An alternative CRISPR-Cas nuclease for **biotech & pharma**

Dr Michael Krohn and **Dr Dirk Sombroek** of **Brain Biotech** introduce a specially developed nuclease, which is the first of a new class of genetics-based cancer therapies tool

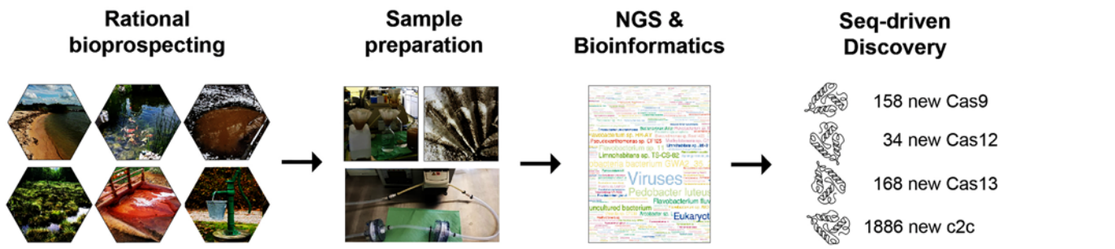

Figure 1 - Workflow that led to identification of more than 2,000 novel Class 2 CRISPR systems

The discovery of gene technology's most precise tool CRISPR (Clustered Regularly Short Interspersed Palindromic Repeats) won a Nobel Prize in 2020. CRISPR is a gene-editing technology originally derived from prokaryotes. What makes it so unique and fascinating is the ease and versatility of its application in life sciences, allowing for the targeting or editing of almost any nucleotide sequence in a given living organism.

This still sounds crazy to molecular biologists, since they dreamed of it for decades. Today modern sequencing and CRISPR methods can be used to read, re-write and control changes in the code of life, making it probably as meaningful as the historic Gutenberg event of book printing.

In this technology, CRISPR-encoded enzymes known as CRISPR-associated (Cas) nucleases are programmed with a small ribonucleotide stretch - a guide RNA (gRNA). This allows the complex of nuclease and gRNA to scan, identify, bind and cut at the site of the gRNA's pendant sequence in the genome of a cell.

Today CRISPR is pervasive in the life sciences and is used in diagnostics and agriculture or for more sustainable microbial production in industrial applications. It is also used *ex vivo* to design or correct patient cells for re-introduction and for curative clinical goals, and it has entered clinical trials and therapies.

However, one facet of CRISPR's inherent potential is still missing: applying it as a smart, programmable anti-cancer cytotoxic drug. The vision is to rely on a patient's genomic make up to selectively destroy cancerous cells identified and targeted by its malicious genomic cancer sequence code. Here we describe such a novel mode of action (MoA) that we believe has the potential to become a programmable anti-cancer drug for oncotherapies.

Diversity is key

The IP landscape for CRISPR applications has been described at best as complex, at worst 'a nightmare'. The major licensors of CRISPR technologies around the Cas9 and Cpf1 (Cas12a) nucleases, BROAD/MIT and ERS Genomics/Berkeley, remain in a multi-year legal battle.

As a result, interested parties often either have to license CRISPR from both institutions or at least face a painful and time-consuming process to identify the right licensor or even the right patents for their business. In addition, the licensing fees are prohibitive for many applications. This was our main motivation for identifying and developing an independent and proprietary CRISPR IP position.

The prime selection parameter for Cas nuclease development was to achieve freedom to operate (FTO). In addition to legal hurdles, there are also limits on what can be done with CRISPR, for which some of our technologies can provide game-changing competitive advantages. Here we focus on G-dase E*, previously known as BEC. This has a novel MoA and is significantly different to classical CRISPR variants like Cas9 or Cpf1. In terms of G-dase E the European Patent Office granted a new patent in September 2023 covering Brain Biotech's G-dase E nucleases as a composition of matter1 and still pending in other jurisdictions.

Advanced metagenomics approach

From a scientific point of view, Cas nucleases are incredibly powerful tools. When the first publications on CRISPR Cas nucleases appeared, Brain immediately began testing and adapting the system for its own basic research.

The functionality was amazing and allowed for new technological applications in the field of biotechnology. However, as explained above, the IP and licensing situation was not acceptable. Therefore, the company initiated a programme using an advanced metagenomics approach to identify new Cas nucleases with the aim of gaining FTO.

Figure 1 shows the workflow. A Nagoya-compliant collection of appropriate samples from carefully chosen habitats, followed by the enrichment of specific microorganisms with the highest potential for novel CRISPR systems, then complete DNA extraction and deep next-generation sequencing. Together with a partner, we screened approximately 100 million genes in a bioinformatics pipeline.

