Editorial

# Identifying therapeutic vulnerabilities in lung cancer: application of a chemistry-first approach

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In the last three decades, 5-year relative survival rates for lung cancer improved slightly, from 14.3%, 15.5%, and 18.4% (1). One of the reasons for these dismal survival rates is that most patients present with advanced-stage disease, a time during which systemic therapies are unlikely to be curative. In addition, the basics of lung cancer treatment, including surgery, chemotherapy and radiotherapy, remained relatively constant. However, a key therapeutic advance in recent years has been the development of molecular-guided drugs against genes such as *EGFR* and *ALK*. These approaches have shown that understanding the molecular landscape of a tumor can identify vulnerabilities and targets for intervention. This evolution in our approach to cancer treatment is a cornerstone of precision medicine.

Precision medicine for patients with advanced non-small cell lung cancer (NSCLC) has now become routine in clinical practice, whereby molecular profiling matches targeted agents to actionable genetic alterations. As mentioned above, these actionable targets are mainly restricted to *EGFR* and *ALK*. An immense challenge in precision oncology has been the ability to discover *new* links between existing chemicals and recurrent genetic alterations in tumors, particularly somatic changes currently not considered to be "actionable." This can be a daunting task when trying to distinguish "driver" and tumor maintenance mutations from the landscape of "passenger" alterations that may be pharmacologically irrelevant. It is particularly monumental in lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD), which are the second and third most highly mutated tumor subtypes reported in The Cancer Genome Atlas, respectively (2-4), with a mean non-synonymous mutation burden of ~250 mutations/tumor. However, as highlighted by Minna, Kim, White and colleagues, recently published in *Cell* (5), a large mutational burden also increases the probability that tumors harbor unique vulnerabilities not present in normal cells, which is something that could be exploited therapeutically. In that paper, Mc Millan *et al.*, elegantly designed and executed a chemistry-first drug screening approach for the identification of undiscovered therapeutic vulnerabilities in lung cancer. As discussed below, their study demonstrates that many undeveloped avenues are open for the productive continued pursuit of tumor-intrinsic precision medicine in NSCLC.

One of the first major drug screening efforts was performed by the National Cancer Institute in the 1980s when it created the NCI-60 Human Tumor Cell Lines Screen (6). The NCI-60 initiative was an endeavor to screen large numbers of known and new compounds across a selection of cell lines representing multiple tumor types. The genomes and transcriptomes of the NCI-60 panel have been extensively characterized and the initiative has culminated in the screening of over 100,000 compounds, which has led to the selection of drugs for preclinical studies and ultimately for clinical trials (7). For example, Bortezomib, a protease inhibitor, demonstrated an unusual

cell line response pattern in the screen, data that encouraged its further clinical development and eventual approval for multiple myeloma (8). Additional drug screening resources for the scientific community include the Genomics of Drug Sensitivity in Cancer (GDSC) and the Cancer Cell Line Encyclopedia (CCLE), which collectively characterized more than 1,000 cell lines and nearly 150 anti-cancer agents (9,10). Other valuable resources for drug screening include the more recently developed Patient-Derived Xenograft (PDX) repositories at the NCI, EuroPDX and elsewhere (11), initiatives that better capture the genetic complexity of human cancers than cell lines.

While high throughput screens using the NCI-60 panel have guided the selection of compounds for preclinical development, the NCI-60 has some limitations. For example, the panel contains only nine lung cancer lines. Four of the cell lines have *KRAS* mutations, but none have *EGFR*, *BRAF*, *RET*, *TRK*, *CMET*, or *FGFR2* oncogenic driver mutations, all of which are clinically relevant targets. In addition to the proven oncogenic drivers in lung cancer, the genetic diversity in LUSC and LUAD is extreme. More recently, efforts have been made to screen panels comprising a large number of cell lines from single or multiple cancer types because they more efficiently capture the genetic and phenotypic heterogeneity within specific tumor types. Moreover, agnostic and unbiased drug screening efforts have been conducted with specific tumor models in mind, some of these drug-repurposing efforts have had promising results.

