Consistency of *in vitro* drug sensitivities within pharmacological classes

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ABSTRACT

Multiple comparative analyses between the common drugs and cell lines of the Genomics of Drug Sensitivity in Cancer (GDSC) and the Cancer Therapeutics Response Portal (CTRP) have previously shown low consistency between the *in vitro* phenotypic measures of a drug in one study with the other. While several potential sources of inconsistency have been tested, the similar targets of tested compounds has yet to be tested as a contributing factor of discrepancy. This analysis includes two methods of reclassifying drugs into classes based on their targets to identify the truer set of consistent cell lines, showing an increased correlation between the two pharmacogenomic studies.

Keywords: Drug sensitivity, pharmacogenomics, cell line studies

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INTRODUCTION

With cancer incidence and prevalence rates increasing alarmingly, oncologic treatment still remains based on pathological examination, symptoms of the disease, and history of medications¹. Given that patients have such differing responses to drugs with widely varying effects, there has been increasing interest in the shift of medicine from a population-level to patient-specific, specifically with a focus on identifying biomarkers of treatment response.

One challenge of oncogenic treatment biomarker identification lies in contradictory results from past studies which have found that the same set of cancer cells can exhibit different behaviours when exposed to the same treatment². Studying pharmacogenomics has allowed further research of identifying genetic variants that cause differential response to particular therapies, or in other words, recognizing an individual's sensitivity to certain anticancer drugs. By understanding how to identify an individual's response to a specific therapy based on their genotype, treatment plans could be aimed at prescribing the right drug at a dose that causes minimal toxicity.

Analyzing the response to a range of anticancer agents at variable doses through drug sensitivity measures, such as the half maximal inhibitory concentration (IC₅₀) or the Area Above the Curve (AAC), allows the quantification of a drug's efficacy in large-scale screenings. By measuring these drug dose responses using cell line studies, the efficacy and potency of drugs can be assessed. These cell line studies characteristically investigate the cell state post-treatment at multiple doses of the drug and include cell line profiling at the DNA, RNA, and chromosomal levels with the goal of accelerating anticancer therapy discovery³.

Large-scale pharmacogenomic studies of cancer cell lines such as the Genomics of Drug Sensitivity in Cancer project (GDSC)⁴ and the Cancer Therapeutics Response Portal (CTRP)⁵ have been profiled with predictions of sensitivity profiles of each cell line to different anticancer drugs. These databases have been used in numerous laboratories to guide research on the molecular mechanisms of cancer, to generate hypotheses for the development of new therapies, and in conjunction with clinical studies⁶. An overlap in common cell lines, drugs, mutations and gene expressions in both the GDSC and CTRP studies has allowed for the assessment of drug sensitivity consistency across the datasets.

Despite the significance of cell line studies in the identification of new cancer therapies, discrepancies between studies in drug response data have been

identified, but the source of the inconsistencies is still uncertain⁷. In the comparison study of GDSC and the Cancer Cell Line Encyclopedia (CCLE)⁸, drug sensitivity measures IC₅₀ and AAC were compared across shared cell lines and drugs. Only a single drug having moderate correlation and another single drug with fair correlation were found out of the 15 shared compounds, showing an alarming discordance between the two datasets⁷. The discrepancy could neither be explained by the choice of drug sensitivity estimator, nor by the tissue source⁷. The disagreements between studies point to the concern that a molecular biomarker of drug response learned from one study may not be predictive of the reported response in another study⁸. Considering that cultured cell lines remain central to cancer research for assessing gene-drug associations and the selection of anticancer drugs, the inconsistencies sound a note of caution on prospective interpretations of the data from large-scale cell line studies.

A possible cause for the discrepancy that has not yet been investigated is the fact that multiple drugs may have the same targets, or alternatively stated, be defined into pharmacological classes (PCLs). A pharmacological/perturbagen class is defined as a grouping of compounds that share the same mechanism of action (MOA) or biological functions⁹. Anticancer therapies can be divided into two main classes: broad-spectrum therapy, which involves combinations of multiple agents that can collectively impact the genesis of cancer¹⁰, or targeted therapies, which are drugs designed to interfere with a specific molecular target that may promote growth and progression of a tumor. Compounds can be further classified into more distinct classes based on their targets. For example, the Connectivity Map (CMAP) defines PCLs by first grouping compounds that share MOA⁹. It further refines the groupings and confirms the shared features through a high-throughput gene expression assay, L1000, to assess whether agents in the same PCL give similar gene expression signatures and confirm the shared activity⁹. CMAP yields 171 high-confidence PCLs corresponding to 930 unique perturbagens, with each PCL ranging in size from 3 to 44 members with an average size of 5.8 members⁹. Most PCLs defined by CMAP are completely distinct, with 95% of agents belonging to just one PCL⁹.