Finally, two enzyme families with different activities were identified. The sequences of the first originate directly from the metagenome (G-dase M), while the enzymes of the second, G-dase E, have been heavily engineered using advanced methods (Figure 2). G-dase E in particular stands out from the crowd as its unusual capabilities open up a whole new range of applications.

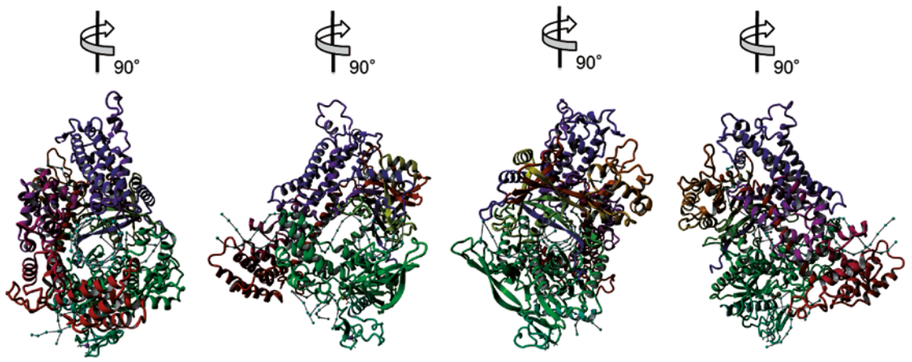

Figure 2 - Homology modelling of G-dase E nuclease

Novel enzymatic mechanism

The Akribion Genomics team has since developed these novel Cas nucleases, known as G-dases, with strong activity in various organisms, including bacteria and mammalian cells. The enzymatic mechanism of G-dase E differs significantly from that of previously known CRISPR systems.

Classical Cas nucleases like Cas9 or Cpf1 target DNA and usually create a double strand break in a genomic DNA sequence that is defined and bound by the so-called spacer sequence of the gRNA. G-dase E, like Cas13, instead targets RNA by binding to a sequence of a RNA biomarker in the cell.

However, upon binding it gets activated and shreds down all RNA- and DNA-type nucleic acids through a constitutive collateral activity, whereas Cas13 only depletes RNA. This mechanism has been described in our patent application and a similar MoA has been published for Cas12a2 nucleases in prokaryotes.[1,2]

The fundamental difference in the MoA enables G-dase E to kill mammalian cells when activated by a targeted RNA biomarker, since these cells cannot cope with the complete destruction of RNA and DNA. This particular ability has not been demonstrated for other Cas nucleases.

Attempts by Kwon *et al.* to test and qualify Cas9 as a programmable cell killing tool revealed that multiple genomic double strand breaks are required for such an application, disqualifying them as tools for cell depletion. Hence the complexity of providing multiple guide RNAs (for extreme multiplexing) and identifying multiple DNA biomarkers is preventive for therapeutics.[3]

However, since G-dase E is a Cas nuclease, its activation is programmable and should be adaptable to the genomic make-up of a cell by employing one designated gRNA. Thus, one RNA biomarker should be sufficient to kill a cell. Based on this concept, G-dase E, which uses guided toxicity in a cellular context based on a known cellular RNA biomarker, could become a novel class of drug that could even become a personalised drug based on a patient's genetic make-up in the future.

Potential in cancer

In general, G-dase E's novel enzymatic mechanism opens up applications in therapeutics and diagnostics. It could also be highly synergistic with other genome editing applications by depleting unedited cells, which could be beneficial, for example in *ex vivo* cell therapies. However, there is a lot of evidence that the 'guided toxicity' mechanism described above or the targeted elimination of cells based on an RNA biomarker has the greatest potential in cancer therapies.

The vast majority of cancer indications are characterised by genetic alterations, such as oncogenic fusions or mutations, when compared to healthy cells. These changes lead to the appearance of RNA molecules with unique sequence motifs that can be targeted and addressed by G-dase

E (Figure 3). As a prerequisite, healthy cells have no such RNA motif and the nuclease should remain silent. In this context, G-dase E represents a completely new class of drugs.

Significant progress has been made in the development of cancer drugs, but there is still room for improvement. Cancer cells are known to develop resistance mechanisms that cause certain treatments to fail. Furthermore, current cancer drugs often have off-target effects that can affect treatment response and quality of life.

