the left, with the number of cell lines with GI\textsubscript{50} values within said intervals in brackets. Each box represents the density of genes appearing in optimized Gaussian SVM models in those functional categories, with darker grey indicating frequent genes in indicated GI\textsubscript{50} threshold intervals, while lighter grey indicates less commonly selected genes. The number of thresholded models used to derive the density plot within each interval is equal (or greater, in the case of multiple equally performing models) to the number of cell lines within that GI\textsubscript{50} interval.

**Supplementary Figure 1.** Classification accuracy of models on TCGA bladder cancer patients treated with cisplatin and/or carboplatin as the resistance threshold is varied. Recurrence and disease-free survival are used as a binary measure to assess performance. The x-axis indicates movement of the resistance threshold, with more cell
lines labeled sensitive on the left and more labeled resistant on the right. Maximal AUC is indicated by the downward arrows.

**Supplementary Figure 2.** Classification accuracy of SVM models for cisplatin, at a range of response thresholds, were assessed using gene expression data for cisplatin-treated bladder cancer patients from Als et al.\textsuperscript{28}. Patients with a ≥ 5 year survival post-treatment were labeled sensitive. Red arrows indicate the SVM models with the highest positive predictive value (PPV) in the accuracy of classification of patient outcome.

**Supplementary Figure 3.** Hyperplane distance calculated by all thresholded SVMs for recurrent (<6 months) TCGA patients. Each diagram represents the predictions of all SVMs for all patients who had recurrence less than 6 months after treatment (N=10). Each point represents an SVM, where the x-axis represents the number of cell lines set to resistant (in order of lowest to highest GI\textsubscript{50}), and the y-axis represents the calculated hyperplane distance. A negative hyperplane distance would represent a prediction of resistance to cisplatin. Despite this, some patients show a strong preference towards predictions of sensitivity (i.e. TCGA-XF-A9SU).

**Supplementary Figure 4.** Hyperplane distance calculated by all thresholded SVMs for sensitive TCGA patients. Each diagram represents the predictions of all SVMs for all patients who had recurrence > 4 years after treatment (top; N=3), or patients who showed no recurrence after 6 years (bottom; N=6). Each point represents an SVM, where the x-axis represents the number of cell lines set to resistant (in order of lowest to highest GI\textsubscript{50}), and the y-axis represents the calculated hyperplane distance. A positive hyperplane distance would represent a prediction of sensitivity to cisplatin.
Cisplatin

SLC31A1 → SLC31A2

SLC22A5 → SLC22A7 → SLC22A10 → SLC22A11 → SLC22A12 → SLC22A13 → SLC22A16 → SLC22A20

ATP7A → ATP7B

SLC22A5 → SLC22A7 → SLC22A10 → SLC22A11 → SLC22A12 → SLC22A13 → SLC22A16 → SLC22A20

ATP7A → ATP7B

MRE11A → POLQ → POLD1 → RAD50 → TP63 → TP53 → MAP3K1 → MAPK8 → MAPK11 → MAPK12 → MAPK13 → MAPK14

BARD1 → NBN → PNKP → PARP → CFLAR → BCL2L1 → BCL2 → MAPK8

FEN1 → FAAP24

GSTO1 → GSTP1 → MT1A → MT2A → MT3 → MT4

GSX1 → GSX2

MAP3K1 → MAPK8 → MAPK11 → MAPK12 → MAPK13 → MAPK14

MT1A → MT2A → MT3 → MT4

CDKN2C

Other Genes associated with cisplatin resistance in the literature:

MAPK3, PRKCA, TLR4

MFA:

| Expression | Copy Number | Both | No MFA Correlation |
|------------|-------------|------|---------------------|
| Higher Levels Correlate with Higher GI50 & IC50 |           |      |                     |
| Lower Levels Correlate with Higher GI50 & IC50 |           |      |                     |
| Resistant | DNA Repair | Cell Growth | Metabolism | Signal Transduction / Apoptosis | Transcription / DNA Binding | Transcription / Translation | Membrane Transport |
|----------|------------|-------------|------------|---------------------------------|-----------------------------|---------------------------|---------------------|
| GI<sub>50</sub> (# cell lines in interval) |
| 3.50 - 3.75 (4) |
| 3.76 - 4.00 (7) |
| 4.01 - 4.25 (8) |
| 4.26 - 4.50 (11) |
| 4.51 - 4.75 (5) |
| 4.76 - 5.00 (8) |

Gene Expression

Frequency of Genes Selected in SVM for GI<sub>50</sub> interval

- ERCC1
- LIG3
- RAD50
- EGF
- VEGFB
- VEGFC
- GSR
- MTHFR
- MTR
- AKT1
- GNRT1
- HRAS
- OPRI1
- RAF1
- KRAS
- NEDD4L
- SGK1
- ETS2
- TP53
- EIF3I
- EIF3K
- EIF4E2
- ATP7B
- SCN10A

Legend:

- 0%
- 20%
- 40%
- 60%
- 80%
- 100%
| GI_{50} (# cell lines in interval) | Apoptosis | Immune System | Metabolism | Signal Transduction | Transcription / DNA Repair | Membrane Transport |
|----------------------------------|-----------|---------------|------------|---------------------|--------------------------|-------------------|
| 3.50 - 4.50 (4)                  |           |               |            |                     |                          |                   |
| 4.51 - 4.80 (7)                  |           |               |            |                     |                          |                   |
| 4.81 - 5.10 (7)                  |           |               |            |                     |                          |                   |
| 5.11 - 5.40 (7)                  |           |               |            |                     |                          |                   |
| 5.41 - 5.70 (14)                 |           |               |            |                     |                          |                   |
| 5.71 - 6.00 (4)                  |           |               |            |                     |                          |                   |

Frequency of Genes Selected in SVM for GI_{50} interval

| BCL2 | CSMD1 | ICAM5 | FCGR2A | HLA-B | HLA-A | MPO | SIAE | PRSS1 | AGXT | NAT2 | NQO1 | PANK3 | SGPP2 | UGT1A1 | NAGK | PROC | APOBEC2 | KIT | KLC3 | BRAF | IGFR1 | KISS1 | NOTCH1 | IGF1 | IGFR1 | KLF3 | HIF1A | NFE2L2 | PARP15 | MSH2 | SLC47A1 | CLCN9 | ABG2 | SLC1B1B | GRTP1 |
|------|-------|-------|--------|-------|-------|-----|------|-------|------|------|------|-------|-------|--------|------|------|---------|-----|------|------|-------|-------|--------|------|------|-------|-------|--------|-------|------|--------|-------|------|---------|-------|

| 0%   | 20%   | 40%   | 60%    | 80%    | 100%  |

Sensitive Resistant