Discovering drugs to overcome chemoresistance in ovarian cancers based on the cancer genome atlas tumor transcriptome profile

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ABSTRACT

Ovarian cancer accounts for the highest mortality among gynecologic cancers, mainly due to intrinsic or acquired chemoresistance. While mechanistic-based methods have been used to identify compounds that can overcome chemoresistance, an effective comprehensive drug screening has yet to be developed. We applied a transcriptome based drug sensitivity prediction method, to the Cancer Genome Atlas (TCGA) ovarian cancer dataset to impute patient tumor response to over 100 different drugs. By stratifying patients based on their predicted response to standard of care (SOC) chemotherapy, we identified drugs that are likely more sensitive in SOC resistant ovarian tumors. Five drugs (ABT-888, BIBW2992, gefitinib, AZD6244 and lenalidomide) exhibit higher efficacy in SOC resistant ovarian tumors when multi-platform of transcriptome profiling methods were employed. Additional in vitro and clinical sample validations were carried out and verified the effectiveness of these agents. Our candidate drugs hold great potential to improve clinical outcome of chemoresistant ovarian cancer.

INTRODUCTION

Ovarian cancer is the leading cause of death among gynecological cancers with an average 5-year survival rate of only 46% [1]. The standard of care (SOC) for ovarian cancer is surgery followed by chemotherapy with a combination of a platinum agent (carboplatin or cisplatin) and a taxane (paclitaxel or docetaxel). Even though the majority (about 80%) of ovarian cancer patients respond to the initial chemotherapy, around 20% patients fail to respond. In addition, more than half of initial responders relapse within 3 to 5 years [2, 3]. Therefore, the identification and development of effective drugs against chemoresistant ovarian tumors is of great importance.
rich molecular profiles available in over 500 high-grade serous ovarian cancer (HGSOC) made TCGA ovarian cancer dataset an optimal dataset to comprehensively examine the molecular landscape of ovarian cancer. However, one drawback of the TCGA data is the lack of clearly reported drug sensitivity data. Our work therefore filled this gap by applying drug prediction methods to TCGA in order to generate predicted drug IC\textsubscript{50} for every ovarian tumor sample.

More importantly, given that both the SOC and drugs that have never been used in treating ovarian cancer have been screened \textit{in vitro}, this allowed us to generate predicted \textit{in vivo} drug sensitivity to a wide range of drugs. By stratifying patients based on their likelihood of responding to SOC chemotherapy, we revealed several drugs that can be more efficacious in tumors that are resistant to SOC. Additionally, \textit{in vitro} and in independent clinical sample validations were carried out to confirm the role of these agents.

**RESULTS**

**Predicting drug sensitivities in ovarian tumors based on their transcriptome profiles**

Using pRRophetic, we generated 1,773 predicted drug IC\textsubscript{50} for all tumors in TCGA ovarian cancer datasets (see Methods; 138 drugs × 598 unique tumor samples). Separate predictions were generated using each of the 4 different transcriptome profiling platforms, including 520 samples for Affymetrix microarray, 574 samples for Agilent microarray, 413 for RNA-Seq, and 266 for RNA-Seq V2 (samples were overlapped among 4 platforms). A high predicted drug IC\textsubscript{50} represented less sensitive/potential resistance, and conversely a low predicted drug IC\textsubscript{50} suggested sensitivity.

