EXPANDING CELLULAR IMMUNOTHERAPY

SPOTLIGHT

Efficient non-viral engineering of immune cells for cell therapy using circular single-stranded DNA

Howard Wu

VIEWPOINT

"The integration of precision gene editing with safe and scalable delivery systems represents a paradigm shift in how we think about and approach immunotherapy."

Cell & Gene Therapy Insights 2025; 11(1), 7–11 · DOI: 10.18609/cgti.2025.002

RESEARCH BACKGROUND

In recent years, gene-editing technologies have become central to advancements in immunotherapy, particularly in engineering immune cells for the treatment of diseases such as cancer and autoimmune disorders. However, traditional methods—especially those relying on viral vectors for gene delivery—have raised significant concerns related to safety, efficacy, scalability, accessibility. These concerns have slowed the widespread adoption of these therapies over the globe. Recently, a Boston, MA, USA-based biotech company Full Circles Therapeutics' achieved technological breakthrough to overcome many of these limitations, signaling a new era in the development of safe, scalable, efficient, and more affordable immune cell therapies.

Although CRISPR/Cas9 technology has been widely used in gene editing, enabling efficient targeted gene knockout and single-base editing, precise and site-specific integration of large gene fragments remains a technical challenge. These current gene delivery technologies face the following issues:

- Limitations of viral vectors: including immunogenicity, which can trigger immune responses; difficulty in production, as the viral preparation process is complex and costly; payload limitations, such as the restricted gene capacity of AAV vectors; and safety concerns, as random gene integration by lentivirus and AAV can pose potential safety risks.
- Drawbacks of non-viral methods: such as low efficiency and high toxicity associated with double-stranded DNA (dsDNA), and challenges in large-scale production of linear single-stranded DNA (lssDNA).

These technological bottlenecks present significant challenges for the engineering of immune cells (such as CAR-T cells) and the clinical application of cancer immunotherapy. Therefore, there is an urgent need for a non-viral, efficient, and safe gene integration technology.

RESULTS AND CONCLUSION

The company's innovation developed a proprietary miniaturized circular single-stranded DNA (cssDNA, trademarked as C4DNA™), a non-viral technology that enables stable genomic integration without relying on viral vectors. They developed a non-viral gene writing technology (GATALYST™) platform based on the C4DNA, which has brought disruptive progress in the field of large gene fragment integration. This platform utilizes a genetically engineered phage system to produce mini circular single-stranded DNA (cssDNA) with a size of up to approximately 20 Kb. It achieves efficient and precise large gene integration through a homologous recombination mechanism.

The cssDNA non-viral vector avoids the genomic instability caused by random integration and has the potential for industrial-scale production, significantly improving manufacturing efficiency. The GATALYST platform can achieve efficient, precise, and safe integration of large genes in various dividing cells. It has demonstrated up to 70% knock-in efficiency in induced pluripotent stem cells (iPSCs), as well as high-efficiency site-specific integration in multiple clinically relevant primary immune cell types. The platform can edit multiple genomic loci relevant to clinical applications and is compatible with various nucleases editing systems.

Using electroporation delivery technology, cssDNA can achieve up to 50% site-specific integration of dual-targeting CAR (CD19/CD22) in CAR-T cells, exhibiting excellent anti-tumor function both *in vitro* and *in vivo*. This provides new avenues for treating relapsed or refractory multiple myeloma (RRMM).

The GATALYST platform, with its flexible, safe, and scalable cssDNA vector, shows great potential in the field of immune cell therapy. This platform is expected to overcome the limitations of current gene delivery methods and provide a novel non-viral solution for gene cell therapies targeting cancer, autoimmune diseases, and genetic disorders, thus advancing the development of precision medicine.

PERSPECTIVE

The non-viral revolution: precision and safety in focus

One of the most significant challenges in the field of gene editing has been ensuring the precise and controlled integration of genetic material into the genome. Full

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Circles' use of C4DNA, a miniaturized form of circular single-stranded DNA, enables targeted, accurate genomic integration. This eliminates the reliance on potentially unpredictable viral systems, providing a safer alternative for patients. The technology significantly enhances scalability, editing efficiency, and overall safety, particularly for CAR-T and CAR-NK cell therapies. These therapies, which have already demonstrated significant promise in treating cancers, could become more accessible and less expensive as the technology scales up. For autoimmune diseases where the safety bar is much higher than end stage cancers, adoption of a non-viral engineering approach is more attractive.

