The Future Of CRISPR Screens In Efficient, Innovative Drug Development

CRISPR gene-editing technology has already done much to advance the cause of genetic screening in drug development. The ability to disrupt gene transcription on a genomic scale has proved invaluable in sharpening the early drug-development stages of target identification and validation.

And not a moment too soon, as recent publications indicate that new drugs supported by genetic evidence of disease impact are twice as likely to end up as approved medicines.¹

Target identification and validation, clarification of mechanisms of action or drug resistance, and biomarker identification are typical applications for established CRISPR screens. “It’s mainly functional screens for target discovery,” explains Vikram Wali, senior scientist and program lead, oncology discovery, for Janssen in the US.

“It could be in vitro or in vivo. It could be genome-wide or more focused. Then we try to generate appropriate models for assay development – that could be knock-in or knock-out strategies in cell-line models. We also do modifier screens, where you can evaluate biomarkers or mechanisms of action.”

At integrated-epigenetics specialist Constellation Pharmaceuticals, CRISPR screening is used at two stages of discovery: target identification and translational biology. In the latter case, “we’re looking for biomarker identification, combination strategies and resistance mechanisms for our existing inhibitors,” comments Andrew Conery, director of functional genomics.

The Joint AstraZeneca – Cancer Research UK Functional Genomics Centre employs CRISPR screening to address three primary challenges in therapy development, notes deputy laboratory head David Walter. The first challenge is identifying synthetic lethal interactions, such as PARP inhibition in BRCA-mutant tumors, which can potentially be exploited therapeutically.

The second is looking for drug-resistance mechanisms. The third is locating potential targets for combination therapies or biomarkers for patient stratification.

CRISPR Handicaps

Standard CRISPR screens, like most biological screens, do have some drawbacks. The basic principle, points out Carlos le Sage, manager, functional genomic screening at Horizon Discovery, is to “hit different gene
targets across a large population of cells. In that setting, it’s quite limiting in terms of the number of readouts we can measure.”

With readouts typically based on a single phenotype, such as growth or viability, there is a need for more detailed resolution of gene-mutated phenotypes that can be measured simultaneously. For example, as le Sage explains, researchers can only get so far by extracting transcriptomic messenger RNAs from screens and comparing these sample populations for variations induced by drug intervention.

“However, the information is again limited, because it’s based on cell growth as a first phenotype,” le Sage comments. “Sequencing pooled transcriptomes will only tell us a little more about very strongly expressed genes and changes in the pool of cells expressing those genes.”

It will not capture subtle but significant changes in gene expression. Also, “there’s no real possibility to link the changes in messenger RNA expression directly to an individual gene mutation introduced in a programmed fashion,” le Sage observes.

As Conery notes, the last few years have seen a number of ancillary technologies emerge to enhance the utility of standard CRISPR screens, such as algorithms for maximizing/minimizing on-target/off-target effects when designing guide RNAs for a screening library. “I think now the standard representation of guides per gene is four or at most eight, as opposed to the early days, when some libraries had 20 guides per gene,” he says.

There has also been considerable evolution in understanding issues around executing screens, such as maintaining population representation. “We know how many cells you need to start with, and what multiplicity of infection you need for viral transduction, so that when you select the cells, you’re not losing guide RNAs from your screen,” Conery explains.

From this platform of acquired knowledge, CRISPR screening has continued to evolve. New technologies that enable standard CRISPR screens to overcome some of their limitations, combined with new iterations of CRISPR, are finding their way into the drug development pipeline.

**Pooled Single-Cell CRISPR Screens**

One new iteration of the CRISPR platform is pooled CRISPR screens with a single-cell whole-transcriptome readout – known variously as Perturb-seq, CRISP-seq or CROP-seq.

With CRISPR screening and a single-cell RNA-seq readout, “we can actually measure the effect of one particular mutation on the whole transcriptome, and with a very high resolution,” le Sage elaborates. That provides “much greater understanding of the relationship between the genotype, the gene mutations made by the CRISPR–Cas9 reagents and the resulting phenotype in any screening assay.”

“You know what guide is in there and, based on the cell barcodes, you know what messenger RNA they express in that particular cell. You can monitor thousands of gene-expression changes.”