As a proof-of-concept, we compared our predicted drug IC\textsubscript{50}s to the patient outcome data (survival) available through TCGA. Here, because of the lack of drug treatment response reported in TCGA, the survival data was used as a surrogate for the measured drug response phenotype. When evaluating predicted vs. actual drug sensitivity (quantified as alive or dead after a given treatment), we observed that in the ovarian cancer patients who were treated with paclitaxel, the predicted drug IC\textsubscript{50}s for paclitaxel were correlated with the patients’ survival outcomes (Figure 1, Student’s \textit{t}-test \(P=0.032\), Wilcoxon rank-sum test \(P<0.0001\)). When a tumor is predicted to be more sensitive to the drug (i.e., a lower predicted IC\textsubscript{50} values), the patient is more likely to be alive. These correlation trends between predicted and observed drug sensitivity were also observed for docetaxel and cisplatin, although not statistically significant. Note that only a subset of TCGA ovarian cancer samples contain treatment information with the highest numbers of patients treated with paclitaxel (\(n=469\)). Given ovarian cancer survival is highly correlated with disease stages, we also fitted a regression model between survival and predicted paclitaxel sensitivity controlling for disease stage. Once again, we observed a significant correlation between the predicted paclitaxel IC\textsubscript{50} and the survival outcomes of those patients underwent paclitaxel treatment (\(P=0.0385\)). Only 112 and 154 patients were known to be treated with docetaxel and cisplatin,
respectively, suggesting that we may be underpowered to observe such an association for these other drugs.

**Identify drug candidates that may work in the SOC resistant ovarian tumors**

For every tumor sample, we averaged the predicted drug IC$_{50}$ cisplatin and docetaxel, the current SOC, as an indicator of individual tumor sensitivity to SOC (See blue line in Figure 2). Given 80% SOC response rate was well documented by literature [2, 3], we stratified the 20% tumor with higher predicted SOC IC$_{50}$ as SOC non-responders (Figure 2). We then performed a Student’s t-test for predicted drug IC$_{50}$ for all other drugs between SOC responders and non-responders. Only those drugs that showed lower predicted IC$_{50}$ in SOC non-responders groups than SOC responders (P<0.05) were further evaluated.

Each of the 4 transcriptome profiles for these samples were analyzed separately to generate predicted drug IC$_{50}$s. Therefore, four sets of candidate drugs were identified using each transcriptome profiling dataset. Specifically, we identified 13, 17, 18 and 12 drugs with the Affymetrix arrays, Agilent arrays, RNA-Seq, and RNA-Seq V2 datasets, respectively. (Details about these drug candidates identified from each platform can be found in Supplementary Table 1). Among them, 5 drugs were identified in all four datasets. They were ABT-888, BIBW2992, gefitinib, AZD6244, and lenalidomide (Table 1). For each candidate drug, the average predicted drug IC$_{50}$ in SOC sensitive and resistant group were reported in Table 1. All 5 candidate drugs had significantly lower predicted drug IC$_{50}$ (suggesting more sensitive) in the SOC resistant group than those in SOC sensitive group (P<0.05, two-tailed Student’s t-test). Significant negative Pearson correlations were shown in Figure 3 between ABT-888 (R$_{s} = -0.164$, P$_{s} = 0.0002$), or BIBW2992 (R$_{s} = -0.148$, P$_{s} = 0.0007$), and SOC when rank order patients based on their predicted sensitivity to these drugs. To ensure the robustness of the results, we also performed Pearson test using the actual predicted drug IC$_{50}$. Again, significant negative correlations were shown between SOC and ABT-888 (R$_{s} = -0.222$, P$_{s} < 0.0001$), or BIBW2992 (R$_{s} = -0.412$, P$_{s} < 0.0001$). These inverse correlations for drug response indicated that our candidate drugs were predicted to be more efficacious in SOC resistant patients. Notably, the average predicted drug IC$_{50}$ of each given drug in the SOC resistant or sensitive groups were very close between different datasets. This suggests that our prediction was highly reproducible even though different technologies were applied to obtain gene expression profiles.

