BASE EDITING: An Innovative Player Driving Cell And Gene Therapy To New Frontiers

One of the fastest moving fields in biology today is genetic engineering. The techniques used to modify genetic sequences, whether for research purposes or therapeutic intervention, are evolving at pace.

The emergence of CRISPR–Cas9 as a gene editing technology is in part responsible for this injection of speed. CRISPR-Cas9 is now well established as part of the drug development pipeline, from target identification and validation to potential cell-based therapeutics. While easy to use and widely adopted, though, CRISPR-Cas9 and other nuclease-based gene editing platforms are not without limitations.

At the heart of nuclease-based gene-editing systems is their ability to generate double-strand breaks (DSBs) in the genome. As the cell fixes these breaks, error-prone DNA repair mechanisms may be employed. These mechanisms can lead to insertions or deletions (indels) of random DNA base pairs that can result in disabled gene expression.

Although very effective at inactivating genes, the random generation of indels can give rise to deleterious off-target effects, such as chromosomal translocations and large genetic rearrangements. These could be an issue in a therapeutic context.

Gene Disruption

There is no doubt that CRISPR-Cas9 has revolutionized the field of gene editing. But in terms of the mechanism by which genes are disrupted, CRISPR can be considered "a relatively 'blunt' tool for knocking out genes", comments Ceri Wiggins, Manager of the Cambridge R&D team at Horizon Discovery Group. "By inserting or deleting DNA base pairs, the result is disruption of the reading frame and a transcript that no longer codes for a functional protein," Wiggins explains.

These unwanted effects are all the more problematic when the goal is to knock out a number of genes in combination – for example, in broadening the use of chimeric antigen receptor (CAR)-T cells. Multiple gene edits are likely to be needed if the aim is to prolong the survival of CAR-T cells in the patient, particularly in the immune-suppressive tumor

microenvironment often encountered in solid tumors.

Moreover, gene edits will be necessary at multiple sites to build in cloaking mechanisms that prevent CAR-T therapies from being challenged by a patient's immune system, cautions Ben Taylor, Associate Director at AstraZeneca. "You can do that iteratively, one gene knockout at a time," Taylor adds. "But really for developing a therapeutic, you would ideally make multiple mutations in one go."

With multiplex gene editing, "every time you break the DNA, you increase the chances of a large-scale rearrangement," Wiggins elaborates. "And the more edits you do, the more unpredictable that becomes." Speed is also of the essence, particularly with autologous CAR-Ts, "where there is obvious time pressure to isolate, edit and re-transfuse patients with their edited T cells".

As Taylor points out, "it all comes back to specificity and control: being able to go in with an entity that will create a specific change, which you know will occur at a high rate of efficiency." Referring to the ability to introduce new DNA sequences by adding exogenous repair templates, Taylor continues: "With Cas9, you can do it, but there is still a lot of work needed to improve how efficiently and precisely this can be achieved".

Base Editing

A more direct means of accessing that precision is through base editing, a recent iteration of CRISPR-Cas9 editing that uses a modified version of Cas9 that only 'nicks' rather than cuts DNA, linked to a deaminase enzyme capable of converting one base into another. This accomplishes gene corrections through single base changes, or gene knockouts by introducing stop codons, while minimizing the potential for DSBs.

"Base editors still make use of short RNA guide sequences to direct an editing complex to your target of interest," Wiggins notes. "But instead of disrupting genes via the creation of indels, a specific base pair is changed through the action of the nickase, the deaminase and parts of the cell's DNA repair mechanisms."

In January 2019, Horizon formed an exclusive partnership with Rutgers University in the US, to pursue further development of a novel base editing technology invented by researchers at the university. A year later, Horizon announced it was seeking additional partners to take the Rutgers base editing technology forward for therapeutic, diagnostic and services applications.

"At that stage, the system was very much an R&D tool, and we've spent the past 18 months really characterizing it, developing the system and transitioning towards something that can be applied to cell or gene therapy," Wiggins says.

Horizon has sought input from AstraZeneca, a long-term partner for CRISPR-based products and services, to expedite development of the technology. "That's on several levels," Wiggins explains. "It's about making the platform as efficient as it can be. It's also about making sure we have a really good understanding of a base editor's off-target profile, and what we can do to make it better."

Advantages, Applications

The enhanced precision of base editors, together with reduced risk of off-target effects by not causing DSBs, provide several advantages over standard CRISPR-Cas9. Multiplex editing to make more efficacious CAR-T cells is one area where base editing could have the edge over nuclease-mediated editing in knocking out several genes rapidly and safely within a patient's cells.

Base editors are also ideally suited to addressing monogenic diseases that require single base changes, such as sickle-cell anemia, beta thalassemia, α1-antitrypsin deficiency or Duchenne muscular dystrophy.

AstraZeneca has been exploring base editors as potential therapeutic tools in α1-antitrypsin deficiency. "We've introduced the human gene into a mouse model, and we've been doing proof-of-concept studies with CRISPR and base editor to directly repair the mutation that drives the disease," Taylor explains. "We've been able to demonstrate very clearly that if you can knock out or mutate that gene, you can get a significant therapeutic effect in animal models of disease."

