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INDUSTRY NEWS

CRISPR-Cas9 for the Treatment of Transthyretin Cardiac Amyloidosis

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1. INTRODUCTION

Transthyretin amyloidosis (ATTR) is a progressive life-threatening disease caused by the accumulation of misfolded transthyretin (TTR) within the heart [1]. ATTR amyloidosis can be categorized into two forms: variant (ATTRv), caused by mutations in the *TTR* gene, and wild-type (ATTRwt) amyloidosis, occurring in elderly individuals without *TTR* gene mutations [2]. Predominantly synthesized in the liver, TTR protein forms a homotetramer complex acting as a transporter for thyroxine and retinol-binding protein [3]. Amyloidogenesis starts with the tetramer dissociation followed by the monomers misfolding and aggregation into oligomers and fibrils, affecting various organs such as the heart and peripheral nervous system (PNS) [4]. The two main clinical phenotypes of ATTR are cardiomyopathy (ATTR-CM) and polyneuropathy (ATTR-PN). Amyloid fibrils can infiltrate every cardiac structure, thus leading to the development of a pseudo-hypertrophic phenotype, heart failure, valvular heart disease (namely aortic stenosis), and arrhythmias. PNS involvement leads to a rapidly progressive sensory-motor axonal neuropathy with symptoms of paresthesias, burning pain, dysesthesias, and dysautonomia, including impotence, diarrhea, or constipation [4]. In ATTRv-CM, orthotopic liver transplantation (OLT) has been the primary treatment approach as it allows to replace mutant TTR with normal TTR. However, this option is limited by donor availability, surgical risks, and complications associated with immunosuppressive therapy. Additionally, disease progression is often observed despite OLT [5]. Recently, the development of TTR stabilizers, such as tafamidis or diflunisal, has provided a targeted therapeutic option for ATTR-CM [6, 7]. Tafamidis binds to the TTR tetramer, stabilizing its structure and inhibiting further formation of amyloid fibrils. Clinical trials have shown that tafamidis can slow disease progression and improve the quality of life in ATTR-CM patients [6]. Another approach currently approved for ATTRv-CM is the use of RNA interference (RNAi) strategies. RNAi utilizes small interfering RNAs (siRNAs, *i.e.*, patisiran) or antisense oligonucleotides (ASOs, *i.e.*, inotersen) to silence the production of TTR [8]. Patisiran and inotersen have shown promising results in treating ATTR amyloidosis, demonstrating reductions in TTR levels and improvements in quality of life and symptoms [9, 10]. Based on current evidence, tafamidis is the only approved treatment for ATTRwt-CM and isolated ATTRv-CM. Isolated ATTRv-PN can be treated with tafamidis, patisiran or inotersen, whereas ATTRv-PN and CM can be treated with tafamidis or patisiran. While these treatment options provide advancements in managing ATTR-CM, challenges remain, including the high cost of therapy, the requirement for long-term administration, and the need for early and accurate diagnosis. Moreover, treatment with patisiran requires premedication with glucocorticoids and antihistamines, whereas inotersen therapy may rarely show serious adverse events, such as glomerulonephritis or decreased platelet counts [11].

The phase 3 APOLLO-B trial (NCT03997383) will also evaluate patisiran in ATTR-CM, both variant and wild type. Furthermore, there are ongoing phase III clinical trials. HELIOS-B (NCT04153149) will evaluate second-generation siRNA vutrisiran in ATTR-CM. A second-generation ASO, eplontersen, will be evaluated for the treatment of ATTRv-PN (NEURO-TTRansform, NCT04136184) and ATTR-CM (CARDIO-TTRansform, NCT04136171). A therapeutic strategy based on monoclonal antibodies (*i.e.* PRX004, NI301A) capable of selectively inducing amyloid removal is being tested in different clinical trials with promising results [12].

Currently, approved treatment options for ATTR amyloidosis have revolutionized the management of the disease; however, these drugs have several limitations, which highlight the need for alternative strategies.