**Validation**

For validation, we applied the same methods to an independent ovarian cancer dataset: the Australian Ovarian Cancer Study (AOSC, n=285). Significant higher predicted sensitivity (lower predicted IC$_{50}$) in SOC resistant ovarian tumors were confirmed for BIBW2992 (P=0.003) using 80/20 (responder vs. non-responder) cutoff. To test if the results were robust to the choice of cutoff, we stratified SOC sensitivity using the 50/50 predicted SOC IC$_{50}$ as threshold (50% sensitive and 50% resistant) as well. As a result, all candidate drugs—AZD6244, gefitinib, BIBW2992, lenalidomide and ABT-888, were significantly more sensitive in the SOC resistant tumors (P<0.05). In addition, we performed correlation analysis between predicted SOC and candidate drug IC$_{50}$. Significant correlations were found for all drugs as ABT-888 (R$_{s} = -0.119$, P$_{s} = 0.023$, R$_{p} = -0.112$, P$_{p} = 0.029$), BIBW2992 (R$_{s} = -0.318$, P$_{s} < 0.0001$, R$_{p} = -0.276$, P$_{p} < 0.0001$), gefitinib (R$_{s} = -0.302$, P$_{s} < 0.0001$, R$_{p} = -0.273$, P$_{p} < 0.0001$), AZD6244 (R$_{s} = -0.272$, P$_{s} < 0.0001$, R$_{p} = -0.259$, P$_{p} < 0.0001$), and lenalidomide (R$_{s} = -0.142$, P$_{s} = 0.008$, R$_{p} = -0.139$, P$_{p} = 0.009$).

For *in vitro* validation, we employed a large scale independent cell line drug sensitivity screening data set – CTRP v2 [14]. In CTRP v2, 4 of the 5 candidate drugs were screened (ABT-888, BIBW2992, gefitinib and AZD6244). The AUCs (area under the dose response curve) were used to define cellular sensitivity to these drugs. Given the small sample size (41 ovarian cancer cell lines in total) in CTRP v2, we stratified 22 cell lines as sensitive to SOC and 19 as resistant based on mean predicted SOC IC$_{50}$. Then, for the four drugs, differences of AUCs between SOC resistant and sensitive group were analyzed using one-tailed t-test. P-values were presented in Table 1. ABT-888 and BIBW2992 showed significant lower AUC (suggesting higher sensitivity) in SOC resistant group (Figure 4A, P=0.011 for ABT-888, Figure 4B, P=0.031 for BIBW2992). There was no significant difference between the SOC resistant and sensitive groups (P>0.05) for gefitinib and AZD6244.

**Identification of pathways associated with the SOC resistance and candidate drugs sensitivity**

To further explore the underlying biology that leads to the effectiveness of the 5 candidate drugs in SOC resistant ovarian tumors, we performed Gene Set Enrichment Analysis (GSEA) using the KEGG pathway gene sets. The pathways listed in the left column of Table 2, were statistically significant positive correlation to SOC sensitivity and negative correlation to sensitivities of the candidate drugs (FDR q-value < 0.25). In other words, the enrichment of KEGG pathways we selected could make the tumors more resistant to SOC, at the same time more sensitive to candidate drugs.

Interestingly, among the pathways that were enriched for SOC resistance and candidate drug sensitivity (Table 2), were the apoptosis and cytosolic DNA sensing pathway. Both apoptosis and cytosolic DNA...
sensing pathways desensitized tumors to SOC, which was supported by literature [15]. ABT-888, a PARP1/2 inhibitor, plays a role in inhibition of DNA repair and introduction of cell death. Gefitinib and BIBW2992 are both EGFR inhibitors, which could induce apoptosis by inhibition of Ras signaling [16].

**DISCUSSION**

Taxane and platinum based chemotherapy was introduced 20 years ago to treat ovarian cancer as standard first line regimen. There has been little progress in the last decade to improve the overall survival for chemoresistant ovarian cancer patients. In this study, we applied a novel transcriptome-based drug sensitivity prediction method to a collection of large *in vivo* and *in vitro* ovarian cancer datasets. Importantly, we identified five agents—ABT-888, BIBW2992, gefitinib, AZD6244, and lenalidomide that exhibit higher sensitivity in SOC resistant ovarian cancers in multi-platform TCGA datasets. We provided further validation of these drugs’ sensitivity in additional clinical samples derived from ovarian cancer patients (through AOCS) and *in vitro* (through CTRP v2).

