



Published in final edited form as:

Nature. 2016 November 30; 540(7631): E1–E2. doi:10.1038/nature19838.

## Consistency in large pharmacogenomic studies

Paul Geeleher<sup>1</sup>, Eric R. Gamazon<sup>1,2</sup>, Cathal Seoighe<sup>3</sup>, Nancy J. Cox<sup>1,2,\*</sup>, and R. Stephanie Huang<sup>1,\*</sup>

<sup>1</sup>Department of Medicine, The University of Chicago, Chicago, IL 60637, USA; <sup>2</sup>Division of Genetic Medicine, Vanderbilt University, Nashville, TN 37232; <sup>3</sup>Department of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, Ireland

Haibe-Kains *et al.*<sup>1</sup> reported inconsistency between two large-scale pharmacogenomic studies (the Cancer Cell Line Encyclopedia<sup>2</sup> (CCLE) and the Cancer Genome Project<sup>3</sup> (CGP)). Upon careful analysis of the same data we arrived at much more positive conclusions. Here, we highlight the most important reasons for this.

To assess the concordance of two large studies of the efficacy of cancer drugs, Haibe-Kains *et al.* compared the correlation in drug sensitivities with correlation in gene expression values measured on the same human cancer cell lines. The authors reported correlation “*between*” cell lines for gene expression but, inconsistently, “*across*” cell lines for drug sensitivity (see Methods). On reanalysis, we found much higher correlations between cell lines than across cell lines for both gene expression and drug sensitivity measures (median  $r_s = 0.88$  between cell lines,  $r_s = 0.56$  across cell lines for expression; median  $r_s = 0.62$  between cell lines and  $r_s = 0.35$  across cell lines for AUC, a drug sensitivity measure). Thus, by correcting this inconsistency, the correlations for expression and drug sensitivity data were far more similar than was originally reported, severely undermining the authors’ interpretation of the relative quality of expression and drug sensitivity datasets.

However, the fundamental issue is that the authors’ reported Spearman correlation coefficients do not fairly reflect the concordance of drug sensitivity between the studies, because of the lack of variability in drug response, which arises due to the highly-targeted nature of many of the drugs assessed. To see why correlation is not an appropriate measure of biological concordance for these data, consider the hypothetical example of a drug that is not effective against any cell lines (a possibility for an experimental drug): In such a case randomly-fluctuating measurement error, inherent in biological assays, will dominate over the (non-existent) biological variability, meaning that there could be no expectation of correlation between repeated measures of drug sensitivity (assuming other experimental variables are held constant). In this study, many of the drugs were highly-targeted agents, which by design require specific (and often rare) molecular targets for response (see Supplementary Table). Consider nilotinib, which the authors reported as exhibiting “poor

\*Address for correspondence and reprints: Nancy J. Cox, 900 E 57<sup>th</sup> street, KCBD room 3100, the University of Chicago, Chicago, IL 60637. Telephone: (773) 702-; Fax: (773) 702-; nancy.cox@vanderbilt.edu, R. Stephanie Huang, 900 E 57<sup>th</sup> street, KCBD room 7148, the University of Chicago, Chicago, IL 60637. Telephone: (773) 702-9363; Fax: (773) 702-0963; rhuang@medicine.bsd.uchicago.edu.

consistency” between CGP and CCLE ( $r_s = 0.1$  for AUC); nilotinib targets *BCR-ABL1*, a fusion-gene. In CGP, *BCR-ABL1* status was reported strongly associated with drug sensitivity ( $P = 2.54 \times 10^{-65}$ ), accurately reflecting the known biology. *BCR-ABL1* status was not reported by CCLE, however, upon re-analysis we identified three *BCR-ABL1*-positive cell lines among the 189 nilotinib-treated cell lines that overlapped CGP and these were also the three most sensitive samples ( $P = 9 \times 10^{-7}$ ). Hence, despite the fact that these drug sensitivity data were accurately recapitulating biological expectations in both studies, the authors’ criteria incorrectly classify nilotinib sensitivity as *discordant*. Of the 577 cell lines screened in CGP 573 do not harbour the nilotinib target, i.e. the *BCR-ABL1* fusion gene. Thus, given (as expected) no drug response in almost all cell lines screened (median AUC across all cell lines = 0.99; AUC of 1 represents no drug response; Fig. 1(a); Supplementary Table), there was little biological variability across the vast majority of cell lines, resulting in low correlation between the repeated measurements made by CCLE and CGP, despite clearly concordant results. Similarly, most other drugs that the authors compared were also targeted agents, meaning this lack of drug response was *common*; indeed, for 10 of the 15 drugs, median AUC was greater than 0.90 in CGP and 8 of these 10 also have median AUC greater than 0.9 in CCLE, resulting in little variability across most cell lines when treated with these drugs. Indeed, we identified a *systematic* relationship between variability in drug response in either study and correlation between the two studies (Fig. 1(b)). A valid comparison of CGP and CCLE must consider the pharmacology of the drugs screened and in particular the differences in the variability induced by different drugs. Nilotinib was not an isolated case; in fact, despite the highly experimental nature of many of the compounds screened by CCLE/CGP, we still identified multiple expected associations that were consistently reported by both studies including *ERBB2* for lapatinib<sup>4</sup>, *NQO1* expression for 17-AAG<sup>5</sup>, *BRAF* mutation for PD-0325901<sup>6</sup>, AZD6244<sup>7</sup>, and PLX4720<sup>8</sup>, *MDM2* for Nutlin-3a<sup>9</sup>, and *MET* for Crizotinib<sup>10</sup> (Supplementary Table). Finally, the utility of these pharmacogenomic datasets is now further supported by findings that models fit using data from CGP could reliably predict drug response in multiple clinical trials<sup>11,12</sup>.

