Drug Selection in the Genomic Age: Application of the Coexpression Extrapolation Principle for Drug Repositioning in Cancer Therapy

Daniel L. Gustafson,^{1,2} Jared S. Fowles,¹
Kristen C. Brown,¹ and Dan Theodorescu²

¹Flint Animal Cancer Center, Colorado State University,
Fort Collins, Colorado.

²Comprehensive Cancer Center, University of Colorado–Denver,
Aurora, Colorado.

ABSTRACT

The use of patient-specific data in drug and dose selection is becoming an increasingly important component in cancer therapy. Basing drug choice on molecular aspects of the tumor is consistent with the identification of cancer as a molecular disease or diseases, even within the same histological type, and its treatment specific to the background from which it arose and exists. Multiple examples exist of single-gene mutations, and over- or underexpression that convey either sensitivity or resistance to a given agent. What about the picture that global gene expression in a cancer presents regarding drug sensitivity or resistance? Coexpression extrapolation (COXEN) is a methodology that acts as a Rosetta stone between patterns of gene expression that correlate to drug responses in vitro and those in tumors of untreated patients to predict chemosensitivity in such tumors even to drugs that are not specifically indicated for that histotype. Further applications of COXEN in drug discovery allow for in silico screens correlating drug response and gene expression in a genetically diverse cell panel to gene expression patterns in a target tumor with the potential for identifying and repurposing compounds. Here we discuss how COXEN is being developed and tested for application in drug selection, repositioning, and repurposing in oncology.

INTRODUCTION

Current clinical practice in oncology bases the choice of systemic therapy on descriptive histologic characteristics. For example, a diagnosis of osteosarcoma would lead to the use of adjuvant anthracycline and platinum drug therapy after surgical resection of the tumor. Why are these drugs used? The simple answer is that studies have shown that

patients receiving these drugs after surgery live significantly longer than those receiving surgery alone.¹ A few studies in human patients have shown that *in vitro* chemosensitivity testing of tumor biopsies can tailor therapy and lead to increased antitumor response,^{2–5} but these specific results and the overall approach are far from thoroughly validated. Nevertheless, the concept of basing therapy on an assessment of drug sensitivity rather than on tumor histotype alone appears to be a promising as yet unproven strategy. Limitations of such *ex vivo* chemosensitivity assessment in clinical practice are the technical difficulties associated with tissue procurement and culturing as well as measures of drug response.

In 2006, two different approaches to predicting tumor chemosensitivity were independently described. The first, called the broad connectivity map (www.broadinstitute.org/cmap), matched patterns of change in gene expression as a measure of mechanistic connectivity between xenobiotics⁶ and cellular gene alterations *in vitro*. The second, called “co-expression extrapolation” (COXEN) algorithm, uses gene expression profiling in patient tumors and cell lines *in vitro* coupled with an informatics approach to project phenotypic data on drug response from cell lines to predict patient drug sensitivity.⁷ Others have used variations and combinations of these ideas. In colorectal cancer, molecular subtypes were identified that may and may not benefit from chemotherapy as well as specific molecular therapies.⁸ Although gene expression profiling by microarrays is now considered routine, and more state-of-the-art approaches such as whole exome and deep sequencing may be more appealing and data-enriched, microarray-based gene expression analysis has the benefit of defined quality control criteria, well-described data analysis pipelines, and assessment of variability and reproducibility that are critical for use as a clinical test.^{9–11} Furthermore, in a study by The Cancer Genome Atlas group combining genomic DNA copy number arrays, DNA methylation, exome sequencing, messenger RNA arrays, microRNA sequencing, and reverse-phase protein arrays to complete a molecular portrait of breast cancer,

Reprinted with permission from *Drug Repurposing, Rescue, and Repositioning*; 1:1;17–21.

Drug Repurposing, Rescue, and Repositioning has merged with *Assay and Drug Development Technologies*.

the authors concluded that “the information content from copy number aberrations, miRNAs and methylation is captured at the level of gene expression and protein function.” In addition, they observed an apparent disconnect between the presence of *PIK3A* mutations in exome sequencing and biomarkers of pathway activation.¹² Supporting the value of gene expression over other assessment techniques in drug sensitivity prediction, algorithms for drug sensitivity using gene variants identified in the NCI-60 panel by whole exome sequencing were largely unsuccessful in predicting response to cytotoxic chemotherapy,¹³ in contrast to approaches using gene expression that have shown success in patients.⁷ Thus, using microarray-based measurement of gene expression seems to be optimal and robust for use in clinical applications because of both the availability of quality

control protocols for use in Clinical Laboratories Improvement Amendments certification as well as the information content associated with these assays.

