The CRISPR revolution arrives in immuno-oncology

CRISPR-cas9 technology is having an exciting impact on drug discovery as a whole, but more specifically CRISPR presents enormous opportunities in targeted screening for immuno-oncology research.

By Helen Stewart-Miller and Thomas Hope

Clustered regularly-interspaced short palindromic repeats (CRISPR) were first detected in the *E. coli* genome in the 1980s, and then in the 1990s in other bacteria and archaea. While this reoccurrence in many organisms hinted of its importance, it was not until the turn of the century when the similarity between these intriguing repeating sequences and fragments of virus and plasmid genes was observed. After that, the understanding of CRISPR's role as a prokaryotic defence mechanism started to blossom.

Within a few years, researchers had harnessed CRISPR and the associated Cas9 enzymes, giving rise to a powerful gene editing technology that would go on to truly revolutionise the research arena in a diverse range of fields, from biomedical science to crop breeding.

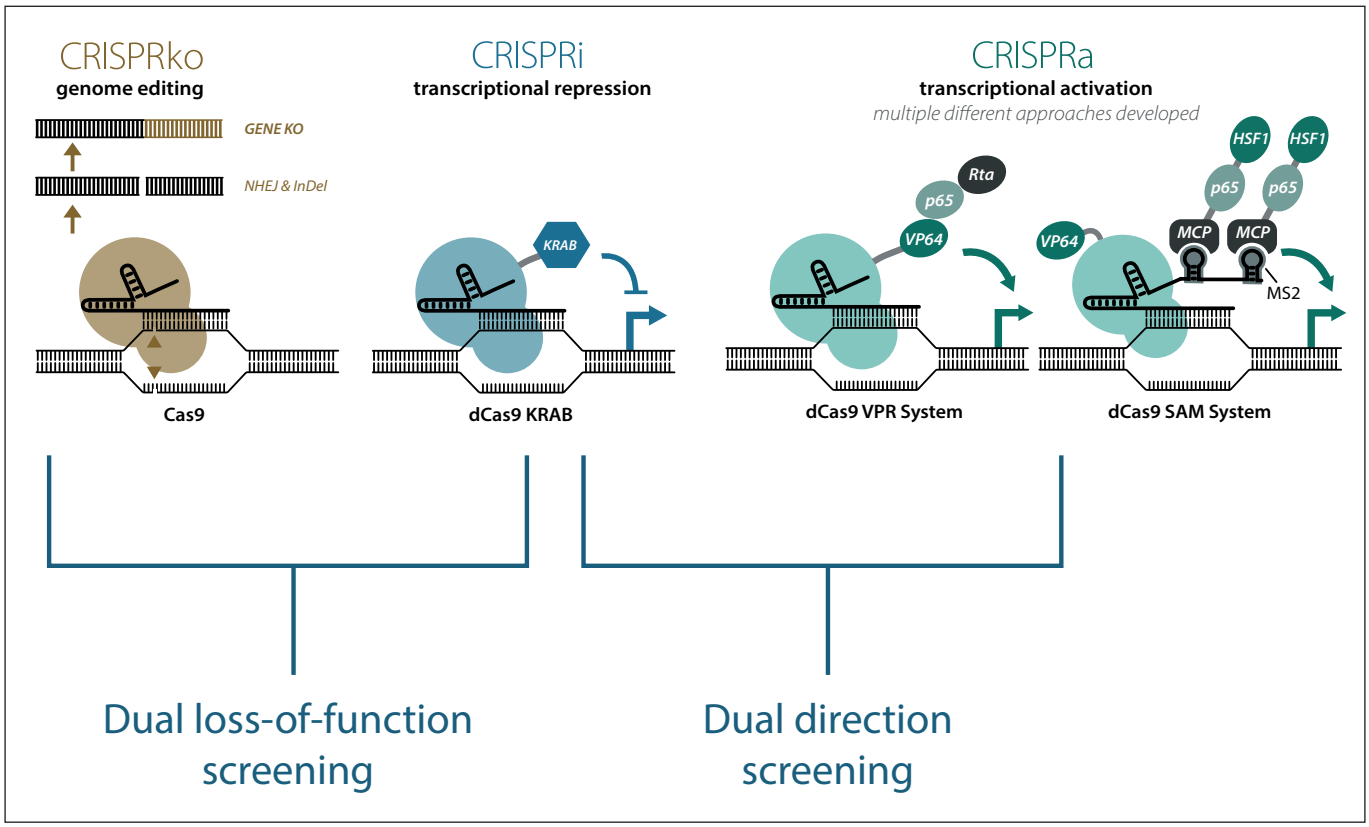
Drug discovery is one area where CRISPR-Cas9 technology is now having a profound impact, and it has a particular suitability for immuno-oncology. With the help of several experts from the cutting-edge of the industry, this article looks at this new trend, and its exciting implications for new drugs and therapies that could bring radical improvements for patient care.

