

IN THE SPOTLIGHT

Teaching an Old Dog New Tricks: Drug Repositioning in Small Cell Lung Cancer

Jing Wang¹ and Lauren Averett Byers²

Summary: Jahchan and colleagues report the use of a biostatistical analysis to identify effective therapeutics for small cell lung cancer (SCLC). Their results reveal a new use for the tricyclic antidepressant imipramine in SCLC and shed light on the therapeutic potential of drug repositioning in cancer and other diseases. *Cancer Discov*; 3(12); 1333-5. ©2013 AACR.

See related article by Jahchan et al., p. 1364 (3).

Small cell lung cancer (SCLC) is an aggressive, high-grade neuroendocrine cancer that makes up 14% of lung cancers. In the United States, 31,000 new cases are diagnosed annually (1). Because it metastasizes early, SCLC is usually advanced at the time of diagnosis, and survival rates with existing treatments are only 6.5% at 5 years for all stages (1). Unlike non-small cell lung cancer (NSCLC), in which the discovery of driver genes (e.g., *EGFR*, *ALK*, *ROS1*, and *RET*) has led to a growing number of successful treatments for specific patient subsets, the standard of care for SCLC has not changed significantly in more than three decades.

For patients with SCLC, there is an urgent, unmet need for active drugs. This is especially true in the second-line setting, where topotecan remains the only U.S. Food and Drug Administration (FDA)-approved treatment, and drug resistance remains a major clinical problem. However, despite substantial efforts by several groups to develop new therapies for SCLC, certain barriers exist that have made translational research in this area challenging. These include the relatively limited availability of tumor tissue for molecular analyses, as, unlike NSCLC, this disease is not treated with surgical resection. Moreover, the distinct morphology of SCLC cells means that a diagnosis can be made from a few cells obtained by fine-needle aspirate (currently insufficient for many molecular profiling platforms). Finally, repeat biopsies of SCLC tumors following the emergence of drug resistance are uncommon, despite their potential value for exploring mechanisms through which resistance develops. Recently, the need for intensified efforts in translational SCLC research has been pushed to the forefront by the Recalcitrant Cancer Research Act of 2012, a bill enacted by the United States Congress last January (2). This law provides a framework for accelerated

research efforts in aggressive cancers with a 5-year survival rate of less than 20%, focusing initially on SCLC and pancreatic cancer.

Despite the challenges outlined above, the rapid growth of high-throughput profiling data, coupled with sophisticated bioinformatics approaches, provide new opportunities for progress in SCLC. In this issue of *Cancer Discovery*, Jahchan and colleagues (3) used a drug-repositioning bioinformatics approach to identify FDA-approved drugs with activity in SCLC. Starting with an *in silico* analysis that was then coupled with further testing and validation in preclinical models, the authors show that tricyclic antidepressants (TCA) and related inhibitors of G-protein coupled receptors (GPCR) are potential “new” treatments for SCLC and other high-grade neuroendocrine cancers. Using publicly available gene expression data, the authors integrated drug signatures (computed by comparing treated versus untreated cell lines) with SCLC signatures to identify drugs predicted to have activity in SCLC. Among the top therapeutic candidates (which included irinotecan, an established chemotherapy for SCLC), they observed an enrichment in drugs targeting neuropeptide and calcium signaling pathways.

On the basis of these results, they selected six drugs targeting these pathways for an initial *in vitro* investigation. Treatment with these drugs produced cytotoxic effects in SCLC cell lines, but not in NSCLC cell lines—supporting preliminary findings from their bioinformatics analysis and prompting further investigation in animal models with imipramine (a TCA), promethazine (a first-generation H1 receptor antagonist with anticholinergic and antiadrenergic activities), and bepridil (a calcium channel blocker). *In vivo*, all three drugs controlled SCLC tumor growth in a genetically engineered SCLC mouse model (Kp1), human orthotopic model (H187), and patient-derived xenograft model (NJH29), as compared with placebo (saline). Tumor growth delay was also observed with imipramine in a model of cisplatin-resistant SCLC, suggesting that it might have activity in the second-line setting following standard treatment with platinum-based chemotherapy.