Although survival information has been collected for almost all TCGA samples, the treatment information for each patient is lot sparse, ranging from 40-97% coverage for each disease type to date. Our work bypassed this issue by generating our own *in vivo* drug sensitivity prediction. This method has been tested previously *in silico* analysis of 3 independent clinical trials and has demonstrated superior power [12]. Furthermore, in our proof-of-concept analysis, we observed a significant different predicted drug IC\textsubscript{50} between dead and alive patients who were treated with paclitaxel. It is ideal to use response to treatment as a phenotype when comparing to our predicted drug IC\textsubscript{50}; however, since such data is not present, we opted to examine relationship between predicted drug sensitivity and survival outcome as a surrogate for treatment response.

BIBW2992 (afatinib) is an irreversible tyrosine kinase inhibitor (TKI) that inhibits ErbB family members including EGFR, HER2, and HER4. Thereby BIBW2992

![Figure 2: TCGA ovarian cancer patients were subgrouped into SOC responders and SOC non-responders. SOC, standard of care.](image-url)
Figure 3: The opposite effect patterns between candidate drugs and SOC. (A) Significant negative Pearson correlation between the ranking of SOC and ABT-888 ($R_p = -0.164$, $P = 0.0002$). (B) Significant negative Pearson correlation between the ranking of SOC and BIBW2992 ($R_p = -0.148$, $P = 0.0007$).
Table 1: Summary of the predicted drug IC$_{50}$ for candidate drugs in SOC responders and non-responders analyzed using different expression profiling platforms

|                      | Affymetrix          | Agilent | RNA-Seq | RNA-Seq V2    | CTRP v2 |
|----------------------|---------------------|---------|---------|---------------|---------|
|                      | Predicted drug IC$_{50}$ | Student's $t$-test | Predicted drug IC$_{50}$ | Student's $t$-test | Predicted drug IC$_{50}$ | Student's $t$-test | Predicted drug IC$_{50}$ | Student's $t$-test | Predicted drug IC$_{50}$ | Student's $t$-test | P value | Correlation analysis |
| SOC Responders       | SOC Non-responders  | P value | SOC Responders | SOC Non-responders | SOC Responders | SOC Non-responders | SOC Responders | SOC Non-responders | SOC Responders | SOC Non-responders | Spearman | Pearson |
| ABT-888              | 5.35                | 5.27    | 3.17x10^-3 | 3.54          | 5.30          | 7.69x10^-3 | 5.35                  | 5.26          | 1.26x10^-4         | 5.35                | 5.25         | 1.17x10^-5           |
| BIBW2992             | 2.27                | 2.14    | 7.95x10^-4 | 2.28          | 2.18          | 2.63x10^-3 | 2.28                  | 2.19          | 1.82x10^-3         | 2.28                | 2.19         | 3.57x10^-4           |
| Gefitinib            | 2.05                | 1.79    | 8.92x10^-4 | 2.04          | 1.84          | 1.00x10^-4 | 2.03                  | 1.90          | 6.62x10^-4         | 2.05                | 1.85         | 1.02x10^-4           |
| AZD6244              | 3.05                | 2.73    | 2.73x10^-4 | 3.04          | 2.80          | 3.74x10^-4 | 3.06                  | 2.72          | 7.44x10^-4         | 3.07                | 2.68         | 7.77x10^-4           |
| Lenalidomide         | 5.40                | 5.30    | 2.71x10^-3 | 5.39          | 5.35          | 1.57x10^-3 | 5.39                  | 5.32          | 1.04x10^-4         | 5.39                | 5.33         | 8.82x10^-4           |

* P value was calculated from Student’s $t$-test by comparing Predicted drug IC$_{50}$ between SOC responders and non-responders.