A real boost for the immune system

The search for effective cancer treatments is particularly complex as the disease is incredibly diverse and highly individualised. In addition, cancerous cells can suppress the immune system to avoid detection, creating further challenges for researchers. However, scientists are looking to harness the power of the immune system. The fundamental aim of immuno-oncology approaches is to equip the immune system with the necessary tools to overcome these evasion mechanisms, and to stimulate a direct response towards the unique antigens expressed by cancer cells.

"The switch from focusing on the molecular basis of the tumour to the cellular basis of the immune system is having great benefits for patients," said Dr David Ferrick, Associate Vice-President, Cell Analysis at Agilent Technologies. "Rather than trying to identify a unique molecular pathway of the pathogenic disease itself, researchers are now intervening to support and re-engage the immune system, because we know that most cancer patients have an intact immune system which is the most natural and specific system for targeting chronic diseases."

"Recent advances in immuno-oncology are guiding the way towards precision medicines with high specificity towards the individual disease," said Dr Emily Leproust, CEO and Co-Founder at Twist Bioscience. As a result, hopes are high that research in this field will provide the next generation of cancer drugs. "The benefits of such precision medicines would be huge: more effective treatment, combined with less side-effects, leading to improved patient outcomes," continued Leproust.



CRISPR's knockout capabilities

The benefits offered by CRISPR in terms of identifying target molecules are revolutionising the drug discovery field. The gene-editing technique is providing researchers with a precision tool with the capability to activate or inhibit genes. In this way, researchers can identify disease-causing proteins and genes, which can then be targeted with drug candidates. "The target specificity of CRISPR-Cas9 is impressive," explained Dr Gregory Alberts, Global Subject Matter Expert at Lonza, "allowing one to examine in detail and modify specific pathways and cellular processes at will. Furthermore, the effects of complete gene knockout can be viewed, rather than partial reductions in gene expression."

In the drug discovery context, these capabilities are transforming functional genomic screening, providing a better means to link genotype to phenotype. "The aim is to understand which genes are driving specific disease phenotypes," said Leproust. "This could be the transformation of cells from normal to malignant or, in the case of immuno-oncology, it could be identifying the genetic aberrations allowing the cancer to evade the immune response, and which scientists might subsequently aim to address."

CRISPR screening for immuno-oncology drug discovery builds on a number of technologies that were already established in the functional genomic screening field, while overcoming the limitations of earlier methods such as the use of pooled lentiviral libraries and shRNA. It enables thousands of genes to be modified and their function assessed in a single experiment. Not only does this decrease the likelihood of off-target effects, and therefore false positives, but it also offers the improved ability to fully silence gene expression, resulting in a similar decrease in the chance of false negatives.

This level of precision and ease-of-use enables the identification and validation of novel drug targets, while facilitating the study of the underlying causes of disease. Excitingly, CRISPR is also being used by researchers to identify the effect of genetic mutations on drug activity, patient responsiveness and resistance.

High-throughput CRISPR-Cas9 ribonucleoprotein delivery can obtain very high levels of gene knockouts (in the range of 85-95%), which is a huge improvement on previous immuno-oncology methods, and curtails specific functional gene expression at the source. "CRISPR truly represents the next evolution in targeted screening for immuno-oncology research," said Dr Gregory

Figure 1
Illustration of the three CRISPR screening mechanisms that can be utilised during drug discovery and development.

Figure 2
Lonza's 384-well HT Nucleofector™ System is an independent platform for high-throughput Nucleofection™ experiments and is perfectly suited for automated screening applications with maximum speed and reproducibility

Alberts at Lonza. "It provides clean analyses with minimal background or residual target activity. This means it becomes significantly easier to spot more subtle phenotypic variations."

The ability to identify specific pathways in disease progression or amelioration, and test the different components of these pathways for effectiveness in phenotypic responses, dramatically expands the potential choices for druggable targets. This precision can streamline the immuno-oncology drug discovery process and lead to a more efficient and targeted application of resources.

One of the biggest therapeutic advantages of CRISPR is for personalised medicine[1]. Determining which genes promote or reduce the activity of a particular immuno-oncology therapeutic enables the stratification of a patient population. In other words, "if someone has a particular gene, you can give them targeted immuno-oncology therapeutics that will ultimately be more effective, rather than going for a more broad-spectrum approach and playing the odds," commented Catherine Tarrade, Product Manager at Horizon Discovery.