The authors then explored the potential mechanism of SCLC growth inhibition and showed that treatment with the TCA caused apoptotic cell death via activation of caspase-3. Interestingly, doses used in their studies were in

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the same range as those used to treat depression—suggesting that their experiments mimicked clinically achievable doses and that the targets modulated by drug treatment were likely similar in both cases. As such, based on previous studies that suggested that the major targets at this dose range were GPCRs (including histamine H1 receptor, muscarinic acetylcholine receptor, 5-HT₂ serotonin receptor, and alpha1-adrenergic receptor), the authors then confirmed their initial findings using selective inhibitors against these GPCRs. Consistent with their hypothesis that the activity of imipramine may be related to its action on GPCRs, GPCR inhibition also led to decreased cell survival and inhibition of downstream signaling, including diminished PKA activity, and activation of stress-induced mitogen-activated protein kinase (MAPK) signaling. These effects were specific to SCLC and were not observed in the NSCLC models tested.

A particularly intriguing finding of the study was that the preclinical activity of imipramine could also be reproduced in other high-grade neuroendocrine tumors, such as Merkel cell carcinoma (an aggressive neuroendocrine tumor of the skin), pheochromocytoma, and neuroblastoma. Interestingly, and in contrast, large cell neuroendocrine NSCLC cell lines were not sensitive to the drug, despite having similar clinical and molecular characteristics to SCLC. These findings could, therefore, provide treatment opportunities for other high-grade neuroendocrine cancers, which are even less common than SCLC but also have limited treatment options.

The computational repositioning approach used by Jahchan and colleagues (3) to identify candidate drugs for SCLC was developed by Sirota and colleagues (4, 5), as described in two 2011 reports. Although the method was published previously, it is novel for SCLC and is a sensible approach for target biomarker discovery. In their algorithm, drug-exposure expression data were used as a reference database and queried with the disease signature by applying a nonparametric rank-based and pattern-matching strategy, originally introduced by Lamb and colleagues. The gene expression data for these experiments were obtained from the National Center for Biotechnology Information Gene Expression Omnibus (GEO), a publicly available microarray gene expression database. A similarity score was then computed that reflected the similarity of the drug and disease signatures. To evaluate the significance of predictions, the investigators applied a permutation approach using randomly generated drug signatures, repeated 100 times. The false discovery rate for individual drug-disease similarity scores/ q values was computed by calculating the expected number of false positives, given the actual distribution of similarity scores and the distribution of scores after randomization for the disease signature (5).

The bioinformatics approach used by the authors is straightforward, statistically sound, and broadly applicable for therapeutic target screening. One potential limitation, however, is the significant technical variation between microarray experiments that can introduce strong batch effects. Therefore, continued refinement of the algorithm—with attention to removing batch effect—could further

strengthen the approach. Furthermore, there are now a number of high-throughput techniques being used to generate molecular data beyond mRNA expression. Adapting this type of bioinformatics approach to an integrated analysis of multiple data types (including siRNA, methylation, sequence, and proteomic data) holds tremendous potential (Fig. 1).

Although drug repositioning (or repurposing) does not occur infrequently within oncology [e.g., approval of imatinib for gastrointestinal stromal tumor after initial development for chronic myelogenous leukemia, or the recent testing of PARP inhibitors in SCLC after initial success in *BRCA*-mutated breast and ovarian cancers (ref. 6; NCT01286987)], it occurs less often across medical specialties. Nevertheless, successful examples exist of drug repurposing from one disease condition to a completely unrelated one. These include the repurposing of thalidomide from a failed antiemetic with an unacceptable risk profile (causing limb malformations in offspring of pregnant women treated with the drug) to a standard-of-care treatment for multiple myeloma (7). As the authors of the current article correctly point out, repurposing of drugs—especially those with existing FDA indications—has several advantages. These include the potential for more rapid and less expensive development due to the existence of an established dose and known toxicity profile. On the other hand, finding clinical-trial funding for older, off-patent drugs may pose certain challenges in the short term.

One important factor in the development of any drug is its side-effect profile. In this case, TCAs are no longer the drug of choice for the initial treatment of depression because of newer medications with more favorable toxicity profiles (e.g., selective serotonin reuptake inhibitors). However, when compared with existing chemotherapies for SCLC and other neuroendocrine cancers (e.g., cisplatin, etoposide, and topotecan), the side-effect profile of a TCA is likely no worse and is possibly better.

