Drug Targets Don’t Pass Muster with CRISPR

The presumed targets of many anticancer drugs may be wrong, concludes a recent study that used the latest CRISPR gene-editing technologies to probe the mechanisms of action of 10 small-molecule drugs in or near clinical testing (Sci Transl Med 2019;11:eaw8412). With all 10, the putative targets proved nonessential both to cell growth and to drug activity—a finding that calls into question some of the common techniques used for target validation in oncology.

“There are some fundamental issues in how new drug targets are studied and how new drugs are characterized,” says Jason Sheltzer, PhD, of Cold Spring Harbor Laboratory in New York, who led the research.

RNAi assays, Sheltzer points out, are prone to off-target effects, and this older approach to evaluating gene dependencies may have frequently led drug developers astray. With more precise CRISPR-based methods, he says, “you do a better job of finding cancer-essential genes, you do a better job validating a drug’s on-target mechanism of action, and that kind of preclinical validation will help clinicians design better clinical trials to decrease the failure rate of new drugs.”

“The depth and scope of this analysis highlights the potentially widespread problem of off-target pharmacology for small-molecule inhibitors,” says Jeff Settleman, PhD, head of oncology research at Pfizer in San Diego, CA. He notes, however, that the study focused only on investigational agents, most of which either failed early testing or had yet to reach the clinic. “For the vast majority of approved oncology drugs,” he emphasizes, “there are abundant data supporting the on-target nature of the observed clinical benefit.”

Sheltzer and his colleagues had previously shown that MELK, a protein once thought critical for the growth of multiple cancer types, was not needed for cell proliferation, and that a putative MELK inhibitor worked through some other pathway (eLife 2017;6:e24179). To test whether similar problems were widespread, his team considered 10 more drugs that collectively target six different proteins.

The researchers selected five drug targets reported to underpin cancer “addictions”—proteins without which the cells cannot survive—as well as one target reported to induce cell death when activated. None of the targets had a known resistance mutation that would definitively demonstrate the drug had on-target activity.

Using gene-editing tools to mutate or silence each protein in a variety of cell lines, Sheltzer and his colleagues showed that the cells’ proliferative capacity was unscathed by CRISPR-induced perturbations. What’s more, the 10 drug inhibitors all continued to block cancer growth in the gene-edited cells, indicating that the molecules “must necessarily be killing the cancer cells through some other unknown, off-target effect,” Sheltzer says.

Follow-up experiments with CRISPR involving one of the drugs helped pinpoint its true function. The preclinical-stage agent OTS964 didn’t work through TOPK inhibition, as its manufacturer OncoTherapy Science had reported (Sci Transl Med 2014;6:259ra145). Instead, it proved to be a CDK11 blocker. Several approved breast cancer drugs target other members of the CDK family protein, but OTS964 is one of the first directed against CDK11, a protein vital for cell-cycle control and RNA transcription regulation.

The paper “definitely speaks to the irreproducibility of science,” says Sourav Bandyopadhyay, PhD, of the University of California, San Francisco, who was not involved in the study. However, the problem is much bigger than any one assay: “You need to do rigorous science, rather than just throw out RNAi and pop in CRISPR,” he says. “CRISPR is not quite a panacea in rescuing every drug target.”—Elie Dolgin

Database Drives Biomarker Research

The Genomics of Drug Sensitivity in Cancer (GDSC) project has added 4 years of additional data to a database of cancer drug screens designed to identify predictive biomarkers for cancer therapies. Available at cancerxgene.org, these data are driving research on the relationship between genomic features and drug responses to better tailor treatment to individual patients.

The GDSC project started 10 years ago and is part of the Cancer Dependency Map, a joint effort between the Wellcome Sanger Institute in Cambridge, UK, and the Broad Institute in Cambridge, MA, to identify vulnerabilities in cancer cells that could become drug targets. “We’ve pulled together one of the largest collections of cancer cell models in the world,” says Hayley Francis, PhD, of the Sanger—one that, according to a 2016 study, accurately captures the genomic landscape seen in tumor cells from patients (Cell 2016;166:740–54). The goal, she adds, is to fully characterize the genomic features of the models and then use the models for research.

In the GDSC project, researchers are performing high-throughput screens and cataloguing drug responses “to try to integrate the genomic information and the drug response information, and look for biomarkers of drug response,” Francis says. To date, the project has released data on 453 cancer drugs tested in 989 cell lines, which include 386,293 assessments of how cell lines respond to different doses and 494,973 associations between drug responses and genomic features such as copy number variations and expression data. The database has been used to identify biomarkers for 37 drugs. “In many cases, the database provides proof of concept,” Francis says. “At the moment, it’s providing information rather than guidance.”—Olivera Finn, PhD
as mutations, gene amplifications and deletions, gene fusions, and changes in gene expression. The project mainly tests approved drugs and those in clinical trials.