2. CRISPR-Cas9 BASED STRATEGIES

Clustered, regularly interspaced short palindromic repeats (CRISPR)-Cas9 discovery by Emmanuelle Charpentier and Jennifer Doudna has profoundly revolutionized biomedical research. It is currently used in many different areas, from controlling pathogens and pests to the creation of transgenic disease models or genetically modified organisms [13]. In medicine, it is currently being studied for the treatment of several diseases, such as cancer, progeria, beta-thalassemia, sickle cell disease, hemophilia, cystic fibrosis, Duchenne's muscular dystrophy, Huntington's disease, malaria, type 1 diabetes, and many others [14]. The first clinical trial on CRISPR-based *ex vivo* genome editing tried to treat human immunodeficiency virus 1 (HIV-1) infection by disrupting *CCR5*, a critical receptor for the viral compartment [15]. CRISPR-edited *CCR5*-ablated hematopoietic stem were successfully transplanted into a 27-year-old patient with HIV-1 infection and acute lymphoblastic leukemia, resulting in incomplete leukemia remission for 19 months. During that time, the *CCR5* disruption ranged from 5.20 to 8.28% in bone marrow cells [15]. Recently, a CRISPR-based approach has also been used in one subject with transfusion-dependent beta-thalassemia and one subject with sickle cell disease, resulting in high levels of allelic editing, increases in fetal hemoglobin, and

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transfusion independence [16]. However, despite no off-target effects being seen, adverse effects such as neutropenic pneumonia, hepatic vein-obstructive infection, and sepsis were observed in both patients [16]. Finally, CRISPR-based genome editing has emerged as a successful and powerful tool to enhance the natural ability of human T cells to fight different types of cancer [17, 18].

ATTR-CM represents the ideal candidate for gene-editing strategies for different reasons: higher reduction in TTR levels is associated with improved clinical outcomes; ATTR-CM is a monogenic disease; the disease-causing protein, TTR, has only limited functions, and its knockdown has therefore a limited impact; TTR is mainly produced by the liver, for which established drug delivery systems exist, and specific target should maximize efficacy while minimizing systemic toxic effects [19].

CRISPR-Cas9 gene editing technology offers exciting opportunities for precise and targeted interventions in the treatment of ATTR-CM. NTLA-2001 is a new CRISPR-Cas9-based *in vivo* gene-editing therapy for the treatment of ATTR-CM. NTLA-2001 consists of a lipid nanoparticle (LNP) delivery system with liver tropism carrying a single guide RNA (sgRNA) that targets human TTR and a mRNA sequence of the Cas9 protein. Once injected intravenously, plasma apolipoprotein E opsonizes the LNP surface in circulation, and the LNP is then actively endocytosed by hepatocytes through the low-density lipoprotein receptor. After the breakdown of the LNP, the TTR-specific sgRNA interacts with the Cas9 endonuclease, thus inducing DNA cleavage in the TTR sequence and preventing further production of TTR (Fig. **1**). Preclinical studies have shown that single NTLA-2001 doses resulted in sustained reductions in serum TTR protein of >95% [20].