High throughput genetic screens have been powerful tools for identifying genes in cancer cell lines that are cancer "drivers" or are responsible for conferring resistance to therapy. These screens involve high-throughput methods for knocking down/out genes by introducing shRNAs or gene edits via CRISPR-Cas9 into cell lines. Genetic screens have been typically restricted to a certain genomic background and/or a specific therapeutic regimen. For example, an shRNA screen of 1,000 genes was recently employed in *ALK* fusion-positive lung cancer cell lines derived directly from patients who developed resistance to the ALK inhibitors, crizotinib or ceritinib (12). The landmark study pointed to activated SHP2, a non-receptor protein tyrosine phosphatase, as a resistance mechanism to ALK inhibitors. Preclinical testing of a small molecule inhibitor of SHP2 suggested that combined ALK and SHP2 inhibition may be a promising therapeutic strategy for recurrent *ALK* fusion-positive NSCLC (12). In a separate study, Neal Rosen, Scott Lowe and colleagues performed an shRNA screen targeting more than 500 kinases on a KRAS-mutant lung cancer cell line treated with the MEK inhibitor, trametinib (13). They found that fibroblast growth factor receptor 1 (FGFR1) contributes to trametinib resistance. A combination of trametinib and ponatinib, a pan-FGFR inhibitor, was effective in treating *KRAS*-mutant xenografts in mice. Given that targeted therapies have not yet been approved for NSCLC patients with *KRAS*-mutant tumors, the study could impact the design of clinical trials for this patient population. Other high throughput screening projects have been larger in scale. For example, project Achilles at the Broad Institute has performed screens on more than 500 cell lines with shRNAs covering >25,000 gene products (14-16). Project Achilles also includes a CRISPR/Cas9 screen targeting >19,000 genes (17). Drug screens have not been limited to cell lines. Novartis published a seminal report in which drug screens were performed in over 1,000 PDX models across many cancer types (18).

Minna, Kim, White and colleagues took a different approach from the studies outlined above. Their study was drug-driven rather than being centered on a specific genomic alteration or resistance phenotype. Moreover, their study was lung cancer-focused. They carefully selected a large panel of deeply annotated NSCLC cell lines that recapitulate the genetic and phenotypic diversity of patient tumors and screened them by following an agonistic chemistry-first approach (5). They matched chemicals with diverse genetic lesions and biomarkers of response in human lung cancer. The approach was elegantly designed to concentrate first on a large set of compounds as a discovery tool for identifying novel chemical/genetic relationships in highly characterized NSCLC cell lines. The output was a large set of potential avenues for further investigation of novel agents and response biomarkers, particularly for compounds that are clinically available but currently lack connections to patient biomarkers of response (5).

The authors assembled a panel of nearly 100 NSCLC cell lines, primarily LUAD, which were representative of the phenotypic variation observed in the human disease. There has been much debate about whether established cell lines recapitulate the heterogeneity of primary tumors, and if it is possible to accurately filter somatic from germline mutations. To address this, the authors performed whole exome sequencing (WES) on matched B cell lines from 34 cell lines and used the matched pairs and public datasets to filter probable germline variations. They also noted a high concordance between transcript profiles from RNA-

 

seq and gene expression arrays that were performed years apart. Armed with confidence in the mutation calls and the accuracy and stability of cell line provenance, the authors performed affinity propagation clustering, which classified more than 15 phenotypic groups. From the groups, they chose 12 cell lines representing overall phenotypic diversity of the cell line panel (5).

The screening process started with over 200,000 chemicals across the 12 selected cell lines. Through a multi-tiered strategy, the authors selected over 200 compounds that were then tested for efficacy across the entire 100 cell line panel. They performed sparse feature selection to find robust chemical/genetic associations. As a compelling proof of concept that clinically relevant associations were discoverable following their approach, they were able to accurately match known compounds with cell lines expected to be sensitive based on genetic composition and known biomarkers of response. For example, the experimental schema linked cell lines with high ALK expression to the ALK inhibitor crizotinib, and mutant or amplified *EGFR* cell lines with the EGFR inhibitor erlotinib (5).

Out of the 200 compounds that were rigorously screened, more than 170 were linked to genomic features. The chemical/genetic relationships spanned a highly diverse set of biological processes. These included host defense pathway activation, nuclear hormone signaling, and ciliogenesis. For example, the authors noted a strong association between the compound SW036310 and mutations in *TTC21*, a gene that encodes for a protein localized to the cilia and required for normal ciliary function (19). The authors provided evidence that the compound deregulated primary cilia biology that is required for cell survival in cell lines that are mutant for *TTC21*. In particular, cell lines that were *TTC21*-mutant had evidence of primary cilia as well as hyper-activation of pro-survival signaling pathways known to be activated by cilia, providing further support for the dependency of the cell lines on the aberrant protein (5).