COEXPRESSION EXTRAPOLATION

The COXEN algorithm generates gene expression signatures to generate panels of genes that can be used as biomarkers for the determination of sensitivity or resistance to specific drugs in patients.⁷ These panels are based on a “Rosetta Stone-like” linkage between tumors of untreated patients in a reference tumor set to that of untreated gene expression profiles of the NCI-60 cell line panel and their drug sensitivity data (<http://dtp.nci.nih.gov/index.html>). The power of COXEN is that it uses the expression profile of the reference set (NCI-60) to sift out a

Fig. 1. Schematic depicting the COXEN method of chemosensitivity prediction. The general steps involved in the process are shown on the left in bold, and the resources required for the analyses shown on the right in italics. COXEN, coexpression extrapolation.

predictive cohort of genes by examining the coexpression pattern with a learning set (i.e., tumors of untreated patients in a reference tumor set) to form the basis of the gene expression model. Depending on the need, any untreated patient's tumor type reference set can be selected. Shown in *Figure 1* is a basic schematic of the COXEN approach with regard to the data utilized for the identification of differentially expressed genes, coexpression analysis, model building, model learning, and model testing and validation. The COXEN approach has been used to accurately predict the sensitivity and resistance of cancer cell lines⁷ as well as patient outcomes of several histological types, including breast,^{14–16} bladder,^{15,17} ovarian,^{18,19} and lung²⁰ with regard to predicted drug response. Methods have also been established using the COXEN approach for the prediction of drug combination chemosensitivity²¹ that is critically important considering that patients are rarely treated with single drugs but are generally treated with drug combinations consisting of multiple agents with differing but presumably complimentary mechanisms of action.

DRUG SELECTION WITH COXEN

The purpose of developing and validating algorithms for the prediction of drug sensitivity or resistance in cancer is to utilize this information to optimize drug selection in individual patients. Thus, a mechanism must exist for translation of gene expression data to a *de novo* prediction in response to a drug or drug combination. The COXEN approach has been used in this manner to

identify compounds from the >43,000 screened with the NCI-60 panel for predicted activity in bladder cancer cell lines with the subsequent identification of a compound (NSC-637993; SymadexTM), an imidazoacridinone that inhibits topoisomerase II,²² validated for the predicted activity.^{7,23} Drug discovery and development (biomarker-selected phase II trials) applications of the COXEN algorithm open up the screening theoretically to any compound that has been tested for drug sensitivity in a suitably large, genetically diverse tumor or cell line panel. Identification of treatment in patient populations can either involve the selection of alternative protocols approved for the same indication such as using MVAC (methotrexate, vincristine, doxorubicin, cisplatin) or GC (gemcitabine, cisplatin) in advanced bladder cancer patients, or screening a tumor gene expression profile versus a much larger panel of FDA-approved anticancer agents. *Figure 2* shows an example where chemosensitivity predictions have been made based on a bladder tumor gene expression profile versus 76 FDA-approved agents for cancer therapy. This second application clearly has the largest hurdle to overcome in terms of clinical practice because of the use of drugs in cancers for which they do not have an indication. In the example shown in *Figure 2*, of the top five agents, two of them, 5-fluorouracil²⁴ and gemcitabine,²⁵ have established activity in bladder cancer and floxuridine is an active form of 5-fluorouracil. Busulfan has no known activity in bladder cancer; however, it does have known toxicity in urothelial cells²⁶ and as such would presumably have cytotoxic activity in cells of urothelial origin. Toremifene, whose

Fig. 2. Plot showing the COXEN-predicted relative sensitivity and resistance of a human bladder tumor to 76 FDA-approved agents for cancer treatment. Individual drugs are listed on the x-axis with the relative sensitivity (*up, blue*) or resistance (*down, red*) represented by bars.

activity is classified as a selective estrogen receptor modulator, presumably would have little activity in bladder cancers; however, recent studies have shown that toremifene has off-target activities, including inhibition of acid ceramidase²⁷ and blocking viral entry into cells,²⁸ which may translate to non-antiestrogenic effects. Nevertheless, plans are currently underway at several centers to evaluate this approach in patients for whom no available standard of care therapy exists and where such experimental approaches are ethically acceptable.