* Correlation analysis was performed between predicted SOC IC$_{50}$ and candidate drug IC$_{50}$ predictions from pRRophetic. The first in vitro validated candidate drug is ABT-888 (veliparib), a potent inhibitor of PARP1 and PARP2. Poly (ADP-ribose) polymerase (PARP) is essential enzyme involved in damaged DNA detection and repair through the base excision repair pathways. The inhibition of PARP can sensitize tumors to cytotoxic agents by blocking DNA repair, followed by cell cycle arrest and apoptosis, and possibly make tumors more susceptible to DNA-damaging agents including carboplatin [22].

PARP inhibitors have shown preclinical activity in cancers that are deficient in DNA repair due to defects in homologous recombination (HR), eg. BRCA-mutated tumors [23, 24]. PARP inhibitors were initially tested in trials as treatment for BRCA mutation-associated ovarian and breast cancers. The TCGA discovery on the defects in the HR pathway commonly existed in more than 50% of high-grade serous ovarian cancers [25], had led to further investigation of PARP inhibitors in a wider population of ovarian cancers. Olaparib, as the first FDA-approved PARP inhibitor, is used to treat recurrent BRCA mutant ovarian cancer patients. ABT-888 is undergoing a number of clinical trials in combination with SOC, and also as maintenance in the first-line treatment of ovarian cancer. In a single-agent Phase I trial [26] designed for platinum-refractory ovarian or basal-like breast cancers, ABT-888 showed higher response rate and clinical benefit rate in BRCA-mutated tumors than BRCA wild-type tumors. Another single-agent Phase II study [27] in BRCA-mutated ovarian cancer patients has also been reported. ABT-888 was well tolerated with a response rate of 26%. It remains unclear whether PARP inhibitors should be utilized as newly-diagnosed or relapsed.
patients, single agent or in combination with SOC or as maintenance treatment. Further investigations will help to answer the questions and unveil its full potential as a treatment option.

Gefitinib, a selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, can competitively inhibit ATP binding on EGFR. Gefitinib was approved by the FDA as monotherapy treatment for patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) after failure of both platinum and taxane based chemotherapies [28]. EGFR is commonly present in 33% to 75% of ovarian cancers and increased EGFR is associated with poor survival in ovarian cancer patients [29]. A Phase II trial with gefitinib in combination with paclitaxel and carboplatin as a second-line treatment for advanced ovarian adenocarcinoma, showed a high rate of 63% overall response [30]. Our study provides additional supporting evidence in further pursuing the evaluation of gefitinib in SOC resistant ovarian cancer treatment.

Another example is AZD6244 (selumetinib), a potent, highly selective MEK1/2 inhibitor which also inhibits ERK1/2 phosphorylation. It has reported single agent activity in several trials on solid tumor including recurrent low-grade serous ovarian cancer (LGSOC). A recent phase II AZD6244 trial in LGSOC showed better efficacy than SOC in terms of response rate and disease stabilization [31]. One of the reasons to platinum resistance has been proposed as through the activation of JNK and ERK cascades by cisplatin-induced DNA damage, where JNK and ERK are required for cell proliferation and differentiation. The inhibition of JNK or ERK cascades can sensitize ovarian cancer cells to cisplatin [32]. Therefore, it is not surprising that AZD6244, as an ERK1/2 inhibitor, may promote sensitivity to cisplatin. The findings along with our work warrants further investigation of this drug in ovarian cancer.

Lenalidomide is an antiangiogenic agent, with capability as an immunomodulator, which can inhibit hypoxia-inducible factor (HIF)-1α, an essential regulator of metastasis. Lenalidomide was approved by the FDA in 2006 to treat patients with multiple myeloma in combination with dexamethasone. Its clinical efficacy has also been reported as a single agent and combined with chemotherapy in solid tumors, including ovarian, prostate, renal cell and hepatocellular cancers [33, 34]. Most of the Phase I trials on ovarian cancer showed an acceptable safety profile, while one of them was terminated because of toxicity. Further studies of lenalidomide may be warranted in this disease setting.

