CELL & GENE THERAPY INSIGHTS

GENE EDITING

SPOTLIGHT

Pioneering the future of non-viral genome engineering

Hao Wu
Full Circles Therapeutics

"With the achievement of large DNA integration in a target-specific manner, many new therapeutic applications will be unlocked."

VIEWPOINT

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INTRODUCTION

On Jul 30, 2024, Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, spoke to Hao (Howard) Wu, Co-Founder and CSO, Full Circles Therapeutics, in a discussion around the emerging innovations and challenges in genome and epigenome editing. This article is based on that conversation.

Full Circles Therapeutics is currently engaged in a monumental endeavor: realizing the final chapter of genome engineering. Their primary focus is on integrating large genetic payloads in a target-specific manner

within the genome, particularly in clinically relevant cell types, both *in vivo* and *ex vivo*. This approach aims to address the genetic root causes of rare genetic disorders, oncology, and autoimmune diseases such as systemic lupus erythematosus and acute myeloid leukemia.

INTRODUCING A NON-VIRAL GENOME ENGINEERING PLATFORM

At the heart of Full Circles Therapeutics' innovation is their viral-free genome engineering platform, a proprietary technology that utilizes a scalable, programmable mini-circular single-stranded DNA, trademarked as C4DNA™. This unique DNA molecule enables the integration of extra-large genetic payloads into the genome in locus specific manner, a feat previously not easy to achieve. C4DNA presents several advantages, includ-ing that it is non-viral, can be scalably man-ufactured to the milligram to gram level, and has a shorter half-life than conventional dou-ble-stranded (ds) DNA, hence lower cytotox-icity to the cells and less concerns for random integration.

C4DNA also enables highly efficient tar-geted genome integration, with a molecular weight of only half of dsDNA counterparts, leading to easy delivery of high copy num-bers. C4DNA's versatility means that it can be used both with CRISPR and with vari-ous other genome editing systems such as transposaons, integrases, transcription acti-vator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs). This adaptabil-ity makes C4DNA a powerful tool across a wide range of genome engineering applications. Moreover, C4DNA's programmability allows for the incorporation of polycistronic cassettes, enabling genetic circuit engineering.

C4DNA can be used with payloads of up to 25 kb, and at Full Circles so far, developers are achieving 10–15 kb docking with satisfac-tory knock-in efficiency. The goal is to cor-rect multiple mutations through the insertion of extra-large payloads. This platform will be useful in working to address diseases or genetic disorders with extra-large genetic root cause genes in a mutation agnostic manner, such as cystic fibrosis, Stargardt syndrome, and otoferlin-related hearing loss, as well as in immuno-oncology applications.

NON-VIRAL VERSUS VIRAL PLATFORMS

Cell and gene therapy is currently dominated by viral modalities, for instance, AAV-based therapies. Many CGT developers are working to engineer the capsid to produce more efficient and specific AAV. Lentivirus and other viral particles have already been used in early clinical trials, with some drug approvals seen. Viral particles are widely used in this way as they allow the efficient delivery of the genetic payload into the cell types used in clinical development.

However, viral modalities have many shortcomings, including their unavoidable immunogenicity, payload limitations, and manufacturing challenges. For example, over 50% of humans already show humoral immunity to AAV, meaning drugs developed using this modality offer limited dosing. AAV has a payload limitation of around 4.5–4.7 kb, which limits the number of genetic disorders that can be targeted. During manufacture, empty or truncated AAV capsids pose challenges for dosing and contribute to adverse effects, compromising the efficacy and safety of the final drug product. Lentivirus has been widely reported to be randomly integrated into genomes posing huge potential safety concerns.

To address these issues, Full Circles Therapeutics has opted to pursue a non-viral approach with C4DNA, eliminating those potential safety concerns, immunogenicity, and other manufacturing challenges, whilst allowing for the integration of much larger genetic payloads. Additionally, non-viral platforms like C4DNA promise to be more cost-effective, reducing manufacturing costs and ultimately making gene therapies more

affordable by reducing the cost of goods to 20% of current costs.

Non-viral platforms are not without their challenges, particularly in the realm of delivery. Effective delivery of nucleic acid modalities to specific tissues remains a significant hurdle. Innovative delivery methods and complementary technologies, such as lipid nanoparticles (LNPs) and ultrasound-guided delivery, are being explored to enhance the targeted delivery of genome editing therapeutics.

OVERCOMING DELIVERY CHALLENGES FOR GENE EDITING THERAPEUTICS

For genome engineering, solving efficient nuclear delivery is key to achieving highly efficient targeted genome integration. There are two levels of target specificity: tissue and genome targeting. Genome targeting specificity may be addressed more easily. For example, viral vectors possess the intrinsic nature of being untargeted, in that they can be integrated anywhere in the genome. Powerful genome engineering tools such as CRISPR, TALENs, ZFNs, and targeted integrases or transposons offer a more precise approach to genome targeting.

