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Review

The Potential of CAR-T cells for Treating Heart Diseases: Current Status and Hurdles in Clinical Translation

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Traditional treatments for heart disease, including pharmacotherapy and surgical interventions, are effective in managing symptoms and preventing complications but often fail to fully restore cardiac function or halt the progression of the disease. Additionally, these approaches are frequently associated with significant adverse effects. Inspired by the success of CAR-T cell therapy in oncology, this review examines the potential of CAR-T cell technology for treating heart diseases, detailing how CAR-T cells, engineered by merging antibody-derived targeting domains with T-cell signaling domains. The technology's core includes an extracellular antigen-binding domain, hinge region, transmembrane domain, and intracellular signaling domain, with the single-chain variable fragment (scFv) playing a crucial role in antigen recognition. The paper delves into the immune mechanisms in cardiovascular diseases like heart failure, hypertension, and myocardial infarction, focusing on the roles of T cells in promoting myocardial fibrosis and the therapeutic potential of regulatory T cells (Tregs) in recovery phases. Additionally, it explores the use of lipid nanoparticles (LNPs) carrying mRNA to produce transient, non-integrative CAR-T cells targeting fibroblast activation protein (FAP) to reduce myocardial fibrosis, a method showing promise in preclinical models by enhancing cardiac function and reducing ventricular fibrosis. Despite its potential, the study acknowledges challenges in clinical translation, such as limited therapeutic effects and severe inflammatory responses, highlighting the need for further optimization and research in CAR-T cell technology for cardiovascular disease treatment.

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Introduction

Current treatments for heart diseases often involve long-term management strategies, including medication, lifestyle changes, and invasive procedures such as surgery (Writing Committee Members et al. 2021). While these treatments can be effective in managing symptoms and preventing complications, they frequently fall short in fully restoring heart function or halting disease progression (Fine et al. 2020). Moreover, the side effects associated with chronic medication use and the risks linked to surgical interventions present significant challenges. This highlights a critical need for innovative therapeutic approaches that can more directly and efficiently address the underlying causes of cardiac conditions.

In 2013, cancer immunotherapy was heralded as the breakthrough of the year by Science magazine, marking the commencement of a new immunological chapter in cancer research and therapy (Couzin-Frankel 2013). Currently, the adoptive transfer of T cells expressing chimeric antigen receptors (CAR) represents a significant direction in immunotherapy, demonstrating promising therapeutic prospects in clinical oncology. CAR-T cell therapy, an innovative approach to immunotherapy, involves isolating T cells from either the patient or healthy donors, genetically engineering them *in vitro* to express CARs that target disease-specific antigens, and expanding these cells to sufficient numbers before reinfusing them into the patient to combat disease

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(Qin et al. 2021; Dabiri et al. 2023). This therapy has shown remarkable clinical efficacy against various hematological malignancies, becoming the first genetically modified cell therapy approved by the U.S. Food and Drug Administration (Wang et al. 2023). Despite the proven effectiveness of CAR-T cells in tumor treatment, recent studies have begun to explore their potential application in treating other diseases, including cardiac damage.

Previous reports indicate that traditional CAR-T therapy can also address cardiac damage (Yang et al. 2024). In a mouse model of hypertensive heart damage, CAR-T cells targeting the fibroblast activation protein (FAP) - a marker of activated fibroblasts - can specifically eliminate these cells, significantly reducing myocardial fibrosis (Rurik et al. 2022). However, as FAP expression is not limited to damaged cardiac tissue but also occurs in other tissue injuries and repair processes, traditional CAR-T treatments pose additional health risks due to prolonged CAR-T cell persistence in the body of cardiac injury patients (Rurik et al. 2022). Researchers from the Perelman School of Medicine at the University of Pennsylvania have developed a novel method for the *in vivo* transient generation of CAR-T cells (Rurik et al. 2022). This technique involves the *in vivo* injection of mRNA to transiently generate CAR-T cells capable of treating cardiac damage.

This review will discuss the implications and prospects of this innovative technology for enhancing CAR-T therapeutic strategies and applications in heart diseases. By advancing our understanding of the interactions between engineered T cells and cardiac tissues, this technology could pave the way for more effective and tailored treatments for a variety of cardiac conditions.

Overview of CAR-T Cell Technology

CAR-T cells are engineered through the fusion of an antibody-derived targeting domain with T-cell signaling domains, enabling them to specifically recognize cardiac cell surface antigens, secrete cytokines, and mediate cytotoxicity (De Marco et al. 2023). A CAR is composed of four distinct parts: an extracellular antigen-binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain, each performing specialized functions (Syed et al. 2022). The antigen-binding domain, typically a single-chain variable fragment (scFv), is crucial for the specific recognition of target antigens (Hanssens et al. 2022). This fragment is a peptide chain formed by linking the variable regions of the heavy and light chains of a monoclonal antibody via a short peptide linker (Muñoz-López et al. 2022).