“I think the key selling point of our project is that we’ve got a huge range of diversity of cancer models that we’re using,” says Elizabeth Coker, PhD, of the Sanger. “You need a relatively small number of models to find the really big, really obvious hits, but you need statistical power to find the rarer biomarkers and the rarer variants, and that comes with an increased scale.” For example, Sanger researchers used the data to establish that Ewing sarcoma cells with the EWS–FLI1 translocation are sensitive to the PARP inhibitor olaparib (Lynparza; AstraZeneca; Nature 2012;483:570–5). Clinical trials are now testing olaparib and other PARP inhibitors against the disease.

Other institutions are querying the database, too: It is accessed by more than 350 people per day. “Because we do have the ability to hold these large numbers of cell models and to conduct these high-throughput drug screens, we are able to be a starting point for other labs to investigate drug response,” Frances says. One research group is developing a method for predicting patient responses to chemotherapy based on gene-expression levels and in vitro drug sensitivity, whereas another is investigating why MEK inhibitors show activity in BRAF-mutant but not KRAS-mutant melanoma, and a third is exploring RANBP2 as a therapeutic target in colon cancers that resemble BRAF-mutant disease but lack a BRAF alteration (Genome Biology 2014;15:R47; Cancer Cell 2014;25:697–710; Cell 2016;163:317–30).

The researchers are now adding more models and drugs to the project. They are generating organoids to represent more cancer subtypes, and they also plan to begin testing drug combinations. “Right now, the majority of patients don’t receive precision cancer medicine, and that is simply because we don’t understand what targeted drugs we should be giving to what patients,” Coker says.

Patricia Jaaks, PhD, also of the Sanger, adds, “We hope that finding new biomarkers will eventually help to position drugs in the right disease or cancer context.” –Catherine Caruso

**Collaborative Review, Concurrent Approval**

In a regulatory first, the FDA has reviewed a cancer treatment with drug agencies from around the world, leading to simultaneous approvals in three countries.

The FDA joined Australia’s Therapeutic Goods Administration and Health Canada in granting conditional approval in September to lenvatinib (Lenvima; Eisai) plus pembrolizumab (Keytruda; Merck) for recurrent endometrial carcinomas that are not microsatellite instability–high (MSI-H) or mismatch repair–deficient (dMMR).

The approvals came 3 months prior to the FDA’s target decision date—and likely much earlier than would happen otherwise in Australia or Canada, where drug launches often lag a year or more behind those in the United States.

The regulatory bodies came to the shared decision as part of Project Orbis, an initiative of the FDA’s Oncology Center of Excellence.

“This is a really important direction,” says Frances Richmond, PhD, chair of the Department of Regulatory and Quality Sciences at the University of Southern California in Los Angeles. Having each agency independently review submissions is redundant “when they’re essentially working from the same songbook,” she notes. “The more they can do to reduce the timeline and the cost of developing these drugs is a good thing.”

Since 2004, members of the FDA’s oncology and hematology division have routinely held conference calls with their counterparts in Europe, Japan, and elsewhere. These virtual meetings have allowed the agencies to share confidential information about ongoing reviews and to identify any regulatory divergences across regions.

As highlighted in an August report from members of the FDA and European Medicines Agency (EMA), such exchanges provide “a form of peer review among fellow regulators,” allowing “for more informed regulatory decision-making, and oversight of regulated industry in areas like inspections and data integrity.” (Clin Pharmacol Ther 2019 Aug 26 [E pub ahead of print]).

Yet, the various agencies involved in these strategic partnerships had never synced the submission, appraisal, or approval of any marketing applications.

Through Project Orbis, review teams from Australia, Canada, and the United States collectively considered data from a phase II trial of 94 women with MSI-H/dMMR–negative endometrial tumors that had progressed following at least one systemic therapy.

In the single-arm study, participants received daily oral doses of lenvatinib, a multikinase inhibitor of VEGFRs and FGFRs, and infusions of pembrolizumab, a PD-1–targeted checkpoint inhibitor, every 3 weeks until unacceptable toxicity or disease progression. The objective response rate was 38%, with 10 patients experiencing complete responses and another 26 exhibiting partial tumor shrinkage. The benefit was generally durable, lasting 6 months or longer in 69% of responders, with a safety profile comparable to that reported previously for both drugs as monotherapies.

A randomized phase III trial evaluating the lenvatinib–pembrolizumab regimen against chemotherapy is currently enrolling patients. Continued approval of the drug combination in all three countries could rest on survival data from this 780-person study.

For now, Project Orbis will primarily consider expanding marketing authorization of new indications for previously approved agents. According to the FDA, future collaborative reviews may involve novel therapies—although because more proprietary information would be involved, sharing data becomes trickier. Future efforts could also involve the EMA, Japan’s