Previous studies^{11,12} have presented that integrating therapeutic agents by chemical structure and features can improve predictive performance. With the goal of observing whether a similar effect could be found by grouping drugs into pharmacological classes, we hypothesize that after reclassifying drugs into PCLs and removing outliers within datasets will yield a higher concordance of drug sensitivity measures across studies. By testing a more specific method of classifying

drugs, this analysis factors out a feasible causal component to the inconsistencies in drug sensitivity measures across pharmacogenomic studies.

METHODS

Data retrieval. Drug sensitivity data from the Genomics of Drug Sensitivity in Cancer Project (GDSC)¹³, and the Cancer Cell Line Encyclopedia (CTRP)¹⁴ were retrieved as PharmacoSet¹⁵ objects. Both PharmacoSets used (CTRPv2_2015⁵ and GDSC_2020(v2-8.2)¹⁶) are available from the PharmacoGx package version 2.0.5, in R version 4.0.0. The drug sensitivity data, specifically measures of IC50 and Area Above the Curve (AAC), were retrieved using the PharmacoGx summarizeSensitivityProfiles function using the intersected CTRP and GDSC PharmacoSets¹⁵.

Defining broad-spectrum and targeted compounds.

To define the common compounds binarily as either broad-spectrum or targeted drugs, PubChem was used and manually inputted into the drug_info.xls spreadsheet¹⁷. In total, 10 compounds were defined as broad-spectrum, and 61 compounds as targeted. Each set of sensitivity measures for each cell line were then subsetted to only include the drugs within the specified group of target type, leaving two newly defined subsets: broad-spectrum and targeted drugs.

Defining pharmacological classes. To define the common compounds into pharmacological classes, the CMAP data was downloaded using the CLUE platform⁹. 17 out of the 71 common compounds did not have available connectivity info from CMAP. Each compound's CMAP-defined PCL and connectivity score were manually inputted into the drug_info.csv spreadsheet. All drugs sorted into a PCL had a connectivity score above 99.0. In total, 34 different PCLs were defined, with only 4 PCLs maintaining the minimum of 3 drugs per class, which were:

- **Aurora kinase inhibitor:** Alisertib, Nilotinib,

Tozasertib

- **IGF-1 inhibitor:** AZD8055, Bms-754807, Linsitinib
- **PI3K inhibitor:** AZD6482, Osi-027, Pictilisib
- **Tubulin inhibitor:** Adavosertib, Docetaxel, Vincristine

Each set of sensitivity measures for each cell line were then subsetted to only include the drugs within the specified PCL, leaving four newly defined subsets: Aurora kinase inhibitor, IGF-1 inhibitor, PI3K inhibitor, Tubulin inhibitor.

Measuring consistency. The Pearson correlation coefficient (r)¹⁸ was used to quantify the degree of linear association between the CTRP and GDSC sensitivity measures. Correlations between the datasets for each cell line and for each drug were computed. The correlation was recomputed between datasets for each subset of drugs (broad-spectrum/targeted, PCLs). Within the subset of sensitivity measures for each drug class, if the correlation for a cell line between the CTRP and GDSC datasets had poor consistency (defined as $r < 0.5$), the cell line was removed from within each dataset. To compute the consistency of drug sensitivity measures between the two datasets, Harrell's C-Index, also known as the concordance index, was measured using the Survival R package's *concordance* function (v. 3.2-3)¹⁹. The concordance for each sensitivity measure of the Pearson correlation (r) between the original datasets and class-subsetted datasets was computed.

Methods summary. A reproducible run of this analysis in R is available to generate all results, figures, and plots of this analysis.

RESULTS

Intersection between CTRP and GDSC. To identify common drugs and cell lines between the two datasets, the PharmacoSets were intersected using PharmacoGx¹⁵ intersectPSets function, with a total of 619 cell lines and 71 drugs in common (Figure 1).

Figure 1 Common cell lines (left) and drugs (right) between GDSC and CTRP.

Figure 2 Pearson correlations (r) of IC50 values for the 15 drugs with largest increases in correlation after filtering out inconsistent cell lines using binary drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on binary drug classification ("binary").

Figure 3 Pearson correlations (r) of IC50 values for the 15 drugs with lowest changes in correlation after filtering out inconsistent cell lines using binary drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on binary drug classification ("binary").

Figure 4 Pearson correlations (r) of IC50 values for the 15 drugs with largest increases in correlation after filtering out inconsistent cell lines using PCL drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on PCL drug classification ("PCL").

Figure 5 Pearson correlations (r) of IC50 values for the 15 drugs with lowest changes in correlation after filtering out inconsistent cell lines using PCL drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on PCL drug classification ("PCL").

Correlation of IC₅₀. To quantify the consistency of sensitivity measurements across studies, the Pearson's correlation coefficient (r) were computed for each drug before and after sorting by pharmacological classes. The original correlation of IC₅₀ values between studies for each drug ranged from r -0.872 (Fulvestrant) to 0.999 (Axitinib).