Commercially available tools and technologies

Several manufacturers operate at the cutting edge of the CRISPR immuno-oncology field, offering a profusion of products and services to suit all requirements:

Horizon Discovery has developed three CRISPR screening mechanisms for researchers: "The most common method is CRISPRko (knockout) which completely inactivates a gene. The other two either activate (CRISPRa) or inhibit (CRISPRi) the expression of a gene without altering the DNA," explained James Goldmeyer, Product Manager at Horizon Discovery. These screening platforms can significantly advance drug development programmes by providing the highest levels of quality and confidence in screening results (Figure 1).

Horizon Discovery has expertise and experience in gene editing as well as cell culture providing researchers with highly-tailored solutions where they can select the relevant cells for their particular disease model, and make edits to any immune cell or cell line using CRISPRko. "We are currently the only company offering pooled and arrayed CRISPRko screening in human primary immune

cells," said Dr James Goldmeyer at Horizon Discovery.

In addition to screening services, Horizon Discovery also offers CRISPR-Cas9 reagents for clients who wish to perform follow up studies or reproduce data.

While CRISPR-Cas9 technology is firmly established as the best in class for screening, it does not mean that the screening of immune cells is without challenges. Immuno-oncology screens should ideally be performed in primary immune cells, which are in limited supply because they have to be isolated from patients.

To address this challenge, scientists may choose not to screen entire genomes, but rather a targeted subset of the genome. However, this requires full confidence in the library used: missing or incorrect guides can result in false negatives and a wasted screen. For these reasons, Twist Bioscience has created Oligo Pools for CRISPR screening, which offer unparalleled uniformity and representation, with an industry-leading low error rate in synthesis.

"Oligo Pools maximise efficiency for screens in very precious samples and also provide unlimited customisation in sgRNA library size, made possible with our unique silicon-based DNA synthesis platform. They offer a high degree of flexibility with regard to library design, with no minimum or maximum oligo number. This means it is possible to work on any scale, from a very small subset of genes all the way up to a genome-wide library with 10 or more guides per gene. This allows scientists to use precious or scarce samples, such as primary immune cells, and feel confident that they are maximising the chances of screening success," said Dr Emily Leproust at Twist Bioscience.

For CRISPR-Cas9 screening, Synthego, a leading provider of genome engineering solutions, also offers screening libraries: "CRISPR-based technology has fast become the standard for loss-of-function high-throughput screens," said Dr Jason Steiner, Chief Strategy Officer at Synthego. "Our arrayed multi-guide sgRNA libraries provide superior screening capabilities over RNAi and CRISPR pooled libraries, enabling a better understanding of gene function and smarter identification of drug targets."

Synthego's libraries for arrayed whole human genome CRISPR-Cas9 screening are supplied ready-to-transfect in any human cell type. They provide comprehensive coverage of the human genome, with several guide RNAs selected per gene. In addition, the latest algorithms are used to optimise knockout efficiency. These qualities make them ideal for the assaying and analysis of complex oncology targets.

One of Lonza's leading products in the CRISPR screening domain solves the problem of primary cell transfection. Primary cells are often the most relevant cell type for large-scale CRISPR screens, but the difficulty associated with their transfection can limit their utility.

"Lonza's Nucleofector™ Technology can help overcome these difficulties," said Dr Gregory Alberts at Lonza, "due to its superb capabilities for the efficient transfection of CRISPR substrates, especially the Cas9 RNP and mRNA into primary cells, making it an excellent choice for non-viral CRISPR applications."

In addition, the Nucleofector™ Platform offers powerful and versatile high throughput options such as the 96-well Shuttle™ Device and the 384-well HT Nucleofector™ System, both of which are suitable for integration into robotic platforms, facilitating the use of Nucleofection™ in arrayed CRISPR screens (**Figure 2**).

Lonza is also well known as a provider of ethically-sourced and validated primary cells for large-scale high throughput CRISPR knockout screening in drug discovery. The company supplies more than 100 different primary cell types, from both healthy and diseased donors.

Drug discovery and gene-editing communities are equally well-served by Agilent Technologies, which offers its well-established CRISPR platform as part of the Agilent SureGuide portfolio. The platform performs stable, on-target CRISPR editing of immune cells, allowing the engineering of highly-specific cell therapies.

"The chemistry and components used to create the template-guiding RNA molecules are key to delivering accurate and stable edits," said Dr David Ferrick at Agilent Technologies. "We have the purest and most stable reagents for RNA synthesis; as a result, we can make the longest template RNAs for gene editing." Moreover, these components can be modified so that the various base pairs can enhance the on-target and minimise the off-target effects, as well as improve the stability of RNA molecules.