Several compounds have been proposed for overcoming ovarian cancer chemoresistance, including bortezomib [4], antiprogestin compounds [5], combined treatment with death ligand TRAIL and antidiabetic acting PPARγ ligands [6], P-glycoprotein (P-gp) inhibitors [7, 8], cancer stem cell targeting agents and autophagy based modulation [7]. Most of them, except for bortezomib, were not screened in the anti-cancer drug sensitivity database pRRophetic was built on. Adam et al. observed that bortezomib combined with paclitaxel act in a

Figure 4: ABT-888 and BIBW2992 are more sensitive in SOC resistant ovarian cancer cell lines tested in CTRP v2. In vitro measured drug sensitivities (AUC) in CTRP v2 are compared between SOC sensitive and resistant cell lines. The higher the AUC, the more resistance the cell line has for a given drug. (A) ABT-888 showed significant lower AUC in SOC resistant group (Student’s t-test P=0.011). (B) BIBW2992 showed significant lower AUC in SOC resistant group (Student’s t-test P=0.031).
synergistic manner. There was more than 2-fold decrease in IC$_{50}$ when treating the cells (SKOV3 and A2780) with the combination as compared to paclitaxel alone [4]. Our screen did not reveal the role of bortezomib in SOC resistant ovarian cancer possibly because our work focused on single agent efficacy rather than drug combination.

Our GSEA results indicated that pathways related to apoptosis and cytosolic DNA sensing were important in SOC resistance. These have been supported by various literature [35-37]. For example, enhanced DNA repair [35], induction of anti-apoptotic protein [37] and activation of the AKT [37] have been demonstrated to be the major contributing factors for chemoresistance in ovarian cancer. At the same time, these pathways were found to be enriched in increasing sensitivity to our candidate drugs. ABT-888, as a PARP inhibitor, could block the repair of single-strand DNA breaks and result in accumulation of single-strand breaks and subsequently double-strand breaks. The inability of DNA repair will cause chromosomal instability, cell cycle arrest and ultimately cell death. It is therefore not a surprise that up-regulated DNA repair and apoptosis pathway could decrease the vulnerability of chemotherapy, however, increase the vulnerability of DNA repair targeted ABT-888.

Although the effectiveness of all 5 candidate drugs was reproduced in AOCs, an independent clinical ovarian cancer study, only 2 of the 5 drugs were validated in vitro using CTRP v2. The reasons may be 1) lenalidomide was not screened in CTRP v2; and 2) much smaller sample size in the in vitro validation dataset when compared to the in vivo validation dataset (41 vs 285).

In conclusion, by applying a novel drug sensitivity prediction approach to a set of large in vivo and in vitro datasets, we discovered and validated several candidate drugs that could be more effective in SOC resistant ovarian cancer patients. GSEA analysis unveiled the pathways that may account for the improved efficacy of our candidate drugs.

### Table 2: Summary of enriched KEGG pathways that were significantly correlated with resistance of SOC and sensitiveness of candidate drugs

| Pathways                                    | SOC  | ABT-888 | BIBW2992 | Gefitinib | AZD6244 | Lenalidomide |
|---------------------------------------------|------|---------|----------|-----------|---------|--------------|
| Allograft rejection                         | 0.45 | -0.861  | -0.808   | -0.797    | -0.755  | -0.602       |
| Graft versus host disease                   | 0.4  | -0.847  | -0.827   | -0.808    | -0.761  | -0.542       |
| Type I diabetes mellitus                    | 0.389| -0.803  | -0.736   | -0.774    | -0.708  | -0.515       |
| Antigen processing and presentation         | 0.372| -0.736  | -0.68    | -0.683    | -0.63   | -0.453       |
| RIG I like receptor signaling pathway       | 0.364| -0.597  | -0.543   | -0.453    | -0.521  | -0.235       |
| Autoimmune thyroid disease                  | 0.354| -0.819  | -0.689   | -0.742    | -0.695  | -0.517       |
| Apoptosis                                   | 0.311| -0.517  | -0.519   | -0.488    | -0.503  | -0.185       |
| Asthma                                      | 0.302| -0.79   | -0.72    | -0.767    | -0.715  | -0.414       |
| Intestinal immune network for IGA production| 0.291| -0.795  | -0.633   | -0.742    | -0.66   | -0.575       |
| TOLL like receptor signaling pathway        | 0.27 | -0.626  | -0.539   | -0.549    | -0.585  | -0.22        |
| Leishmania infection                        | 0.26 | -0.712  | -0.614   | -0.7     | -0.661  | -0.223       |
| NOD like receptor signaling pathway         | 0.232| -0.677  | -0.565   | -0.586    | -0.631  | -0.29        |
| Cytosolic DNA sensing pathway               | 0.216| -0.686  | -0.576   | -0.538    | -0.588  | -0.31        |
| Natural killer cell mediated cytotoxicity    | 0.159| -0.668  | -0.515   | -0.622    | -0.611  | -0.404       |