In both methods used to subset inconsistent cell lines (broad-spectrum/targeted vs. PCL) using IC₅₀ values, Bms-754807, Osi-027, Venetoclax, and Nelarabine were in both groups of the 15 drugs with largest increases after each drug filtering method (binary and PCL). Pearson correlation for the drug Trametinib had the largest decrease after both drug filtering methods. Erlotinib, Molibresib, Olaparib, Dabrafenib, and Trametinib were in both groups of the 15 drugs with lowest changes after each drug filtering method.

Correlation of Area Above the Curve (AAC). The original correlation of AAC values between studies for each drug ranged from r -0.368 (Tozasertib) to 0.806 (Dabrafenib). In both methods used to subset inconsistent cell lines (broad-spectrum/targeted vs. PCL) using AAC values, Alpelisib was the only drug

in both groups of the 15 drugs with largest increases after each drug filtering method (binary and PCL). Pearson correlation for the drug Trametinib had the largest decrease after both drug filtering methods. Temozolomide was the only drug in both groups of the 15 drugs with lowest changes after each drug filtering method.

Concordance after subsetting methods. The concordance index between the correlation of all common cell lines between studies and the correlation of subsetting cell lines based on each of the two filtered subsets was computed. For IC₅₀ values, the concordance between the original r and the r of subsetting cell lines after filtering by binary drug classification produced a concordance index of 0.717. The concordance with subsetting cell lines after filtering by PCL classification produced a concordance index of 0.578 (Supplementary Figure 5). For AAC values, the concordance between the original correlation and the subsetting cell lines after filtering by binary drug classification produced a concordance of 0.939. The concordance with subsetting cell lines after filtering by PCL classification produced a concordance index of 0.794 (Supplementary Figure 6).

Figure 6 Pearson correlations (r) of AAC values for the 15 drugs with largest increases in correlation after filtering out inconsistent cell lines using binary drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on PCL drug classification ("binary").

Figure 7 Pearson correlations (r) of AAC values for the 15 drugs with largest decreases in correlation after filtering out inconsistent cell lines using binary drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on PCL drug classification ("binary").

Figure 8 Pearson correlations (r) of AAC values for the 15 drugs with largest increases in correlation after filtering out inconsistent cell lines using PCL drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on PCL drug classification ("PCL").

Figure 9 Pearson correlations (r) of AAC values for the 15 drugs with lowest changes in correlation after filtering out inconsistent cell lines using PCL drug classification method. The bars represent the Pearson's correlation coefficient (r) computed between studies with all cell lines ("orig") and after filtering out inconsistent cell lines based on PCL drug classification ("PCL").

DISCUSSION

Based on original correlations of sensitivity measures including all common cell lines between studies, AAC is much more concordant than IC₅₀ values. After removing inconsistent cell lines using both drug classification methods, the same pattern was still shown (Supplementary Figures 5, 6). This may be explained by certain cells never reaching the 50% inhibition level through all tested doses, which may give null reported IC₅₀ values, whereas AAC can be calculated without a certain inhibition level needed to be reached.

Using the binary drug classification method showed more positive changes in correlation for drugs compared to the PCL drug classification method. This

is understandable since the drugs fit into so many different PCLs and only 4 out of 34 PCLs had the actual minimum of 3 drugs within each class.

Generally speaking, the reasonably high concordance statistics measuring the consistency of sensitivity measures across studies points to the fact that classifying drugs by their mechanism and PCL are good models for filtering out inconsistent cell lines for both IC₅₀ and AUC.

CONCLUSION

The results of this analysis show that the consistency of drug sensitivity measurements across the CTRP and GDSC studies increased after taking into account the

similar targets of drugs; however, no consistent pattern could be found for all 71 drugs in common nor for the two drug sensitivity measures that were investigated. This reanalysis between the two datasets indicates the inconsistencies between the drug sensitivity measures between studies once again, but factors out the possibility of similar drug targets being the sole cause of discrepancies in measured phenotypes. The difference in trends between the two methods of classifying drugs used in this analysis may be used to develop better classification rules for compounds.

Although the concordance between studies showed some increase by taking similar drug targets into account, the cause of the discrepancies shown in the previous analyses still cannot be fully explained. With the lack of standardization in assay and computational methods between studies, the discrepancies of the drug sensitivity data emphasize the need for better standardization to better predict drug response.

While the traditional oncogenic drug development and treatment plan models should utilize the increasing amount of information provided by pharmacogenomic studies like the CTRP and GDSC, the possible sources of discrepancy are critical to note. By raising awareness on the importance of standardizing pharmacological assays and computational methods as well as standardizing drug classification terminology, pharmacogenomic studies can be more reliably and optimally be used in research.

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DATA AVAILABILITY

- Shared Directory: [Google Drive URL](#)
- Figures, Tables and Supplementary Information: <https://www.overleaf.com/read/kcxvsjfbktx>
- Computer Code: <https://github.com/honcasey/consistency-bcb330>
- Container: <https://codeocean.com/capsule/7594181/tree>

COMPETING INTERESTS

No competing interests declared.

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