Agilent Technologies also manufactures RNA for research, from clinical to Good Manufacturing Practice grades. One of the benefits of this is that the source materials for editing guide RNAs do not have to be revalidated and reoptimised at different stages of the R&D process.

Further improvements in a rapidly changing field

Given the continually-evolving nature of CRISPR-Cas9 and its application in the immuno-oncology field, new tools and technologies are transitioning from R&D to reality on a regular basis. While CRISPR-Cas9 has without doubt transformed gene editing through its ease-of-use and precision, that does not mean there is no room for improvement.

A promising development in this regard is prime editing, which uses a modified Cas9 protein, known as a nickase, that does not introduce a double-stranded DNA break (DSB), but instead merely nicks the DNA. In this approach, the Cas9 molecule is fused with an additional functional molecule, in this case, a reverse transcriptase. This new complex nicks DNA at an appropriate target site, converts the RNA into the new DNA template, and inserts it into the genome.

Recent studies show that prime editing is highly accurate and obtains much higher integration frequencies than conventional CRISPR. It could represent a new way of repairing or replacing DNA in cells and facilitate the clinical use of CRISPR for the treatment of genetic diseases with fewer off-target effects.

Lonza Nucleofector™ Technology is ideal for these applications. "Nucleofection™ technology works very well for the transfection of the CRISPR Cas9 RNP into cells, and it is likely that the prime editing complex will also transfect well. Target validation would therefore be more straightforward, regardless of the choice of substrate," commented Dr Gregory Alberts at Lonza.

The importance of minimising DSBs, which have an impact on genome stability, was echoed by Catherine Tarrade at Horizon Discovery: "We are currently exploring the potential of base editing, a novel genome editing method to directly install point mutations into cellular DNA or RNA without making DSBs, which utilises CRISPR components." This technology could be incorporated into next-generation gene editing platforms to enable the development of novel therapeutics that rely on the engineering of a patient's cells, either in the body (gene therapy), or externally before transplanting them back into the patient (cell therapy).

Researchers at Twist Bioscience are looking towards the modification of Cas9 as an area that

holds great potential for advances in immuno-oncology research: "The Cas9 protein continues to be adapted, which means that the types of screens that can be performed are continuously expanding," commented Dr Emily Leproust at Twist Bioscience. "The application of these orthogonal approaches will help build confidence and statistical significance in identified targets for drug development."

Another emerging trend is an increasing focus on cell-centric studies, from the targeting of the immune system and cellular networks, to the engineering of cells and the microenvironment in which cells interact. There is a need to understand and model how cells co-operate in their native environments, to follow them for longer periods of time and to have more relevant systems for modelling intervention. "To adapt to these emerging requirements, we are focusing on live cell solutions that can 'follow' the biology for days, rather than acute or end point assays. To do this the solutions must be kinetic, non-perturbuing or label-free detection and digitally savvy to handle the data processing, visualisations and analyses. Agilent Technologies has assembled innovative products that are capable of providing the necessary qualities that will be critical for success in this cell-centric era of life sciences," said Dr David Ferrick at Agilent Technologies.

According to Dr Jason Steiner at Synthego, one of today's fundamental challenges in research and therapeutic development is the lack of high-quality, relevant models for translational medicine. "Our work aims to accelerate the discovery, research and development of therapeutics through the creation of cellular models that reflect the genomic variation of disease. At Synthego, we focus on providing products and services that leverage the power of CRISPR-based gene editing to enable scientists to create models for thousands of disorders, including oncology diseases."

Conclusion

The benefits of CRISPR screening are clear and the technology is having a particularly dramatic impact on immuno-oncology. It enables researchers to engineer cells with extremely high fidelity, facilitating the editing of exact sequences of interest in cells or pathways, in precisely the same manner, to up-regulate or down-regulate, to add or replace sequences. These capabilities provide a unique opportunity to probe gene function, gene-protein interactions and immuno-oncology therapeutic mechanisms of action. CRISPR screening also facilitates the understanding of which genetic pathways are influenced by immuno-oncology therapeutics, and helps improve therapeutic efficacy and patient stratification to ensure immuno-oncology therapeutics are effective.

Given that the pharmaceutical industry is striving to improve efficiency at all stages of its pipeline, scientists must have access to the best possible screening tools to ensure the highest levels of confidence in drug development targets. This efficiency drive is fuelling the development of ever-increasingly precise and effective tools, such as prime and base editing, which will complement and may even supersede existing CRISPR gene-editing tools. DDW

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Reference

1 CRISPR screening service drives drug discovery. A conversation with Dr James Goldmeyer, Product Manager, Horizon Discovery.