With greater resolution comes a need to know in advance which genes are worth querying. One preparatory step is to run a whole-genome CRISPR screen, then narrow down a list of promising candidates for the single-cell screen. An alternative is to select candidates for single-cell screening through a bespoke RNA-seq experiment, aimed at understanding the cellular response in, for example, tumor development.

So far, single-cell CRISPR screening has been used mainly as a validation tool to interrogate subsets of genes and responses. le Sage sees it as an important driver of patient stratification and personalized medicine in disease areas such as oncology, “especially in the context of the heterogeneity we see in tumors and between cancer patients.”

Single-cell CRISPR screening can “define the expression profile of cancer cells literally as they traverse the various stages of the disease,” he adds. “That helps to identify a treatment regimen for each individual step,
but also provide biomarkers to help understand what stage a particular tumor is at.”

For Horizon, single-cell CRISPR screening is “really the next level in pooled screening,” le Sage says. “You still get the overall population response, but now you can also see the effect at an unprecedented cell-by-cell resolution. So, it’s really possible to assess how a drug changes the gene-expression profile and understand the pathways that drive, say, resistance or sensitivity to a drug.”

**Primary Cell CRISPR Screens**

Another avenue for development is CRISPR screening in primary cells. “There is a lot of interest from the immune-oncology angle,” Walter notes. “We are looking at screens in primary T cells, as key mediators of anti-tumor function. The problem is that they are often dysfunctional in tumors. Being able to manipulate T cells to make them active again at targeting the tumor is a really powerful proposition.”

Primary cells “are more relevant clinically and closer to clinical systems,” Wali points out. As Sylvie Laquerre, senior director of biology and head of discovery solid tumor targeted therapy at Janssen in the US, says, “As we advance in drug discovery, we get more and more targets that might not have the best animal model to reproduce toxicology. We can start taking advantage of this technology to understand the effects of targeting some of those genes in the human system.”

**Scale And Cost**

None of these newer CRISPR iterations are universally applicable, of course, and they come with their own challenges. Foremost among these are scale and cost. “You can’t do single-cell CRISPR screens on a genome scale at the moment, because the sequencing cost is just prohibitive,” Walter comments. All the same, sequencing costs “are coming down and will decrease further,” he adds. “And there are targeted approaches focusing sequencing coverage on genes of interest, thereby significantly increasing the sensitivity of single-cell CRISPR screens.”

One more hurdle is navigating the huge volume of data generated by platforms such as single-cell CRISPR screening. “We definitely have to change our pipelines and implement an RNA sequence-based approach to understand the large data sets we’re getting,” le Sage acknowledges.

“But it’s relatively straightforward to query them. There are ways to normalize and standardize these data sets, and to see what clear outliers there are in those experiments.”

For some researchers, these issues put CROP-seq and related technology in the “wait and see” basket. “It’s just not there yet for drug discovery,” Conery feels. “And the challenge with the single-cell-based approach is that single-cell RNA-seq itself is still developing. Then CRISPR screening comes along, and you’re trying to work with two things that are still evolving.”

However, combining rapidly evolving approaches can contribute substantially to innovation in the CRISPR screening field.

**Appetite For Innovation**

There is indeed continuing appetite for innovation in CRISPR screening, such as better capability to multiplex approaches, combinatorial screening (targeting more than one gene in a single cell) and working in more patient-relevant systems like acquired-resistance models, organoids or patient-derived xenografts (PDXs).

“What we also want is technology that we might not best develop internally, like CRISPR in 3D, CRISPR in vivo,” Laquerre comments. “And CRISPR has some
limitations: there are a lot of big knock-outs. What would be good is a different degree of knocking out, as a small-molecule inhibitor would do.”

For Conery, screening in PDXs “would be really useful. If you could do it in a setting that’s obviously closer to the disease, then potentially you could even model that in a mouse. That’s as close to the human setting as you could get.”

Walter also believes CRISPR screens will move “more and more away from 2D cancer cell lines toward disease-relevant models and in vivo models.” Combinatorial screening “will also be very important,” he suggests. “There are a lot of redundancies in cancer, and ablation of paralog gene pairs can uncover genetic interactions that we don’t identify with monogenic CRISPR screens due to genetic buffering.”

For now, the future of CRISPR screening is assured, whether through the continuing viability of current applications or the continuous stream of new variations on the technology. “There’s a huge interest in these technologies,” Walter says. “It’s just such a clean system, compared with previous technologies like RNA interference. It’s much more robust.”

REFERENCE