Achieving targeted tissue specificity remains a significant challenge, including for non-viral delivery systems. The field is witnessing substantial efforts from both academic and industry researchers to develop innovative approaches that can specifically target defined tissues or cell types. For instance, recent pioneering work from institutions like the Massachusetts Institute of Technology and various biotech companies has led to the development of LNPs designed to target non-hepatic tissues, such as the spleen and lungs.

Addressing these delivery challenges requires a multifaceted approach that takes into account both the chemical and formulation aspects of delivery systems. The strategy to overcome these challenges must be disease-dependent, considering the specific needs of different therapeutic indications. For example, certain genetic disorders in ophthalmology, such as Stargardt syndrome and retinitis pigmentosa, may not require highly innovative delivery methods, as they can be treated with targeted microinjections. The same principle applies to auditory conditions like otoferlin-related auditory neuropathy spectrum disorder or stereocilin-related hearing loss, where local delivery via microinjections of LNPs could be sufficient.

However, for most other indications, innovative targeted delivery methods are essential. Fortunately, the field has seen significant progress, particularly with the success of mRNA vaccines for COVID-19 and subsequent research into delivery technologies like LNPs. Emerging technologies such as ultrasound-guided delivery hold promise for achieving tissue-specific delivery of nucleic acids, offering a non-viral approach to advancing genome engineering therapies.

NOVEL TOOLS IN GENOME EDITING

One trend in the field of genome engineering is to identify smaller versions of genome editors such as mini versions of Cas9, integrase, or recombinase, for example. Companies like Metagenomi, Arbor Biotech, and Mammoth Biosciences have been at the forefront of this effort, uncovering novel, compact editing systems.

In this era of machine learning and AI, not only can we use generative AI and identify those smaller versions by mutagenesis, but we can also use the large databases being built to identify naturally occurring enzymes with potential applications in genome editing. Moreover, these tools could even help us discover unique editing systems within the human genome itself, which may have the added benefit of minimizing immune responses—a critical factor in the development of safer and more effective gene therapies.

Currently, the only approved drug in the field of genome engineering is CRISPR Therapeutics' and Vertex Pharmaceuticals' Casgevy, targeting sickle cell disease and beta thalassemia. There is confidence that this field will advance rapidly. Base editing and prime editing represent groundbreaking emerging technologies within genome editing. Base editing is capable of correcting single point mutations, while prime editing extends this capability by allowing the insertion of short DNA sequences, typically fewer than a couple of hundred nucleotides. Innovations such as genome writing and synthetic DNA, which allow for the insertion of large or extra-large payloads beyond the limitations of AAV payloads, are also gaining attention. Despite these advances, there remains a need for more innovative platforms, as the field is evolving swiftly.

Another area of interest is targeted transposase systems. Traditional transposon systems allow for semi-targeted or semi-random integration of large payloads, which could enable the integration of substantial genetic material. However, the non-targeted nature of this integration limits its transformative potential, although companies like Poseida Therapeutics are actively working to refine this technology. While promising, this technology is still in its infancy and has so far shown efficacy primarily in bacterial systems, with further data and testing needed in mammalian systems.

The field of genome engineering is in constant need of new technologies, and the current developments, though still in their infancy, are promising. The wide array of toolboxes emerging from numerous labs and biotech companies indicates a bright future for the field.

EXPLORING EPIGENOME EDITING

Unlike other genome engineering technologies, epigenome editing does not involve dsDNA breaks or alterations to the DNA sequence. Instead, it leverages a conservative mechanism of gene regulation to harmonize gene expression at the transcriptional level. This allows for the potential to unlock gene expression where it has been silenced in disease settings or to suppress disease-causing genes, such as oncogenes or those involved in neurological disorders. There is a broad spectrum of applications provided we can achieve precise, targeted epigenome editing, whether through DNA methylation or histone modifications.

However, there are significant challenges that need to be addressed. The first challenge is off-target effects at the genomic level. Similar to genome engineering, epigenome editing requires precise targeting within the genome. If the editing machinery affects regions outside of the intended target, it could lead to undesirable phenotypes, such as the inappropriate suppression or activation of other genes. This necessitates careful assessment and regulation, as in genome engineering.

The second challenge is tissue targeting—how do we ensure that the epigenome editor machinery is delivered specifically to the tissue of interest? For example, when targeting the liver to treat a disease like Pompe disease, addressing the *GAA* gene, it is crucial that the delivery system accurately targets the liver. If you deliver systemically, alterations to *GAA* in other tissues may lead to unwanted side effects.