One of the most compelling findings from the study was that the chemistry-first approach identified pharmacological liabilities that would be missed with traditional genomic testing. Remarkably, the authors identified a dozen compounds that were highly sensitive in a subset of cell lines overexpressing various known drug metabolism enzymes. The compounds' metabolites were not generally toxic. Rather, the sensitive cell lines had a selective vulnerability to metabolic byproducts of the administered compounds. Thus, the compounds could be highly effective in subsets of tumors expressing the biomarker(s) with potentially low toxicity in the patient. Although the mechanisms of action and biological activities of these metabolites varied and were not completely determined in the study, the overall theme pointed to the potential of this approach to detect pharmacological liabilities related to drug metabolism, with several possibilities highlighted that could be further developed in preclinical trials (5).

The most salient finding made by McMillan *et al.*, was the identification of actionable targets in a subgroup of *KRAS*-mutant NSCLCs. Nearly one-third of LUADs harbor an oncogenic *KRAS* mutation (2). Despite success in targeting other oncogenic alterations in LUAD (e.g., EGFR, ALK) targeting KRAS has remained enigmatic. Direct inhibitors of KRAS are currently unavailable in the clinical setting. Efforts to inhibit signaling pathways downstream of KRAS in the clinic have failed—this has been attributed to the diversity of co-occurring mutations that mediate resistance to treatment. Precision medicine for KRAS-mutant NSCLC therefore calls for more "precise" measures that consider the remainder of the genomic landscape in these tumors. The authors observed that *KRAS*-mutant cell lines displayed diverse and discordant responses in the chemical screen as expected. However, when they grouped the *KRAS*-mutant cell lines by mutations in other genes, significant chemical/genetic associations were seen. Most noteworthy, *KRAS/KEAP1* double mutant cell lines were sensitive to SW157765, a compound that the authors found to target the non-canonical glucose transporter GLUT8. The authors went on to show that *KRAS/KEAP1* double mutant cells have a strong dependency on GLUT8 for glucose intake, which is required for the production of purines and thymidines. The reason for this higher dependency was not entirely clear, but the authors provided evidence suggesting that it was through NRF2, a regulator of the antioxidant response that is triggered by KEAP1 inactivation (5). The discovery highlights the value of a chemistry-first approach in deeply annotated cell lines, because the chemical relationship between SW157765 and GLUT8 in *KRAS/KEAP1* mutant tumors would not have been apparent by traditional genetic screens. Though *KEAP1* and *KRAS* co-mutations are not common in other cancer types with a high frequency of *KRAS* mutations, such as pancreatic cancer, this study demonstrates a proof of principal for other cancer types.

In summary, McMillan *et al.*, uncovered a compelling diversity of otherwise unappreciated targets within the landscape of genetic lesions of lung cancer. They showed

 

that the identified targets are pharmacologically addressable in the *in vitro* setting, with a high potential for further development in preclinical studies. Of highest relevance, the chemical vulnerabilities were linked to recurrent mutations in lung tumors that are currently not clinically "actionable". The targets highlighted in the paper are recurrent mutations in lung cancers from smokers, and as such have the potential to have substantial impact on the burden of lung cancer mortality—*EGFR* and *ALK* alterations are most common in tumors from never smokers. They made the data publicly available so that others can mine. Thus, the findings provided in this groundbreaking study have the potential to reach far beyond the initial report, as we continue our pursuit to uncover novel vulnerabilities for guiding preclinical trials and for advancing tumor-intrinsic precision oncology.

As mentioned above, the last decade has seen significant advances in molecular-guided treatment approaches for lung cancer, specifically in terms of *EGFR* and *ALK* altered tumors. These approaches have extended survival for lung cancer patients, but in most cases, resistance emerges. The key findings by McMillan and colleagues re-emphasize the need for detailed and individualized scrutiny of tumors, showing that even low frequency alterations can be vulnerable if we take the right approach. As we move further into the era of precision medicine and the discovery of new molecular-guided tumor vulnerabilities, the hope is that some of these discoveries will not just extend patient survival but decrease mortality also.

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## Footnote

*Conflicts of Interest*: The authors have no conflicts of interest to declare.

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