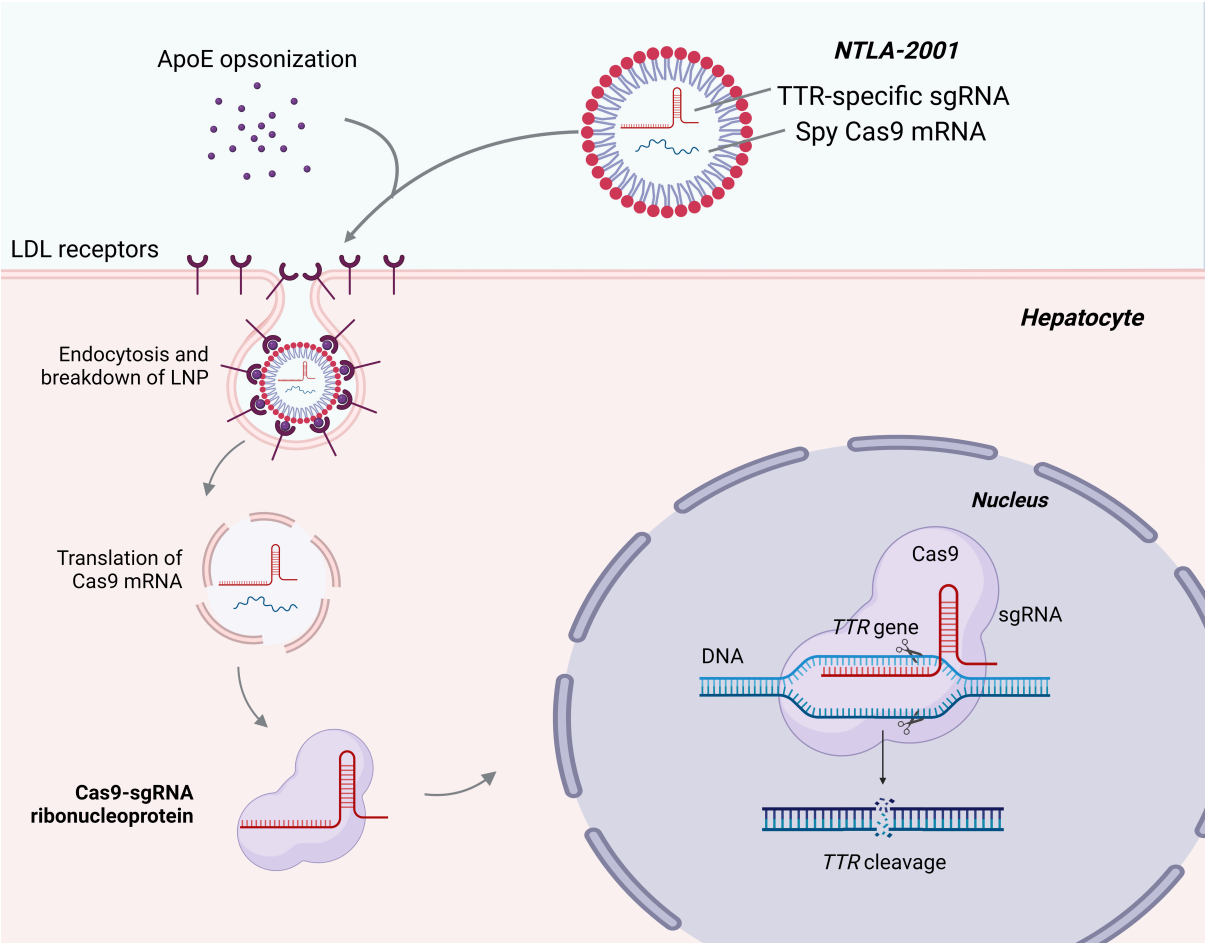

Fig. (1). NTLA-2001 mechanism of action. NTLA-2001 consists of a lipid nanoparticle (LNP) delivery system carrying a single guide RNA (sgRNA) that targets human TTR and an mRNA sequence of the Cas9 protein. Once injected intravenously, plasma apolipoprotein E opsonizes the LNP surface in circulation, and the LNP is actively endocytosed by hepatocytes through the low-density lipoprotein receptor. After the breakdown of the LNP, the Cas9 mRNA is translated, and the so-obtained Cas9-sgRNA ribonucleoproteic complex enters the nucleus. The complex recognizes the TTR gene, thus inducing a specific cleavage. **Abbreviations**: LDL, low-density lipoprotein receptor; LNP, lipid nanoparticle; mRNA, RNA messenger; sgRNA, single guide RNA; Spy, Streptococcus pyogenes; TTR, transthyretin. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