In summary, our analysis shows that Haibe-Kains *et al.*’s conclusions are unsubstantiated and we propose that a fair assessment of concordance between large pharmacogenomic datasets will require the development or adaptation of methods that account for the issues raised here, although great care will be required to ensure that such methods do not introduce their own unforeseen biases.

## Methods

In CGP and CCLE, using ordered data common to both studies, gene expression and drug sensitivity (AUC) values can be arranged in  $n_1 \times m$  and  $n_2 \times m$  matrices respectively where  $m$  is the number of cell lines,  $n_1$  is the number of genes and  $n_2$  is the number of drugs common to both studies. Correlations “between” cell lines are calculated by the correlation of matching columns of CGP and CCLE matrices (vectors of length  $n_1$  for expression or  $n_2$  for AUC). Correlations “across” cell lines are the correlations of matching rows (vectors of length  $m$  for both data).

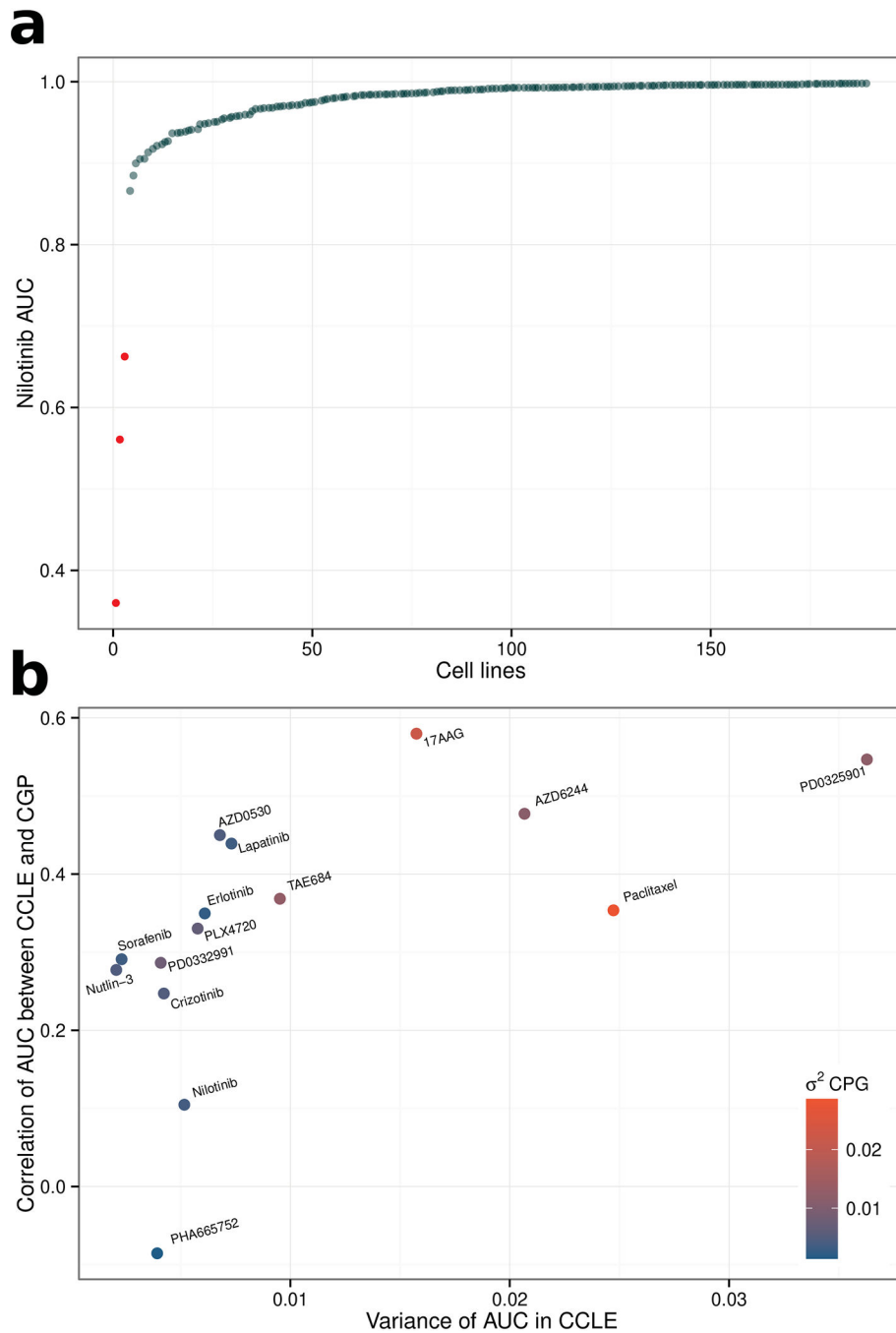
To achieve easy reproduction of our results, we have made the source code for our analysis available in a GitHub repository ([https://github.com/paulgeeleher/nature\\_bca](https://github.com/paulgeeleher/nature_bca)).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## References:

