Potential of gene editing for clinical use highlighted at ASGCT 2021

By Samantha Black, PhD, ScienceBoard.net Editor in Chief

The Science Advisory Board

See Original Article

May 13, 2021 -- From base editing to in vivo CRISPR therapeutics and CRISPR-modified bacteriophages, scientists discussed innovations in preclinical research that have allowed them to advance these unique products to the clinic during a scientific symposium at the 2021 American Society of Gene & Cell Therapy (ASGCT) virtual meeting.

Targeting cardiovascular disease

One ASGCT presentation discussed a potential therapy for the genetic form of atherosclerotic cardiovascular disease, familial hypercholesterolemia, which results in the high accumulation of deadly low-density lipoprotein cholesterol (LDL-C). Nearly 31 million people are affected by the genetic variant of the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene that causes familial hypercholesterolemia.

One potential way to address this disease is using base editing strategies that can with a single administration turn off the gene responsible for LDL-C accumulation and provide lifelong protection. Verve Therapeutics is working toward this goal through the development of its lead candidate, VERVE-101. Dr. Andrew Bellinger, PhD, chief scientific officer at Verve, described the development work on the candidate.

VERVE-101 is a lipid nanoparticle containing messenger RNA (mRNA) encoding an adenine base editor (ABE) and guide RNA (gRNA) targeting PSCK9, which is designed to use a Cas9-like nuclease to change an adenine to guanine and effectively turn off the gene. Studies in nonhuman primates demonstrated that the in vivo treatment was 67% effective, providing 89% reduction in blood PCSK9 protein levels, and 59% reduction of blood LDL-C levels. Researchers at the company showed that this effect was sustained over a period greater than 10 months.

To address the safety of the product, the Verve team used mouse models of the genetic form of familial hypercholesterolemia to determine delivery efficacy and off-target editing. They found that VERVE-101 stays predominantly in the liver and was well tolerated in nonhuman primates. ONE-seq and ABE digenome-seq in vitro screening was used to identify potential sites where the base editor could make a change. When these sites were compared to the treated monkey genomes, they found only a single site, not present in humans, where an off-target change was made.

Therefore, Verve is confident that VERVE-101 will be very safe to use in humans. The company anticipates filing an investigational new drug application and beginning clinical trials for the candidate in 2022.

Targeting liver disease

Another presentation at ASGCT 2021 discussed familial transthyretin amyloidosis, a rare inherited condition characterized by progressive fatal buildup of amyloid, caused by mutations in the TTR gene. Dysfunctional transthyretin protein (encoded by the TTR gene) can fold to form amyloid, where buildup can cause nerve and tissue damage. This disease affects an estimated 200-500 million individuals worldwide.

Intellia Therapeutics is approaching the treatment of this disease by knocking out the TTR gene in the liver. According to Laura Sepp-Lorenzino, PhD, Intellia's chief scientific officer, the idea is that the elimination of the TTR gene, which produces circulating protein, can potentially halt disease progression and reverse disease. The company's lead candidate, NTLA-2001, is designed as a single-dose lipid nanoparticle that contains mRNA encoding CRISPR/Cas9 and gRNA targeting the TTR gene.

Preclinical evaluation of this therapeutic candidate was focused on providing evidence of transient expression of the Cas9 protein, efficacy of knockout, and safety. Using human hepatocytes in in vitro and mouse disease models, Intellia researchers looked at the accumulation of the gene in different organs and confirmed that the treatment led to effective knockout of the TTR gene. With nonhuman primate testing, they showed a reduction of TTR protein reduction that was sustained for over a year. Furthermore, the lipid nanoparticle and mRNA cargo was eliminated within 24 to 48 hours after administration.

Because CRISPR systems make double-stranded breaks in DNA, Intellia scientists considered the potential unwanted changes not only to gene sequence, but also in DNA structure. Similar to Verve, Intellia conducted a battery of tests to assess off-target effects of NTLA-2001, including Site-seq and Guide-seq. These methods provided a list of potential off-target sites, of which seven sites were validated as off-target loci. Further testing in hepatocytes revealed that editing at all of the identified sites were below the level of detection.

Intellia is currently conducting a phase I clinical trial evaluating the in vivo knockout strategy for treatment of familial transthyretin amyloidosis in the U.K. and New Zealand.

Targeting antibiotic-resistant bacteria

Lastly, and with an entirely unique approach, Locus Biosciences presented how its recombinant bacteriophage is being developed for the treatment of dangerous pathogens in the gastrointestinal tract, primarily those causing urinary tract infections.

Locus' platform is capable of producing bacteriophages (bacterial viruses) containing CRISPR-Cas3 materials, which it calls CRISPR phage (crPhage), explained Dave Ousterout, PhD, co-founder and chief scientific officer of Locus. The technology exploits the natural ability and specificity of bacteriophages to deliver a range of therapeutic proteins.

Importantly, the Cas3 protein differs from its relative, Cas9, by generating single-strand breaks that result in large deletions of targeted DNA (up to 100,000 kilobase pairs). Self-targeting sequences contained in the CRISPR-Cas3 machinery essentially eliminates the instructions that bacteria need in order to replicate, thereby causing cell death.

The company has largely focused on modifying the engineering of the therapy to enhance the bactericidal activity of bacteriophages.

Paired in vitro and in vivo testing of *Escherichia coli, Clostridium difficile*, and *Pseudomonas aeruginosa* demonstrated the highly potent and efficient killing of these bacteria with the use of

crPhage compared to wildtype bacteriophages. Locus is currently evaluating its crPhage therapy for the treatment of urinary tract infection caused by *E. coli* in a phase I clinical trial and anticipates starting a phase II trial this year.