VALIDATING THE COXEN APPROACH IN SELECTING DRUG THERAPY

While there is significant retrospective data showing the ability of COXEN-derived biomarkers to predict outcome in patients,^{7,14–20} specific predictions for selecting first-line therapy using the COXEN approach require prospective validation if it is to replace standard-of-care systemic therapy assignment principles. For locally advanced bladder cancer, a national clinical trial has been recently approved in Southwest Oncology Group (S1314: “A Randomized Phase II Study of CO-eXpression Extrapolation [COXEN] with Neoadjuvant Chemotherapy for Localized, Muscle-Invasive Bladder Cancer”) that is prospectively evaluating the value of COXEN prediction for response to MVAC and GC in patients randomly selected for either regimen. The primary objective of this trial is to characterize correlations between MVAC- and GC-specific COXEN-derived biomarker scores and stage (pT0 rate) at cystectomy. The results of this trial will determine if COXEN-derived biomarkers can subsequently be used to select patients who will maximally benefit from neoadjuvant therapy (i.e., achieve pT0 disease) while sparing others the unnecessary toxicity and delay of chemotherapy.

Should the COXEN approach only be limited to situations where it can be used to discriminate between drugs or drug combinations already approved for a given tumor type? Second-line and salvage therapies often rely on the use of drugs that have low response rates and limited activity in that setting. A COXEN-based approach could open up drug selection in these cases to a much larger number, facilitating repositioning and repurposing of these active agents. As shown in *Figure 2*, response predictions can be made across a large panel of FDA-approved anticancer agents. How can these predictions be validated to support testing in clinical trials? The use of patient-derived xenografts (PDXs) in immunodeficient mice has the potential to serve as an intermediary to provide some proof of principle on predicted drug activity and inactivity.²⁹ The use of PDXs, with careful drug dose selection to ensure human equivalent exposures, would allow for the genetic diversity of tumors to apply COXEN and accuracy of correlation between antitumor activity and clinical response³⁰ to determine potential benefit.

CONCLUSION

The use of molecular signatures in tumors to determine drug therapy is occurring, and approaches where drug therapy is dependent on molecular aberrations across diverse tumor types have shown success with molecularly targeted agents.³¹ These same molecular aberrations and mutational spectrum do not seem to have the same predictive power for drug response in cytotoxic agents as they do for targeted agents.¹³ However, cytotoxic chemotherapy does and will continue to play an important role in cancer treatment, and approaches using gene expression data to predict response to these agents can continue to refine and improve cancer drug therapy.

DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Eilber FR, Rosen G. Adjuvant chemotherapy for osteosarcoma. *Semin Oncol*. 1989;16:312–322.
2. Herzog TJ, Krivak TC, Fader AN, et al. Chemosensitivity testing with chemofx and overall survival in primary ovarian cancer. *Am J Obstet Gynecol*. 2010;203:68.e1–e6.
3. Wakatsuki T, Irisawa A, Imamura H, et al. Complete response of anaplastic pancreatic carcinoma to paclitaxel treatment selected by chemosensitivity testing. *Int J Clin Oncol*. 2010;15:310–313.
4. Ugurel S, Schadendorf D, Pfohler C, et al. *In vitro* drug sensitivity predicts response and survival after individualized sensitivity-directed chemotherapy in metastatic melanoma: A multicenter phase II trial of the dermatologic cooperative oncology group. *Clin Cancer Res*. 2006;12:5454–5463.
5. Staib P, Staltmeier E, Neurohr K, et al. Prediction of individual response to chemotherapy in patients with acute myeloid leukaemia using the chemosensitivity index ci. *Br J Haematol*. 2005;128:783–791.
6. Lamb J, Crawford ED, Peck D, et al. The connectivity map: Using gene-expression signatures to connect small molecules, genes, and disease. *Science*. 2006;313:1929–1935.
7. Lee JK, Havaleshko DM, Cho H, et al. A strategy for predicting the chemosensitivity of human cancers and its application to drug discovery. *Proc Natl Acad Sci USA*. 2007;104:13086–13091.
8. Sadanandam A, Lyssiotis CA, Homiczko K, et al. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med*. 2013;19:619–625.
9. Canales RD, Luo Y, Willey JC, et al. Evaluation of DNA microarray results with quantitative gene expression platforms. *Nat Biotechnol*. 2006;24:1115–1122.
10. Daly TM, Dumaual CM, Dotson CA, et al. Precision profiling and components of variability analysis for affymetrix microarray assays run in a clinical context. *J Mol Diagn*. 2005;7:404–412.
11. Dumur CI, Nasim S, Best AM, et al. Evaluation of quality-control criteria for microarray gene expression analysis. *Clin Chem*. 2004;50:1994–2002.
12. Network CGA. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490:61–70.
13. Abaan OD, Polley EC, Davis SR, et al. The exomes of the nci-60 panel: A genomic resource for cancer biology and systems pharmacology. *Cancer Res*. 2013;73:4372–4382.
14. Lee JK, Coutant C, Kim YC, et al. Prospective comparison of clinical and genomic multivariate predictors of response to neoadjuvant chemotherapy in breast cancer. *Clin Cancer Res*. 2010;16:711–718.

15. Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. *Cancer Res.* 2009;69:8302–8309.
16. Shen K, Song N, Kim Y, et al. A systematic evaluation of multi-gene predictors for the pathological response of breast cancer patients to chemotherapy. *PLoS One.* 2012;7:e49529.
17. Smith SC, Baras AS, Dancik G, et al. A 20-gene model for molecular nodal staging of bladder cancer: Development and prospective assessment. *Lancet Oncol.* 2011;12:137–143.
18. Ferriss JS, Kim Y, Duska L, et al. Multi-gene expression predictors of single drug responses to adjuvant chemotherapy in ovarian carcinoma: Predicting platinum resistance. *PLoS One.* 2012;7:e30550.
19. Kim Y, Guntupalli SR, Lee SJ, et al. Retrospective analysis of survival improvement by molecular biomarker-based personalized chemotherapy for recurrent ovarian cancer. *PLoS One.* 2014;9:e86532.
20. Nagji AS, Cho SH, Liu Y, et al. Multigene expression-based predictors for sensitivity to vorinostat and velcade in non-small cell lung cancer. *Mol Cancer Ther.* 2010;9:2834–2843.
21. Havaleshko DM, Cho H, Conaway M, et al. Prediction of drug combination chemosensitivity in human bladder cancer. *Mol Cancer Ther.* 2007;6:578–586.
22. Skladanowski A, Plisov SY, Konopa J, et al. Inhibition of DNA topoisomerase II by imidazoacridinones, new antineoplastic agents with strong activity against solid tumors. *Mol Pharmacol.* 1996;49:772–780.
23. Smith SC, Havaleshko DM, Moon K, et al. Use of yeast chemigenomics and coxen informatics in preclinical evaluation of anticancer agents. *Neoplasia.* 2011;13:72–80.
24. Arena MG, Sternberg CN, Zeuli M, et al. Carboplatin and 5-fluorouracil in poor performance status patients with advanced urothelial cancer. *Ann Oncol.* 1993;4:241–244.
25. Stadler WM, Kuzel T, Roth B, et al. Phase II study of single-agent gemcitabine in previously untreated patients with metastatic urothelial cancer. *J Clin Oncol.* 1997;15:3394–3398.
26. Stella F, Battistelli S, Marcheggiani F, et al. Urothelial cell changes due to busulfan and cyclophosphamide treatment in bone marrow transplantation. *Acta Cytol.* 1990;34:885–890.
27. Morad SA, Levin JC, Tan SF, et al. Novel off-target effect of tamoxifen—inhibition of acid ceramidase activity in cancer cells. *Biochim Biophys Acta.* 2013;1831:1657–1664.
28. Johansen LM, Brannan JM, Delos SE, et al. FDA-approved selective estrogen receptor modulators inhibit ebola virus infection. *Sci Transl Med.* 2013;5:190ra79.
29. Siolas D, Hannon GJ. Patient-derived tumor xenografts: Transforming clinical samples into mouse models. *Cancer Res.* 2013;73:5315–5319.
30. Wong H, Choo EF, Aliche B, et al. Antitumor activity of targeted and cytotoxic agents in murine subcutaneous tumor models correlates with clinical response. *Clin Cancer Res.* 2012;18:3846–3855.
31. Tsimberidou AM, Iskander NG, Hong DS, et al. Personalized medicine in a phase I clinical trials program: The MD Anderson Cancer Center initiative. *Clin Cancer Res.* 2012;18:6373–6383.

Address correspondence to:

*Daniel L. Gustafson, PhD
Flint Animal Cancer Center
Colorado State University
300 W Drake Road*

Fort Collins, CO 80523-1620

E-mail: daniel.gustafson@colostate.edu