The numbers in this table indicated the Enrichment Score (ESs).
drugs comparing with SOC. Clinical trials evaluating the effectiveness of some of these candidate drugs either alone or in combination with chemotherapy are ongoing in ovarian cancer. They should also be carefully examined in the SOC resistant setting.

MATERIALS AND METHODS

Overview

pRRophetic method simultaneously constructs prediction models using transcriptome and drug sensitivity data derived from the Cancer Genome Project (CGP) and apply it to the datasets that contain transcriptome information (i.e., the TCGA) to generate predicted drug IC\(_{50}\)s.

Given that there are 138 drugs screened in CGP [9], we were able to generate predicted drug IC\(_{50}\)s of all 138 drugs in each tumor sample. In this study, we generated predicted drug IC\(_{50}\)s in \textit{vivo} for two independent large ovarian cancer datasets: the TCGA and Australian Ovarian Cancer Study (AOCS) [38]. Furthermore, to validate our prediction, we generated predicted drug IC\(_{50}\)s in another large \textit{in vitro} cancer cell line drug screening dataset, the Cancer Therapeutics Response Portal v2 (CTRP v2) [14]; and compared the predicted drug IC\(_{50}\) to the experimentally measured cellular sensitivity (eg. AUC) to drugs.

Specifically, TCGA ovarian cancer gene expression profiles were downloaded from TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). There are four sets of transcriptome profiles for ovarian cancer: two generated using microarrays—Affymetrix HT Human Genome U133 and Agilent 244K Custom Gene Expression G4502A-07; and the other two with RNA-seq. The Affymetrix platform contained expression profiles of 520 patients, and Agilent platform contained expression profiles of 574 patients. Illumina HiSeq 2000 RNA Sequencing platform contained 413 patients’ tumor expression profile, and Illumina HiSeq 2000 RNA Sequencing Version 2 has expression profiles for 266 patients. Although the samples from these four expression datasets were highly overlapped, because the different transcriptome profiling technologies have different specificity, sensitivity and dynamic range, we examined each dataset separately as technical replications. Findings from each analysis were compared and only those drugs that predicted to be more sensitive in SOC resistant tumors by all 4 analyses were further evaluated.

Transcriptome data for AOCS was generated using Affymetrix U133 plus2 microarray and obtained through GEO (GSE9891, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE9891). This includes gene expression data for 285 ovarian tumor samples.

For prediction validation, we employed CTRP v2 [38], in which area under the dose response curve (AUCs) for 481 compounds were available in 860 cancer cell lines. The drug sensitivity data was downloaded from CTD\(^2\) DATA PORTAL (https://ctd2.nci.nih.gov/dataPortal/). The mRNA expression (Affymetrix U133+2 array) data of the cell lines was obtained from Cancer Cell Line Encyclopedia (CCLE) data portal (http://www.broadinstitute.org/ccle).

