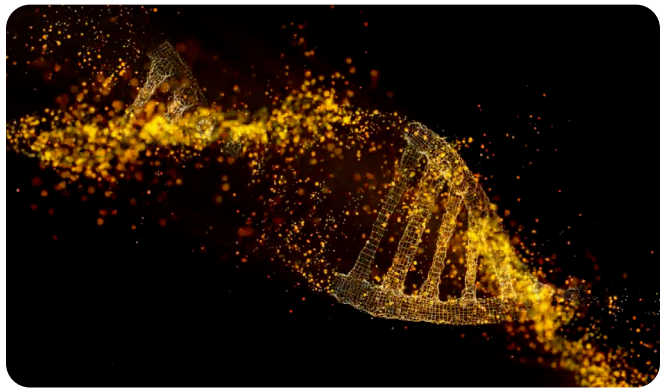
A breakthrough aptamer-mediated base editing platform for simultaneous knock-in and multiple gene knockout

Introduction

CAR-T cell therapy may represent a promising frontier in the realm of immunotherapy, harnessing the potential of genetically engineered T cells to combat cancer. However, the path to realizing its full potential is laden with complexities, including intricate manufacturing processes, and the downstream potential for treatment delays.

In the quest to overcome the complex challenges associated with CAR-T cell therapy, a compelling avenue under exploration involves the utilization of T cells obtained from healthy donors to generate an allogeneic therapy derived from cells that are genetically dissimilar to the patient. The challenge lies in alloreactivity, which happens when the recipient's immune system recognizes the infused allogeneic CAR-T cells as foreign invaders and mounts an immune response against them (host-vs.-graft disease) or vice versa when the infused immune cells attach the patient tissue (graft-vs.-host disease [GvHD]). This recognition can lead to a variety of complications, reducing the effectiveness and safety of the therapy.

One way to overcome this problem is by knocking out key proteins that contribute to the recognition of the CAR-T therapy as "non-self" and proteins involved in the immune response against the host, potentially reducing the risk of alloreactivity and improving the safety and efficacy of the therapy. Central to this approach are gene editing technologies, which play a pivotal role in shaping the future of these therapies.



Instead of relying on conventional protein knockout methods that make use of first generation CRISPR-Cas9, which induce genetic alterations through DNA double-strand breaks (DSBs), a novel and more refined approach has emerged: base editing.

This paper delves into an application of gene editing that diverges from the conventional paradigm. Rather than introducing mutations through sequential or multiple DSBs, this innovative method achieves simultaneous gene knock-in and knockout in a single engineering step. Beyond offering enhanced safety measures, this groundbreaking technique simplifies the process of effecting complex genetic changes in a streamlined workflow.

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Aptamer-recruited base editing

Base editing is a next-generation gene engineering method that allows for targeted modifications in a cell's genome without relying on detrimental double strand breaks in the DNA. With base editing, a single C to T (via cytidine deaminase) or A to G (via adenine deaminase) can be introduced to cause knockout by introduction of premature stop codon or splice site disruption, or can be used to correct a single nucleotide to restore gene function. The base editing machinery consists of an RNA guided nuclease, such as a modified nickase Cas9 protein (nCas9), a deaminase enzyme, and a guide RNA (gRNA).[1]

In an aptamer-recruited system such as the Pin-point™ platform, the three components of the base editing machinery are brought together through the aptamer-containing gRNA component that both engages with the RNA-guided nuclease and recruits the deaminase enzyme (modified to include an aptamer binding protein) to the target site through the aptamer and aptamer binding protein interaction. When introduced into target cells, these components are recruited to the target site by the specificity of the gRNA targeting sequence to introduce single base changes in the genome.[1]

During this editing process, the deaminase enzyme precisely converts one DNA base into another, allowing for base changes without relying on double strand breaks in the DNA. The resulting edited DNA sequences can be confirmed using methods like DNA sequencing. Aptamer-recruited base editing provides accuracy and carries minimal risk of affecting unintended targets, making it an incredibly valuable tool for applications such as CAR-T cell therapy and advanced biopharmaceutical research.

The Pin-point system is an aptamer-recruited base editing platform that embodies precision and adaptability, offering a multitude of benefits for advanced cell therapies. One of the platform's most remarkable features is its ability to seamlessly execute gene knock-ins and knockouts, simplifying the complex task of multiple genetic modifications into a single step.[2] This advancement not only enhances safety measures, a critical factor in engineered cell therapies, but also expedites the process.

