PHARMACEUTICALS

A CRISPR path to drug discovery

Gene editing is quietly revolutionizing the search for new drugs.

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CRISPR–Cas gene editing, once considered arcane, is fast entering mainstream use in research. Most people with an interest in science have probably heard about the technique, which uses a combination of a synthetic guide RNA molecule and an enzyme (typically Cas9) from the bacterial immune system to edit DNA with unprecedented ease and precision. It is a flexible tool with a variety of applications. Most of the media interest in the CRISPR–Cas system has focused on its potential for treating diseases with a genetic basis. Yet CRISPR–Cas also has a big part to play in drug discovery, which could prove to be as important as its therapeutic use — if not more so.

In a comprehensive 2017 review, scientists from the University of California, Berkeley, including co-discoverer of CRISPR–Cas Jennifer Doudna, emphatically concluded that this type of gene editing is "ready to have an immediate impact in real-world drug discovery and development"[1].

Christof Fellmann, a biotechnologist and co-author of the review, explains that the ability of CRISPR–Cas to help identify target molecules will have a crucial impact on drug discovery. By using the system to deliberately activate or inhibit genes, researchers can determine the genes and proteins that cause or prevent disease, therefore identifying targets for potential drugs. CRISPR–Cas is also making it easier to create cellular and whole-animal model systems that precisely mimic diseases. This is enabling scientists to more accurately verify the safety and efficacy of drugs, which ensures that such models are better predictors of what will happen in clinical trials. As these uses are pursued, researchers are also refining and extending the capabilities of CRISPR–Cas to make it an even more powerful gene-editing tool.

"It makes everything easier," says Jon Moore, chief scientific officer at biotechnology company Horizon Discovery in Waterbeach, near Cambridge, United Kingdom. In March 2016, at an event at the Science Museum in London, Moore declared, "The targets we're finding with CRISPR–Cas9 are going to guide the drugs coming out in the 2020s." He stands by that assessment two years on. "If it's not right, then I'll be in trouble," he laughs.

A KNOCK-OUT TOOL

The mechanism that underlies CRISPR–Cas gene editing is relatively simple. A short strand of RNA, tailored to target a specific sequence of DNA, is linked to an enzyme that is capable of cutting double-stranded DNA. Cas9, the enzyme to which Moore refers, is the most widely used, but other enzymes are being explored. After the RNA and enzyme are delivered to the cell nucleus, the RNA binds to its complementary DNA sequence, acting as a guide for the enzyme that then chops the DNA. After that crucial cut is made, DNA-repair enzymes in the cell fix the break in a way that either disables or modifies the targeted gene — its activity can be turned up or down, mutations can be introduced, or sections can be inverted.

The simplicity of using guide RNAs to target any location in the genome is making gene editing accessible to many more researchers. "CRISPR–Cas has taken gene editing out of the hands of those specialists who are expert in complicated molecular biology," says Moore.

Researchers engaged in drug discovery are eagerly exploiting CRISPR–Cas to switch off — or 'knock out' — specific genes to see what they do. Methods of introducing such knock-out mutations have been in use since about 2000, but these earlier approaches, which rely on engineered enzymes to cut DNA, often only partially knock out genes, commonly produce unwanted effects on unintended targets, and lead to inconsistent results between similar studies. CRISPR–Cas avoids these deficiencies and, since rising to prominence in 2012, has made it straightforward to knock out genes of choice. "The difference is in the quality of information you can get," says Moore. CRISPR–Cas is better at knocking out the targeted gene more fully, as well as avoiding unwanted effects, which has made large-scale gene-function experiments much more reliable, he explains.

Knock-out screening to identify genes involved in drug resistance is fast becoming one of the most widely used applications of CRISPR–Cas gene editing in drug discovery. Researchers expose large numbers of cells to a pool of CRISPR–Cas systems carrying guide RNAs that target various genes. This allows them to generate and select individual cells that each have a specific gene knocked out. The cells are then exposed to chemicals or drugs of interest. Genes that confer resistance to drugs can be identified through cells that become sensitive to such compounds after the CRISPR–Cas treatment. These genes, or the proteins they encode, can then be targeted with other drugs to get around the problem of resistance.

Identifying genes that promote disease uncovers some obvious targets for drug development. The simplest candidate drugs bind to and interfere with the proteins encoded by these genes, rather than affect the genes directly. But more-subtle targets for drugs can be revealed by a better understanding of the importance of multiple genes and proteins, their interactions and their mutual regulatory effects. Many diseases, for example, arise when things go wrong in a regulatory pathway that involves a complex network of intracellular interactions. Using CRISPR–Cas to identify, with ease and accuracy, combinations of genes involved in these networks should offer a more sophisticated approach to treatment.