In addition, cancer is a complex disease with multiple molecular and genetic alterations leading to extensive heterogeneity - a challenge for conventional treatments. Finally, therapeutic approaches with greater flexibility and rapid adaptation to new tumour markers make it possible to envisage personalised medicine, where treatment is tailored to individual patients based on their specific tumour characteristics and genetic profile.

The current gold standards in the field of guided toxicity are antibody-drug conjugates and cell therapies like CAR-T: highly toxic and specific, but with a target range limited to cell surface markers or displayed epitopes. A major drawback is a lack of flexibility to address a new target. A complete drug development programme must be initiated for each new marker or indication.

Other CRISPR-like nucleases provide high specificity and flexibility, but are not toxic enough to kill cells. G-dase E offers the combination of specificity, toxicity and flexibility. Due to the molecular mechanism of target recognition, this nuclease is extremely precise and at the same time highly toxic to cancer cells.

Targeting RNAs directly

Direct cell killing by DNA degradation may have a chance to overcome some of the escape mechanisms of cancer cells since G-dase E uses new biomarkers and does not rely on the typical pathways to kill cells. This is why there is enormous potential, especially in combination with established therapeutic approaches.

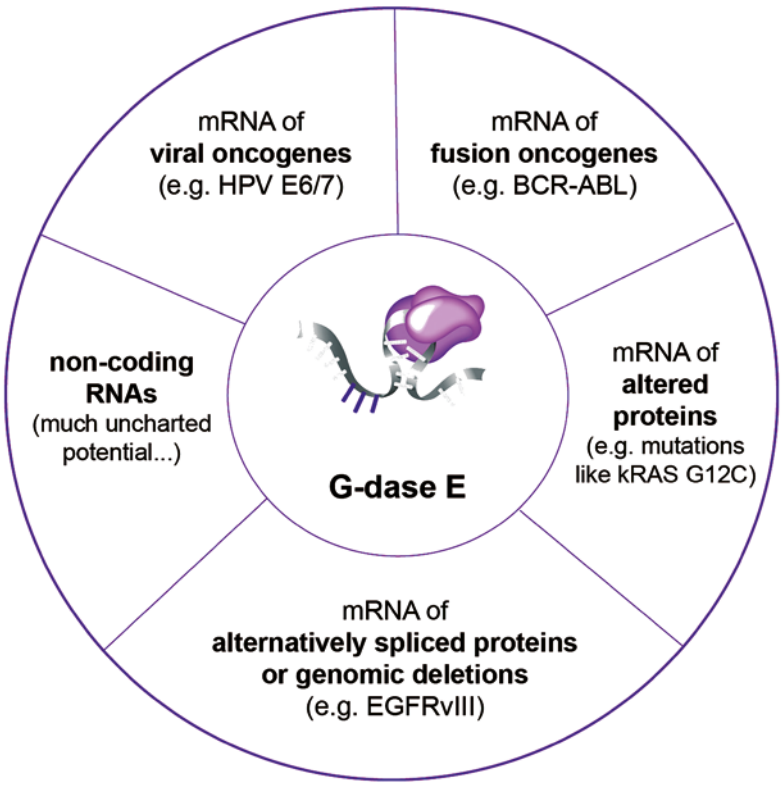

Figure 3 - Addressable molecular target space for cytotoxic nuclease G-dase E

G-dase E has the potential to target cancer-specific RNAs directly, ranging from mRNAs of viral oncogenes to fusions or mutations and non-coding RNAs, which to the best of our knowledge have not been targeted like this in cancer therapy.[4] For this reason, it should have the potential to treat previously untreatable types of cancer.

Finally, in theory G-dase E can be easily programmed to the next biomarker - and thus the next indication - by simply adapting 21 base pairs of the guide RNA, providing its platform technology capability. Like probably other Cas nucleases, G-dase E has the potential to be used in personalised medicine in the future.

Next steps

The efficient delivery of G-dase E into cancer cells is currently under development. The toxic nuclease requires a suitable delivery system for use in upcoming proof-of-concept studies for anti-tumour efficacy and later in clinical studies and treatment.

Fortunately, G-dase E technology can benefit from a wide range of technologies that have been established in recent years for various active ingredients as so called payloads. Advantageously, G-dase E as a payload itself should be highly specific and tightly regulated by the RNA biomarker interaction.

Nevertheless, delivery remains a challenge. Since no available system is suited to target all cancer types, and G-dase E is to be used as a platform, we are looking for partners who are interested in combining their delivery system with a next generation payload. In addition, we are looking for strategic partners for the development of new cancer therapies. ●

* - G-Dase E is a registered trademark of BRAIN Biotech

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