1. Haibe-Kains B et al. Inconsistency in large pharmacogenomic studies. *Nature* 504, 389–93 (2013). [PubMed: 24284626]
2. Barretina J et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483, 603–7 (2012). [PubMed: 22460905]
3. Garnett MJ et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* 483, 570–5 (2012). [PubMed: 22460902]
4. Konecny GE et al. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res.* 66, 1630–9 (2006). [PubMed: 16452222]
5. Kelland LR, Sharp SY, Rogers PM, Myers TG & Workman P DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J. Natl. Cancer Inst.* 91, 1940–9 (1999). [PubMed: 10564678]
6. Solit DB et al. BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 439, 358–62 (2006). [PubMed: 16273091]
7. Dry JR et al. Transcriptional pathway signatures predict MEK addiction and response to selumetinib (AZD6244). *Cancer Res.* 70, 2264–73 (2010). [PubMed: 20215513]
8. Tsai J et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc. Natl. Acad. Sci. U. S. A* 105, 3041–6 (2008). [PubMed: 18287029]
9. Müller CR et al. Potential for treatment of liposarcomas with the MDM2 antagonist Nutlin-3A. *Int. J. Cancer* 121, 199–205 (2007). [PubMed: 17354236]
10. Timm A & Kolesar JM Crizotinib for the treatment of non-small-cell lung cancer. *Am. J. Health. Syst. Pharm* 70, 943–7 (2013). [PubMed: 23686600]
11. Geeleher P, Cox NJ & Huang RS Clinical drug response can be predicted using baseline gene expression levels and in vitro drug sensitivity in cell lines. *Genome Biol.* 15, R47 (2014). [PubMed: 24580837]
12. Falgreen S et al. Predicting response to multidrug regimens in cancer patients using cell line experiments and regularised regression models. *BMC Cancer* 15, 235 (2015). [PubMed: 25881228]



**Fig 1.** Highly targeted agents (nilotinib) highlight a major limitation of the authors' test for concordance. Scatterplot showing the nilotinib AUC values (in CGP) for the 189 cell lines that were screened by both CGP and CLE. Only a very small proportion of cell lines achieve a response, e.g. the three BCR-ABL1 positive cell lines highlighted in red. This almost complete lack of biological variability renders a Spearman correlation ineffective as a means to assess concordance.

**(b) The authors' test for concordance is confounded by variability in drug response.**

Scatterplot showing the strong association between “Spearman’s correlation of AUC between CCLE and CGP” and “variance of AUC in CCLE”. Drugs whose AUC is more variable are more likely to be highly correlated between CCLE and CGP ( $r_s = 0.83$ ,  $P = 1.9 \times 10^{-4}$ ). The points have been color coded by their “variance of AUC in CGP”, which is also significantly associated with both “variance of AUC in CCLE” and “Spearman’s correlation of AUC between CCLE and CGP”.