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Researchers are also using CRISPR–Cas and the DNA-repair processes of the cell to incorporate — or 'knock in' — selected sections of DNA. This can introduce mutations

that transform the protein encoded by the targeted gene, leading to beneficial effects that a drug could then be designed to induce more simply. Some variants of CRISPR–Cas systems can make changes that either inhibit or promote the activity of a gene without changing its actual function. Turning gene activity up or down is a subtler way of investigating the importance of genes and proteins that could be activated or inhibited by drugs to treat disease.

"CRISPR–Cas is enabling nearly unlimited genetic manipulation," says Fellmann, and it is bringing researchers much success. "We have already found exciting new targets using CRISPR–Cas technology," says Moore. He will not reveal what the target molecules are, but does say that his company's research involves mutations in "undruggable" tumour-suppressor and cancer-causing genes that other researchers have been unable to target.

MODEL MAKING

Cell and animal models of human disease are crucial elements of drug development. The initial stages of testing candidate drugs for efficacy and toxicity can rarely be done in people, for ethical reasons. However, many of the disease models that are available to researchers are far from perfect. The main problem has been the complexity — and therefore the time and expense — of building superior models for the huge variety of human diseases that exist. "In industry, speed and cost are as important as feasibility," says Fellmann. If it would take too long and cost too much to make a great model, a less perfect one might be preferred. Yet the developers of drugs would like to avoid such a compromise.

Both Moore and Fellmann agree that the simpler and more reliable gene editing made possible by CRISPR–Cas has enabled researchers to create models of disease more quickly and cheaply. "We can now, pretty much, change any gene in whatever way we want to mimic a disease," says Fellmann. He also emphasizes that the "surgical precision" of CRISPR–Cas gene editing means that little or no trace remains of the editing process. With older genetic-engineering techniques, extra changes to the DNA sequence can be left in or around the altered genes, similar to a surgeon leaving instruments inside a patient after an operation. The precision of CRISPR–Cas greatly reduces the chances of the gene-editing tool having an undesired effect.

WAYS TO IMPROVE

Fellmann and his colleagues are now trying to find and develop innovative versions of the existing CRISPR–Cas tools that might bring further flexibility and precision. Part of this effort is exploring other bacterial gene-editing systems, and alternatives to Cas enzymes have already been found[2] (see *Nature* **536,** 136–137; 2016). An enzyme known as Cpf1, for example, can cut DNA at sites to which CRISPR–Cas is unable to bind. Other such enzymes, including Cas13, can cut RNA — the intermediary between DNA and protein — rather than DNA. This opens up flexibility in the options for modifying the activity of genes, beyond their basic editing.

A more adventurous approach is to engineer the genes from bacteria that encode the enzymes used in existing CRISPR–Cas systems, to add extra abilities. For example, a team of researchers at the University of California, Berkeley, has added a binding site for the hormone oestrogen to the enzyme Cas9 (ref. 3). This demonstrates the possibility of subtly controlling the activity of the gene-editing system through an external signal such as the level of a hormone or its analogue. As an example of its utility in drug development, Fellmann says that the technique could be used to control the timing of gene editing to more closely mimic the timing of the effects of drug molecules in disease models. These engineering efforts are at an early stage, but should eventually lead to a range of innovative functions.

Drug development is a long process: it can take more than a decade for researchers to move from the discovery of a target molecule to the production of a clinically approved drug. So it could be some time before the first drugs to be developed using CRISPR–Cas gene editing hit the market. "But," says Fellmann, "people are already using it now, and in the long term it will definitely have a significant impact."

Jonathan Wrigley, associate director of the Innovative Medicines and Early Development Biotech Unit at AstraZeneca in Cambridge, offers a similarly confident outlook from big pharma. "We are applying CRISPR–Cas technology across our drug-discovery pipeline," says Wrigley. He says that teams at AstraZeneca have generated more than 100 disease models with the aid of CRISPR–Cas in the past three years, and are constantly finding ways to improve the technology. "It has proved transformative in the generation of cellular models to support drug-discovery projects," Wrigley adds. Engineered cell-based models with precise genetic modifications were previously rare in drug discovery, owing to the challenging and time-consuming techniques that were required to generate them. "The CRISPR–Cas technology has enhanced both the feasibility and speed of this process, thereby enabling such models to become integral tools in the early stages of our drug-discovery projects, in a manner not seen before," Wrigley says.

His is one of countless research groups throughout academia and the pharmaceutical and biotechnology industries that now use CRISPR–Cas tools in the search for drugs. Predicting when gene editing will bear fruit is difficult; the drug-development pathway is long and clinical trials are laced with uncertainty, no matter the tool. But with so much ongoing activity, Moore's prediction that CRISPR–Cas will transform drug discovery seems unlikely to get him into trouble. "There's been a massive investment in CRISPR–Cas by pharma," he says. "I am not alone." ■

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2. Murovec, J., Pirc, Ž. & Yang, B. *Plant Biotechnol. J.* **15,** 917–926 (2017).
3. Oakes, B. L. *et al. Nature Biotechnol.* **34,** 646–651 (2016).