A major step forward in the application of gene therapy has been represented by the first in human *in vivo* genome editing trial, published in the New England Journal of Medicine in August 2021. In this study, Gillmore and colleagues have recently provided interim data from part 1 of a two-part, global, phase 1, open-label, multicenter study evaluating single ascending doses of NTLA-2001 for the treatment of ATTRv amyloidosis with polyneuropathy (ATTR-PN).19 Six patients were involved, 3 in each of the two initial dose groups (0.1 mg per kilogram and 0.3 mg per kilogram). Exclusion criteria were represented by non-ATTR amyloidosis, known leptomeningeal ATTR amyloidosis, and a history of treatment with RNA-silencing therapy. Previous use of TTR stabilizers was permitted with a washout period (3 days for diflunisal). Serial assessments of safety during the first 28 days after infusion in patients revealed only mild infusion-related adverse

events in 3/6 patients. At day 28, the mean reduction from baseline in serum TTR protein concentration was dose-dependent, being 52% (47 to 56) in the group that received a dose of 0.1 mg/kg and 87% (80 to 96) in the group that received a dose of 0.3 mg/kg [19].

In November 2022, interim data from the cardiomyopathy arm of the same ongoing part 1 of the study were presented at the American Heart Association Scientific Sessions 2022. Twelve patients with ATTR-CM and a New York Heart Association (NYHA) class I-III were treated with single doses of 0.7 and 1.0 mg/kg of NTLA-2001. Administration of NTLA-2001 led to deep and sustained TTR reduction of > 92% through patient follow-ups ranging from four to six months. Only mild infusion-related reactions were reported (3/12 patients) [20].

CONCLUSION

CRISPR-Cas9 has emerged as a promising alternative to TTR stabilizers and RNAi-based therapeutics [21, 22]. The study by Gillmore *et al.* represents the first-in-human clinical trial of a systemically delivered CRISPR/Cas9-based therapy. NTLA-2001 led to decreases in serum TTR protein through targeted knockout of the TTR gene while being associated with only mild adverse events. For this reason, NTLA-2001 has the potential to become a single-dose treatment to inactivate the TTR gene and reduce TTR production permanently. As compared to current treatment strategies for ATTR amyloidosis, NTLA-2001 has the advantage of inducing a deep and permanent reduction in serum TTR protein concentrations by requiring just a single intravenous infusion to inactivate the *TTR* gene at the root of the disease. On the other hand, some disadvantages must be acknowledged, such as the high therapy costs and the potential for immune responses and off-target effects, as well as the current lack of larger studies to assess its safety and efficacy. In the future, ATTR amyloidosis treatment may be represented by both blocking TTR production with gene editing or RNAi strategies and removing tissue amyloid with specific antibodies; the first approach would drastically reduce circulating TTR levels and avoid the need for TTR stabilizersHowever, several challenges need to be addressed to optimize the safety and efficacy of CRISPR-Cas9-based therapies in clinical settings. These include ensuring long-term stability and monitoring eventual off-target effects. Moreover, the extremely high costs of gene therapies may hinder their sustainability. Phase 1 of the trial is currently ongoing, and dose escalation will continue with a goal of producing maximum reductions in serum TTR in both ATTRv-PN and ATTR-CM, which will likely result in improved symptoms and clinical outcomes in these patients.

LIST OF ABBREVIATIONS

ASO	=	Antisense Oligonucleotides
ATTR	=	Transthyretin Amyloidosis
ATTRv	=	Variant Transthyretin Amyloidosis
ATTRwt	=	Wild-type Transthyretin Amyloidosis
CM	=	Cardiomyopathy
CRISPR-Cas9	=	Clustered Regularly Interspaced Short Palindromic Repeats-Cas9
HIV-1	=	Human Immunodeficiency Virus 1
LNP	=	Lipid Nanoparticle
NYHA	=	New York Heart Association
OLT	=	Orthotopic Liver Transplantation
PN	=	Polyneuropathy
PNS	=	Peripheral Nervous System
RNAi	=	RNA Interference
sgRNA	=	Single Guide RNA
siRNA	=	Small Interfering RNA
TTR	=	Transthyretin

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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