The company's approach directly addresses the scalability problem, which has been a major bottleneck in the widespread use of gene-edited immune cells. One of the key challenges for current therapies, such as CAR-T, is the complexity and cost involved in producing personalized therapies for each patient. Full Circles' non-viral method could lead to the development of off-the-shelf cell therapies with non-viral precise genome engineering, meaning that engineered immune cells could be produced in bulk and stored for rapid use in patients, much like other biologics, making treatments more affordable and time efficient. Full Circles is now leveraging the multiplex editing approach by C4DNA to manufacture the allogeneic CAR-NK product for autoimmune diseases.

A new path for clinical application

Full Circles' recent patent approval in Japan, a leading global market for biopharmaceuticals, is a significant milestone for the company and for the field at large. The granting of the patent underscores the technology's clinical potential, particularly in markets where gene therapies are already a focus of intense research and development. With Japan at the forefront of oncology, autoimmune disease research, and genetic metabolic disorders, the impact of this innovation could be transformative.

The granted patent, which covers the use of universal circular single-stranded DNA for targeted genomic integration, highlights the broad utility of this technology in genome editing. Importantly, this technology is designed to work in a nuclease-editor agnostic manner, a feature that improves its adaptability, programmability and target specificity.

Expanding the therapeutic landscape

The broader implications of Full Circles' breakthrough extend beyond the immediate application to CAR-T and CAR-NK therapies. The potential for using this non-viral gene-editing technology could dramatically expand the range of diseases that can be treated with engineered immune cells. By enhancing the efficacy, scalability, and cost–effectiveness of immune cell therapies, Full Circles has positioned itself to address complex and hard-to-treat conditions—such as cancers, chronic autoimmune diseases, and perhaps even genetic disorders caused by ultra-large root cause genes with scattered mutation haplotypes—that have long eluded conventional treatments.

Dr Richard Shan, the Chairman of Full Circles Therapeutics, emphasized that the company aims to make these therapies more accessible and affordable to a broader patient population. In an era where the cost of biologics and personalized medicine remains a critical barrier to widespread adoption, the scalability of Full Circles' non-viral approach offers a promising solution. By making cell therapies off-the-shelf, the company could facilitate faster and more efficient deployment of treatments, ultimately accelerating the clinical translation of these breakthrough technologies.

Looking ahead: the path to widespread adoption

The recent recognition from Novo Nordisk Bio innovation Hub, which awarded Full Circles the 'Pathbreaker' award, underscores the company's commitment to collaborating with industry partners and accelerating the accessibility of breakthrough therapies. The award is a testament to the importance of forging strategic partnerships to drive the next generation of therapeutic innovation. By focusing on patent licensing and collaborative development, Full Circles aims to expand the reach of its technology and increase its impact in the global healthcare landscape.

Ultimately, Full Circles' non-viral immune cell engineering technology could become a cornerstone in the future of cell and gene therapy, enabling a broader range of patients to benefit from cutting-edge treatments. The integration of precision gene editing with safe and scalable delivery systems represents a paradigm shift in how we think about and approach immunotherapy. With further advancements and clinical validation in the near future, we are confident that this technology could play a key role in shaping the next generation medical treatment for a wide array of diseases.

This VIEWPOINT is based on the following original publication: Xie K, Starzyk J, Majumdar I, *et al.* Efficient non-viral immune cell engineering using circular single-stranded DNA-mediated genomic integration. *Nat. Biotechnol.* 2024; published online Dec 11. https://doi.org/10.1038/s41587-024-02504-9.

BIOGRAPHY

Howard (Hao) Wu has almost 20 years of 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. Dr 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, Dr Wu was leading multiple discovery biology programs and disease prioritization in the genetic disease space at Fulcrum Therapeutics Inc. (NASDAQ:FULC), a 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, Dr 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), 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. Dr Wu has more than 30 journal publications, patents, and research and industry grants. For his work, he has received a fellowship award from the Human Frontier Science Program (HSFP) and the NARSAD Young Investigator Award. He was also awarded the Alfred Blalock Young Investigator Award from JHMI and President's award from Whitehead Institute, MIT.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author is a paid employee and equity shareholder of Full Circles Therapeutics.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

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Article source: Invited.

Revised manuscript received: Dec 13, 2024.

Publication date: DATE.