The third challenge, which is somewhat unique to epigenome editing, concerns the formulation and the packaging of the editing machinery. Epigenome editing typically involves fusion proteins that are often large and bulky, such as those based on dCas9, TALENs, or ZFNs fused with effector domains like DNA methylation editors or histone modifiers. Delivering these large molecules into cells is a significant hurdle.

Fortunately, progress is being made, with companies like Chroma Medicine, OMEGA Therapeutics, and Tune Therapeutics demonstrating promising preclinical results. One trend in the field is the development of miniatures, such as a compact dCas9 versions

of these affected domains, which retain full functionality or even enhance genome targeting efficiency. AI and machine learning are also poised to play a crucial role in epigenome editing.

Another promising avenue that has been somewhat overlooked involves the use of small molecules in epigenome editing. Pyrrole-imidazole polyamides are small molecules with sequence-specific DNA-binding capabilities that have been studied for over a decade. These small molecules can be utilized in epigenome editing or even genome engineering without leveraging the external nuclease enzyme editor system. They can be covalently linked to small molecule binders that recruit endogenous enzymes like DNA methyltransferases or histone modifiers, creating a new class of programmable, small-molecule-based epigenome editors. This approach could offer an alternative to enzyme-based systems to potentially overcome some of the existing challenges. Whether using small molecules or traditional enzyme editors, off-target effects remain a critical concern that must be thoroughly evaluated to ensure safety.

THE FUTURE OF GENOME EDITING

One of the most important challenges in the life sciences field is genome engineering for large payload integration. The genome engineering field has already accomplished gene ablation by creating simple indel-like CRISPR technology, single-point mutation corrections by base editor, as well as tens of nucleotide insertions by prime editing.

The next chapter of genome engineering could revolve around large payload integration in a target-specific, safe, and efficient manner. Over the next 5–10 years, emerging technologies will be coming to play here. With the achievement of large DNA integration in a target-specific manner, many new therapeutic applications will be unlocked. This includes cystic fibrosis, a disease caused by the CFTR gene, which is >5 kb. Over the patient population, the disease has over 900 different mutation haplotypes. If a therapeutic strategy to insert a large payload the full length of the CFTR gene was developed, a universal approach to address all patients with cystic fibrosis could be established. This could also drive down costs and increase speed in bringing those medicines to patients.

Another trend in clinical applications, particularly on the regulatory side, is the need to tolerate n=1 clinical trials. Genome engineering is especially powerful for those genetic disorders for which there are no available treatments, usually in ultra-rare diseases. Within 5–10 years, there may be potentially 5–6 new drugs approved with this technology.

BIOGRAPHY

HAO (HOWARD) WU PhD has almost 20 years' experience in gene editing technology and new drug discovery. He is specialized in overseeing R&D programs, new lab and research team set up in the biotech start-ups.

Wu is the co-founder and CSO of Full Circles Therapeutics, Cambridge, MA, USA, where he is dedicated to developing curative gene editing based gene/cell therapy. He is responsible for generating revenue through collaboration with MNC and biotech partners. Before founding Full Circles Therapeutics, Wu was leading multiple discovery biology programs and disease prioritization in the genetic disease space at Fulcrum Therapeutics Inc. (NASDAQ:FULC), a Cambridge small molecule drug discovery biotech company. He had been with the company through the full development phases starting from the start-up, expansion, until post-IPO development, during which he led a cross-functional team for portfolio disease selection and prioritization of multiple disease programs including neuromuscular disease, cardiac disease, hematological, and metabolic diseases.

Before joining Fulcrum, Wu was a senior research fellow at Whitehead institute, MIT. His research focused on neurological disorders utilizing a combination of CRISPR/Cas9 mediated genomic and epigenomic editing technology and stem cell technology. He did his PhD in Biochemistry and Structural Biology at Hongkong University of Science and Technology (HKUST), New Territories, Hong Kong, and Bachelor's degree in Chemistry from Fudan University, Shanghai, China, before he did his postdoctoral research at Johns Hopkins University School of Medicine, Baltimore, MD, USA, and Howard Hughes Medical Institute, Chevy Chase, MD, USA. Wu has more than 30 journal publications, patents, and research and industry grants. For his work, he has received fellowship award from human frontier science program (HSFP) and NARSAD young investigator award. He was also awarded the Alfred Blalock Young Investigator Award from JHMI and President's award from Whitehead Institute, MIT.

AFFILIATION

Hao (Howard) Wu,
Co-Founder and CSO,
Full Circles Therapeutics,
Cambridge, MA, USA

AUTHORSHIP & CONFLICT OF INTEREST

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