Unlike physiological T cell receptors (TCRs), which require major histocompatibility complex (MHC) presentation for effective antigen binding, CARs, composed of scFv, can bind directly to cell surface antigens independently of MHC (Chmielewski et al. 2013). Additionally, scFv-based CARs can recognize soluble ligands within the cardiac microenvironment, such as transforming growth factor-beta (TGF-β), thereby mitigating the immunosuppressive effects mediated by these molecules (Ramírez-Chacón et al. 2022).

However, the specificity and affinity of scFv are critical not only for targeting CAR-T cells to heart diseases but also for influencing the functional activity of the CAR-T cells (Zhang et al. 2023). An scFv with adequate affinity is fundamental for the CAR's specific recognition and binding to target antigens, yet excessively high affinity can lead to activation-induced cell death (AICD) and toxic side effects (Rafiq et al. 2020). Moreover, even scFvs targeting the same antigen with similar affinities can have diverse effects on CAR-T cells, reflecting the complexity of CAR structural design (Stoiber et al. 2019).

Recent developments in the design of CAR-T cell structures have focused on optimizing the specificity and efficacy of the antigen-binding domain, particularly the single-chain variable fragment (scFv) (Jayaraman et al. 2020). Certain scFvs can also induce antigen-independent effects in CAR-T cells, as demonstrated in early clinical trials where natural scFv induced terminal differentiation, exhaustion, and AICD in T cells (Rafiq et al. 2020).

In addition to scFv, many other molecules can be engineered into the antigen-binding domain for use in CARs. Preclinical and clinical studies of CAR-T cells targeting molecules such as IL-13Rα2, granulocyte-macrophage colony-stimulating factor (GM-CSF), and natural killer (NK) cell receptor D (NKG2D) ligands are ongoing (Khawar and Sun 2021).

The extracellular antigen-binding domain and the intracellular signaling domain of a CAR are connected via a hinge region and a transmembrane domain (Sterner and Sterner 2021). The length and composition of the hinge region can influence CAR's antigen recognition and signal transduction, thereby affecting cytokine secretion and AICD (Zhang et al. 2021a). Although sequences within the hinge region reported in the literature can downregulate CAR-T cell function, they facilitate close contact with proximal antigen peptides (Hanssens et al. 2022). Common hinge region sequences include those from CD8, CD28, and IgG1 and IgG4 (Li et al. 2023). However, peptides derived from IgG can interact with Fcγ receptors *in vivo*, potentially compromising CAR-T cell counts (McCue et al. 2022).

The transmembrane domain, primarily derived from T-cell proteins such as CD3ζ, CD8α, CD4, and CD28, plays a crucial role in the stability of the CAR structure and T-cell activation (Śledź et al. 2023). Studies have shown that CAR-T cells with a CD28-based transmembrane domain can express the CAR gene more stably and efficiently, whereas those with a CD3-based domain can mediate dimerization of the CAR structure, enhancing T-cell activation (Fujiwara et al. 2020; Muller et al. 2021). Moreover, CAR-T cells using CD8α as both hinge and transmembrane domains exhibit reduced secretion of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), thereby reducing sensitivity to AICD (Ba et al. 2023).

The intracellular signaling domain consists of one or

more co-stimulatory signaling structures and an activation domain (Sievers et al. 2020). Most CARs activate CAR-T cells through phosphorylation of tyrosine residues within the immunoreceptor tyrosine-based inhibition motifs (ITAMs) of CD3ζ (Smirnov et al. 2024). However, this alone is insufficient for effective stimulation of CAR-T cell activation and proliferation *in vivo*, necessitating the introduction of co-stimulatory signaling molecules such as CD28 and CD137, both of which significantly enhance the clinical efficacy of CAR-T therapy (Weinkove et al. 2019; Alnefaie et al. 2022). Each mediates different functions and metabolic pathways. CD28-structured CAR-T cells predominantly differentiate into effector memory T cells relying on aerobic glycolysis, while those with CD137 structure tend to become central memory T cells, favoring oxidative metabolism and promoting mitochondrial biogenesis (Zhang et al. 2021b). Additionally, preclinical studies have shown that co-stimulatory molecules like MYD88, CD40, OX40 (CD134), ICOS (Inducible T cell co-stimulator), and CD27 can significantly enhance CAR-T cell function, though clinical advancements are yet to be seen (Moreno-Cortes et al. 2023).