In \textit{vivo} identification of drugs that may work in SOC resistant ovarian cancer patients

We chose to analyze TCGA ovarian tumors as our discovery dataset because it is one of the largest ovarian cancer datasets where algorithms such as pRRophetic could be employed, while AOCS dataset was used as independent \textit{in vivo} validation dataset. For each tumor sample in TCGA and AOCS, we generated 138 predicted drug IC\(_{50}\)s. Specific for TCGA, predicted drug IC\(_{50}\) was generated independently using each set of the four transcriptome profiling datasets separately. Note that lower predicted drug IC\(_{50}\) indicated higher sensitivity to such a drug.

We calculated ovarian cancer patients’ SOC IC\(_{50}\) by averaging the predicted drug IC\(_{50}\) to cisplatin and doctaxel for every patient. Furthermore, with the commonly observed 80% response rate to SOC in ovarian cancer [3, 4], we classified TCGA ovarian cancer samples as either resistant to SOC (as the top 20% ranked SOC response) or sensitive to SOC (the bottom 80% ranked SOC response). We then evaluated the predicted drug IC\(_{50}\)s of other 136 drugs to discover drugs showing opposite predicted efficacy profile to that of SOC using Student’s \(t\)-test. In another word, we aimed at identifying drugs with lower predicted drug IC\(_{50}\) (meaning higher sensitivity) in SOC resistant patients. The same selection method was applied to all 4 TCGA gene expression datasets. The candidate drug lists were overlapped to generate the final candidate drug list. To avoid findings dependent on the arbitrary cutoff used in defining SOC response, in addition to the 80/20 (responder vs. non-responder) cutoff, we also evaluated the 50/50 (responder vs. non-responder) cutoff as well as employing correlation analysis between SOC and candidate drugs. P values less than 0.05 were considered significant.

In \textit{in vitro} and \textit{in vitro} validation of candidate drugs

The findings from TCGA were first validated through the AOCS dataset. Given CTRP v2 has measured hundreds of drugs’ sensitivities in ovarian cancer cell lines (including some of the candidate drugs we predicted to be more sensitive in TCGA and AOCS), we performed separate predictions using CTRP v2 ovarian cancer cell lines. Specifically, gene expression profiles was available in 41 of the 43 ovarian cancer cell lines in CTRP v2. Once again, cell lines sensitivity to SOC were generated by averaging the predicted drug IC\(_{50}\).
of cisplatin and docetaxel. CTRP v2 measured and reported AUC as drug sensitivity with the higher the AUC, the more resistant the cells to any given drug. Then we performed Student’s t-test on candidate drug AUCs between SOC sensitive cell lines and SOC resistant cell lines.

Gene set enrichment analysis (GSEA)

To further explore the potential mechanism underlying the observed opposite therapeutic effect of our candidate drugs and the SOC, we performed GSEA analysis (GSEA software v2.2.2, www.broadinstitute.org/gsea) using the KEGG pathway gene sets. We input TCGA-Agilent expression dataset and predicted SOC IC$_{50}$s and those of the candidate drugs as phenotypes. The association between drug sensitivities and gene expression was run separately for each phenotype following the developer’s protocol (http://www.broad.mit.edu/gsea/). FDR q-value <0.25 was used to define significantly regulated pathways. The positively SOC-correlated pathways were overlapped with pathways that were significantly and negatively correlated with all candidate drugs.

Abbreviations

TCGA: The Cancer Genome Atlas.
SOC: standard of care.
CGP: Cancer Genome Project.
CTRP v2: Cancer Therapeutics Response Portal v2.
AOCs: Australian Ovarian Cancer Study.
AUC: area under the dose response curve.
CCLE: Cancer Cell Line Encyclopedia.
GSEA: Gene Set Enrichment Analysis.

Author contributions

FW and RSH conceived the study. FW collected data and performed the analysis and wrote the paper with the help of JTC and RSH. ZZ, GM, AN and SB assisted with analysis. RSH supervised the study.

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CONFLICTS OF INTEREST

The authors have declared that no conflicts of interest exists.

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