As we delve into the realm of cancer treatment, the Pin-point platform showcases its robustness by ensuring the effectiveness of cells in killing cancer cells. Its capacity to revolutionize gene editing precision aligns seamlessly with the evolving demands of cell therapies in the fight against cancer.

Experimental results

In this evaluation of the Pin-point base editing system, critical aspects were investigated to shed light on its clinical and therapeutic potential, particularly in the context of allogeneic CAR-T cell therapy.

Multiple gene knockouts

A pivotal facet of the study revolves around elucidating the outcomes of gene knockouts. The Pin-point base editing system underwent rigorous testing to achieve highly efficient gene knockouts in human primary T cells. By leveraging mRNA reagents and meticulously designed gRNAs, genes such as B2M, TRAC, PDCD1, and CD52 were successfully knocked out.[2] These tailored gRNAs introduced splice site disruptions or premature stop codons in their respective target genes, showcasing the efficacy and precision of the Pin-point system in executing multiplex gene knockouts.[2]

Evaluation of efficiency and specificity

Efficiency and specificity stand as critical determinants in advanced cellular therapies. This investigation assessed the efficiency of the Pin-point system in facilitating C to T conversions, the genetic requirement for causing gene knockouts with a cytidine deaminase. Notably, the Pin-point system exhibited impressive efficiency levels, ranging from 76% to 85% conversion from C to T across the four target genes without additional enrichment.[2]

Specificity was also assessed and it was observed that editing outcomes at the intended targeted sites are highly controllable with the Pin-point base editing system. With base editing, only the intended bases are modified, which is both more predictable and specific when compared to the use of DSB based technologies where the editing outcome is highly heterogeneous and difficult to control.

When comparing first generation CRISPR-Cas9 and base editing technologies at known off-target editing sites, both the level of editing is lower, and the genetic outcomes at the off-target sites are potentially less detrimental with base editing.

Knock-in results

Beyond gene knockouts, the study delved into the potential of the Pin-point system to be utilized for targeted gene knock-ins. Leveraging the flexibility of the system's aptamer-mediated deaminase recruitment, simultaneous multi-gene editing, including the insertion of the CD19 CAR at the TRAC locus, is demonstrated. This simultaneous approach to knock-ins and knockouts showcased the system's versatility and efficiency, achieving levels of protein depletion and knock-in comparable to those attained with first generation CRISPR-Cas9.[2]

Potential clinical and therapeutic benefits

The findings hold significant promise for therapeutic applications. The remarkable efficiencies achieved in gene knockouts and knock-ins position the Pin-point system as a valuable tool for developing cellular therapies. By precisely targeting genes such as B2M, TRAC, PDCD1, and CD52, the Pin-point system opens avenues for personalized cellular therapies tailored to each patient's unique requirements.[2] Furthermore, the system's ability to minimize unintended gene modifications, reduce occurrence of post-editing chromosomal abnormalities, and mitigate adverse impacts on cell health further enhances its potential as a therapeutic tool.

Implications of simultaneous gene editing for CAR-T cells

Allogeneic CAR-T cell therapy has long been an aspirational goal in immunotherapy. However, challenges related to gene editing and the maintenance of cell fitness have posed significant obstacles. One standout feature of the Pin-point system is its capacity to edit genes without compromising cell viability. This breakthrough technology empowers the creation of genetically modified cells efficiently, paving the way for safer and more effective allogeneic transplants. Moreover, by minimizing off-target effects and chromosomal abnormalities, the Pin-point system demonstrates its suitability for advancing the field of CAR-T cell therapy, offering a promising path forward in the realm of immunotherapy.

Closing

CAR-T cell therapy shows potential in the field of immunotherapy, especially for treating blood cancers. However, there are challenges in the manufacturing process and delays in treatment that need to be addressed. One solution gaining attention is gene editing in CAR-T cells using base editing technologies such as the Pin-point platform. This system revolutionizes gene editing by providing precision and adaptability making complex genetic modifications simpler and more efficient.

These advancements hold promise for cellular therapies that cater to individual patient needs while minimizing genetic alterations and ensuring cellular well-being. Importantly, the Pin-point systems' remarkable ability to edit genes without compromising cell fitness represents a breakthrough in CAR-T cell therapy. It provides more effective complex engineered cell therapies by minimizing off-target effects and abnormalities in chromosomes.

The Pin-point platform is positioned to revolutionize cell therapies by offering precision, efficiency, and safety while advancing the field of CAR-T cell therapy.

Reference

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