Further down the line, the technology may be applicable to high-burden medical conditions. Ultimately, genetic alterations and therapies "could be used as effective treatments for a great many diseases", Taylor believes.

Wiggins cites a recent announcement by Verve Therapeutics, detailing a study where base editing was used to disrupt genes associated with heart disease in non-human primates. "They were able to deliver the base editor directly into the liver. And this 'one-shot' base editing led to measurable decreases in the levels of triglycerides and cholesterol circulating in the blood, comparable to statins."

New iterations of CRISPR-Cas9 also mesh strongly with the 5Rs research framework adopted by AstraZeneca in 2011. "We've been investing heavily in understanding the right targets for a particular dis-

ease indication," Taylor says. "To support that work, we've been building up pooled and arrayed CRISPR screening technologies."

AstraZeneca is now "starting to move into base editing as well, to create new ways of screening preclinical models," Taylor continues. "We're using this to identify the sorts of targets we wouldn't previously have identified, because they're not particularly well characterized, or they have no known association with a certain disease."

Expanding The Toolbox

None of this implies that base editing will replace CRISPR-Cas9. Rather, Wiggins suggests, it is expanding the available toolbox of gene-editing technologies to continue driving innovation in both the screening and therapeutic spaces.

At AstraZeneca, the approach to any given disease indication is "modality-agnostic", Taylor emphasizes. "We've been working very hard over the last four or five years to understand how we can use therapeutic gene editing and Cas9-based therapies to treat patients and cure diseases." With more tools and specialization, "we've brought base editing into the R&D space and used that for model development, getting a really good understanding of what its behaviours are", he notes.

CRISPR-Cas9 is still "a fantastic tool", Taylor stresses. "You can pretty much target anywhere you want in the genome. There's been a lot of work across the globe to understand its safety, improve its fidelity, reduce off-targets, and expand its ability to target new areas of the genome that you couldn't target before, with all the different protospacer adjacent motif, or PAM, variants."

With base editing, "you need to limit it to the genetic changes it can make" Taylor points out. "And it is limited by the variant of Cas9 used. Your standard Cas9s have their PAM requirements, so you can only see certain regions of the genome. Within those regions of the genome, they can only make certain mutations at certain sites."

"Using the increasingly sophisticated tools available to us now, you can understand what off-target editing you're going to get. With that knowledge, you can go through iterative cycles of redesign to overcome these issues."

Base Editing Challenges

There are other challenges to overcome. "As with CRISPR, you have off-target effects associated with Cas9 itself, if you misdirect the complex to different parts of the DNA," Wiggins observes. "The deaminase enzymes also have some inherent off-target activity that must be considered."

Taylor cites reports of base editors "erroneously editing the transcriptome". The technology will benefit, though, from the considerable efforts made to enhance the safety profile of CRISPR-Cas9, he notes. "Using the increasingly sophisticated tools available to us now, you can understand what off-target editing you're going to get. With that knowledge, you can go through iterative cycles of redesign to overcome these issues."

Delivering base editors as therapeutics is another significant obstacle. "It's how you get these components into a cell without damaging the cell or the person, and doing it in an efficient way," Taylor says.

Base Is The Place

Meanwhile, the gene-editing toolbox will continue to expand rapidly. Next-generation technologies, such as prime editing, already offer more flexibility than base editors in terms of being able to cover the full spectrum of monogenic mutations with known clinical impacts. However, that potentially comes at the cost of reduced efficacy and higher indel rates, Wiggins says.

In the meantime, base editing is the place to be. "We've made a lot of progress with base editors in the past few months," Wiggins comments. "We have a very good relationship with AstraZeneca, and we're confident that their broad knowledge in genome editing is really going to help catapult forwards what we're doing with base editing."

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Benjamin Taylor is an Associate Director in Discovery Sciences, AstraZeneca, leading the Cell Biology team in Cambridge UK. His team develops novel cell models and assays to help uncover disease biology and drive drug discovery projects. Much of this work is focussed on using and driving innovation in CRISPR genome modification technologies. Benjamin is an expert in cellular engineering, transcriptomic and next-generation sequencing technologies. Prior to joining AstraZeneca, he obtained a PhD in molecular biology at Imperial College London, UK, before joining the MRC Laboratory of Molecular Biology, Cambridge UK, as a Postdoctoral fellow focussing on mutational mechanisms in cancer.

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Ceri Wiggins is an R&D manager at Horizon Discovery. Upon joining the company in 2011, she played a significant role in establishing new assay capabilities that are now a key offering in the company's services portfolio. Today, Ceri leads a scientific team focused on the development and commercialization of a novel base editing platform, as well as supporting multiple client, CRISPR-based, target validation programmes. Before joining Horizon, Ceri worked at the MRC National Institute for Medical Research in London under Dr. Steve Ley, having gained her doctorate at the University of Cambridge.