Immunological Mechanisms in Heart Diseases

The intricate interplay of immune mechanisms significantly contributes to the pathogenesis and progression of cardiovascular diseases such as heart failure (HF), hypertension, and myocardial infarction (MI) (Jaén et al. 2020). These conditions share common immunological disruptions that can precipitate and exacerbate disease states. Understanding these commonalities offers insights into potential therapeutic targets (Zheng et al. 2020; Passaro et al. 2021).

Cellular Immunity Across Cardiovascular Diseases

In HF, the role of T cells is critical, with CD4+ T cells promoting myocardial fibrosis through activation of fibroblasts, leading to collagen deposition (Bradshaw and DeLeon-Pennell 2020). This fibrotic process is often exacerbated by an imbalance in T cell subsets, favoring pro-inflammatory Th1 and Th17 cells over anti-inflammatory Th2 and regulatory T cells (Tregs) (Drescher et al. 2020). Similar mechanisms are observable in hypertension and MI, where heightened inflammatory responses contribute to vascular and myocardial remodeling (Saheera and Krishnamurthy 2020). In hypertension, T cells, through their interaction with angiotensin II, enhance inflammatory responses and structural changes in the heart and vessels (Norlander et al. 2018). Post-MI, an influx of T cells into ischemic myocardial tissue aids in the initial inflammatory response crucial for healing, yet prolonged inflammation can lead to adverse remodeling and heart failure (Frangogiannis 2014).

Integrative Immune and Neuroendocrine Mechanisms in Cardiovascular Diseases

T cells, particularly CD4+ and CD8+ T cells, trigger acute immune reactions following myocardial injury, releasing pro-inflammatory cytokines that exacerbate myocardial fibrosis and adverse remodeling (Zaidi et al. 202l; Liu et al. 2024). In contrast, Tregs act to suppress this inflammation by secreting anti-inflammatory cytokines such as IL-10 and TGF-β, which inhibit the activity of effector T cells and macrophages, promoting the resolution of inflammation and facilitating tissue repair (Lei et al. 2015). These functions are essential for maintaining immune homeostasis and preventing the chronic inflammation that can lead to heart failure.

B cells also play a dual role in these cardiovascular diseases, acting through both antibody production and cytokine secretion (García-Rivas et al. 2020). Regulatory B cells (Bregs) can potentially ameliorate inflammation depending on the microenvironmental cues, which is crucial in HF and MI recovery phases (Catalán et al. 2021). Pro-inflammatory cytokines such as TNF-α and IL-6 are central to the development of HF and in the exacerbation of hypertensive heart disease (Papamichail et al. 2023). They decrease myocardial contractility and promote cell apoptosis, contributing to the progressive nature of these diseases (Besse et al. 2022).

NK cells are another key component of the immune response in cardiovascular diseases, particularly noted in HF where their altered function correlates with disease severity (Kucuksezer et al. 2021). These cells are less studied in the contexts of hypertension and MI but are known to contribute broadly to immune surveillance and cytokine production (Ravaud et al. 2021).

The neuroendocrine system interacts closely with immune responses in cardiovascular diseases (Taub 2008). Systems such as the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) not only regulate cardiovascular function but also modulate immune activity (Miller and Arnold 2019). In HF and hypertension, an overactive RAAS promotes inflammation through cytokine production and immune cell activation, further deteriorating cardiac function (Satou et al. 2018). Similarly, the SNS influences immune cell distribution and function, contributing to inflammation and remodeling in the heart (Pongratz and Straub 2014).

The involvement of pattern recognition receptors (PRRs) like TLRs and NLRs links innate immunity with long-term cardiac changes in hypertension and MI (Jaén et al. 2020). These receptors detect damage-associated molecular patterns (DAMPs), leading to activation of NF-κB and other transcription factors that drive inflammatory and fibrotic responses (Li and Wu 2021). Inhibiting these pathways offers a promising approach to mitigate cardiac remodeling and hypertrophy.

Specific to MI, Tregs have a profound impact on healing and remodeling by modulating the balance between

pro-inflammatory and anti-inflammatory responses (Lu et al. 2021). They decrease the production of inflammatory cytokines and promote the resolution of inflammation, which is critical to prevent the transition from acute MI to chronic heart failure (Halade and Lee 2022).

The common thread across heart failure, hypertension, and myocardial infarction is the pivotal role of the immune system, influencing disease outcomes through a variety of cells and signaling pathways (Feng et al. 2022). Targeting these immune components holds potential for therapeutic strategies that could modify disease progression and improve cardiovascular health (Jyotsna et al. 2023). Understanding and manipulating these immune responses in a targeted manner could lead to significant advances in the management of cardiovascular diseases.