On the basis of the results described in this issue of *Cancer Discovery* from Jahchan and colleagues (3), a phase IIa clinical trial of the TCA desipramine has been initiated for SCLC and other high-grade neuroendocrine cancers (NCT01719861). However, despite encouraging preclinical data, certain questions remain. For example, although the preclinical data for imipramine is strong, promethazine (a common antiemetic medication) also showed similar *in vitro* and *in vivo* activity in several of the studies. The authors attempted to find patients with SCLC treated with promethazine at Stanford, but did not have sufficient numbers to draw conclusions about its possible clinical activity. However, it seems unlikely that a connection between promethazine and SCLC response would not have been previously described if the drug truly has single-agent activity. In contrast, previous preclinical studies of TCAs support their activity against certain cancers, including glioma, but retrospective analyses have been inconclusive about their anticancer activity. Therefore, as with other drugs that have shown preclinical promise in SCLC, the clinical trial will be critical for assessing the activity of TCAs within this setting.

In summary, Jahchan and colleagues (3) have illustrated how sophisticated bioinformatics can inform the

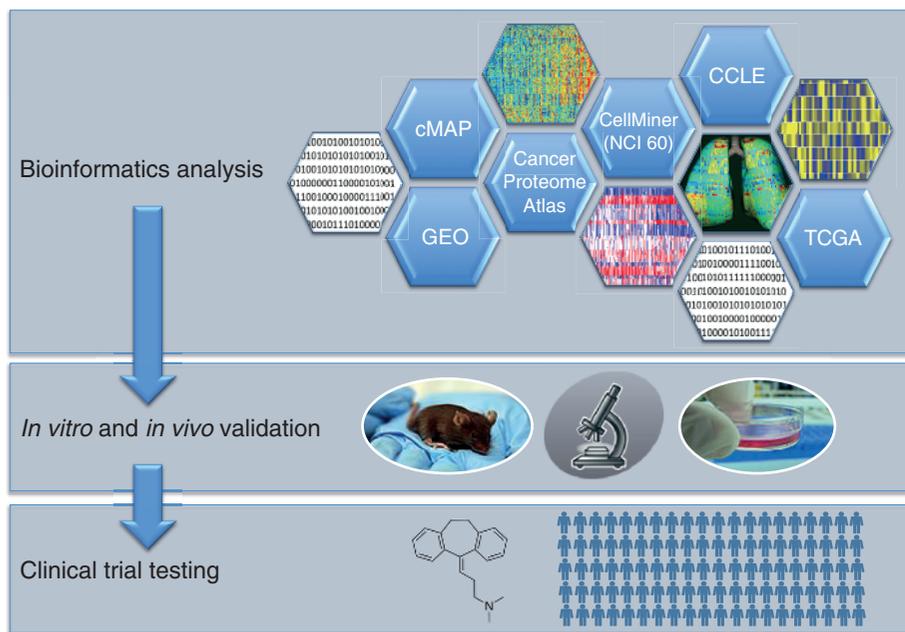


Figure 1. An integrated approach to drug repositioning. Publicly available datasets for initial computational screens include the Connectivity Map (cMAP), GEO, the Cancer Proteome Atlas, CellMiner, the Cell Line Encyclopedia (CCLE), and The Cancer Genome Atlas (TCGA). NCI, National Cancer Institute.

prioritization of candidate drugs for further investigation. The potential impact of computational approaches—paired with appropriate laboratory validation—has recently been recognized with the 2013 Nobel Prize in Chemistry awarded to Martin Karplus, Michael Levitt, and Arieh Warshel for their development of computer models to understand and predict chemical processes, and in a TED talk on drug repositioning by Francis Collins, Director of the NIH (Bethesda, MD; ref. 8). Continued growth and refinement of the methodologies highlighted in this issue (and the training of bioinformaticians with the expertise to lead these efforts) are particularly important given ongoing public, academic, and private-sector efforts that are building major repositories of molecular cancer data (such as The Cancer Genome Atlas, the Cell Line Encyclopedia, and the Cancer Proteome Atlas; refs. 9, 10). These *in silico* experiments are especially critical for diseases like SCLC in which molecular tumor data are more limited, and they can complement current efforts to generate drug response data for SCLC by the National Cancer Institute’s Developmental Therapeutics Program (Frederick, MD; ref. 11). Ultimately, these integrated, cross-disciplinary investigations will help guide clinical trial development and hopefully lead to new therapeutic options for patients with SCLC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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