Application of CAR-T Cells in Heart Diseases

Currently, heart disease remains a leading cause of mortality worldwide, with limited treatment options available (Gaidai et al. 2023). Activated cardiac fibroblasts excessively deposit extracellular matrix proteins, leading to myocardial fibrosis, which can severely progress to fatal heart failure (Liu et al. 2021). However, therapeutic options specifically targeting this myocardial fibrosis are scant. This paper reports a novel approach utilizing engineered T cells to eliminate activated fibroblasts (Aghajanian et al. 2019). Lipid nanoparticles (LNPs) carrying mRNA encoding chimeric antigen receptors (CARs) are used to generate CAR-T cells in mice, which can eradicate pathogenic activated fibroblasts and mitigate myocardial fibrosis (Rurik et al. 2022).

A recent preclinical study have demonstrated the potential of FAP-targeted CAR-T cells to reduce myocardial fibrosis and improve cardiac function (Bughda et al. 2021). This study continues to target FAP by packaging mRNA encoding FAP CAR into LNPs that home to T cells (Kitte et al. 2023). Upon injection into mice, these LNPs bind to T cells and release the FAP CAR mRNA into the cytoplasm, where it is translated to produce FAP CAR-T cells (Rurik et al. 2022). Importantly, the cytoplasmic mRNA does not integrate into the T cell genome, thus avoiding potential adverse reactions due to genomic alterations (Acevedo-Whitehouse and Bruno 2023). Moreover, as cells divide, the concentration of mRNA in the cytoplasm rapidly decreases until it disappears, providing a transient presence suitable for short-term disease treatment (Reid et al. 2014). Researchers report that FAP CAR-T cells appear in mice 24 hours after LNP injection and decline to undetectable levels within seven days (Li et al. 2022). Further evaluation of FAP CAR-T's therapeutic effects on cardiac injury was conducted. In mouse models of hypertensive heart injury and fibrosis, transient FAP CAR-T cells significantly reduced ventricular fibrosis and improved various cardiac functions (Morfino et al. 2023). These encouraging preclinical findings demonstrate the effectiveness of this *in vivo* transient CAR-T generation method in treating cardiac injuries, with the potential for minimal systemic adverse effects due to the short-lived nature of the CAR-T cells (Dalal et al. 2022). Undoubtedly, these studies provide experimental evidence for the *in vivo* generation of CAR-T cells and suggest that CAR-T cell therapies can be applicable beyond cancer to treat other diseases.

In addition to LNP-based mRNA delivery, other targeted mRNA delivery methods have shown promise. For instance, *in vitro* lentiviral-based CAR-T engineering is another effective approach for generating CAR-T cells (Labbé et al. 2021). This method involves using lentiviral vectors to introduce CAR genes into T cells *in vitro*, followed by the expansion of these engineered T cells before reinfusion into the patient (Michels et al. 2023). Lentiviral-based CAR-T cells have been extensively studied in oncology and have demonstrated high efficiency in gene integration and stable CAR expression, which could potentially be adapted for cardiac applications (Rossi and Breman 2024). Comparing these methods, lentiviral-based engineering offers long-term CAR expression, whereas LNP-based delivery provides a transient, safer option with fewer potential genomic alterations. Combining the strengths of both approaches might yield an optimized strategy for cardiac disease treatment.

In addition, non-viral delivery methods such as electroporation and lipid-based nanoparticles (beyond LNPs) have shown potential for mRNA delivery to T cells (Khawar et al. 2024). Electroporation involves using an electric field to introduce mRNA into cells, providing a direct and transient expression of CAR genes (Campillo-Davo et al. 2021). Moreover, polymer-based and hybrid nanoparticle systems are being developed to improve the stability and targeting specificity of mRNA delivery (Yang et al. 2023). These methods, while still under investigation, offer alternative avenues for generating CAR-T cells with the potential to be tailored for specific therapeutic needs, including cardiac applications.

Challenges and Barriers in Clinical Translation

The research from the University of Pennsylvania introduces a novel CAR-T technology, providing a new therapeutic option for treating cardiac injuries. Utilizing mRNA LNPs for *in vivo* CAR-T generation offers several advantages (Rurik et al. 2022). Firstly, the success of mRNA vaccines for COVID-19 underscores that LNP-based mRNA delivery is both feasible and effective, eliminating the need for *ex vivo* preparation of CAR-T cells. This method allows for direct *in vivo* generation of CAR-T cells through mRNA delivery, offering a fresh approach to clinical applications.

Additionally, this technology is a non-viral method of CAR-T generation, avoiding the risks associated with viral integration and potential genomic alterations in T cells. It also allows for the transient generation of CAR-T cells, making it suitable for diseases that require short-term treatment strategies, and presents a more economical and

streamlined approach to CAR-T therapy compared to traditional methods, which are often complex and costly.

Despite these benefits, there are challenges in applying this technology to treat cardiac injuries and other diseases. The limited number of CAR-T cells reaching the site of injury can restrict therapeutic effectiveness (De Marco et al. 2023). While CAR-T therapy has been highly effective in treating hematological malignancies, its efficacy in cardiac injuries is hindered by the limited infiltration of immune cells into the affected areas (Mitra et al. 2023). Exploring the interactions between FAP CAR-T cells and activated fibroblasts could clarify the role of antigen-specific CAR-T cells in cardiac injuries and potentially identify new therapeutic targets (Abdalla et al. 2024). This could optimize *in vivo* CAR-T therapy for cardiac injuries, enhancing CAR-T cell infiltration and function at the injury site, and providing valuable insights for extending this therapy to other diseases.

Moreover, CAR-T therapy often triggers severe inflammatory responses due to the release of large amounts of cytokines by activated cells, leading to cytokine release syndrome and potential organ damage (Brudno and Kochenderfer 2016). The transient expression and short-term action of mRNA LNP-generated CAR-T cells offer a way to manage these effects, though further optimization might be achieved by designing molecular 'switches' to control the activation and deactivation of target genes, enhancing the therapeutic outcome (Ongun et al. 2022).

Drawing on the extensive experience gained from traditional CAR-T development, which has involved designing and modifying CAR structures to enhance activity, similar principles could be applied to optimize mRNA LNP in vivo CAR-T technologies. By studying the pathogenesis and progression of non-tumoral diseases like cardiac injuries, it's possible to optimize antigen target selection. Integrating cytokine, chemokine, or receptor mRNAs could improve T cell proliferation and specific homing to cardiac injury sites, enhancing the effectiveness of cardiac repair. Additionally, understanding the local microenvironment changes in cardiac injuries is crucial for defining advantageous therapeutic strategies for CAR-T cells. Combining the strengths of traditional CAR-T and mRNA LNP *in vivo* CAR-T technologies aligned with disease progression patterns could lead to the development of a comprehensive treatment strategy combining both short-acting broad targets and long-acting specific targets for enhanced therapeutic efficacy.

Conclusion

In conclusion, the advent of novel immunotherapy strategies, particularly CAR-T cell technology, has ushered in promising avenues for treating heart diseases - a realm traditionally dominated by therapies that manage symptoms without fully rectifying underlying pathologies. This paper illustrates the potential of engineered T cells to specifically target and mitigate myocardial fibrosis by eradicating activated fibroblasts, a significant advancement over conventional treatments that often involve long-term medication or invasive procedures with considerable side effects. The novel approach of *in vivo* transient generation of CAR-T cells, employing lipid nanoparticles to deliver mRNA encoding chimeric antigen receptors directly into T cells, represents a significant leap in therapeutic strategy. This method not only circumvents the complexities and risks associated with viral vector-based CAR-T cell generation but also offers a rapid, scalable, and potentially safer alternative by limiting the duration of CAR-T cell presence, thereby reducing the likelihood of prolonged immune activation or other adverse effects.

Furthermore, the research underscores the critical role of fibroblast activation protein as a therapeutic target, an area that previous studies have explored but not without limitations. By fine-tuning the application of CAR-T cell therapy to target specific disease markers such as FAP in cardiac tissues, this innovative approach could substantially reduce myocardial fibrosis and improve heart function. This promising development in cardiac repair highlights the versatility of CAR-T cell therapy, initially developed for oncology applications, and its potential expansibility to treat other chronic conditions that involve inflammatory and fibrotic processes.

The successful application of this technology in preclinical models offers a hopeful outlook for its clinical translation, suggesting that mRNA-based transient CAR-T cell therapy could soon be a viable option for cardiac patients. It provides a robust foundation for further research, potentially leading to broader applications of CAR-T technology beyond oncology, including chronic inflammatory and fibrotic diseases. As this technology continues to evolve, it holds the promise of revolutionizing the treatment of heart diseases, providing patients with more targeted, effective, and safer therapeutic options.

Author Contributions

Ruxia Zhang: Investigation and Writing – Original Draft. Ganggang Si, Jianjun Li and Xiangbing Li: Investigation. Huahua Cui and Sancong Pan: Writing – Review & Editing and Supervision.

Conflict of Interest

The authors declare no conflict of interest.

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