Targeting the Kidney: The Promise of RNA-Based Therapeutics

When Yessenia Gutierrez was just nine years old, her kidneys failed; at 10 years old, she received her first kidney transplant. Gutierrez was born with polycystic kidney disease (PKD), a genetic condition that makes fluid-filled cysts grow on the kidney and can lead to kidney failure.

Growing up, Gutierrez had to stop attending school due to her frequent doctor visits and three-times-a-week dialysis treatments, which each lasted three hours. While the transplant was initially successful, within two years, Gutierrez's lab results showed a massive decrease in kidney function caused by a common virus, causing her to lose the kidney and go back on dialysis. Stories like Gutierrez's are not uncommon, and fatal conditions like chronic kidney disease (CKD) affect around 850 million people worldwide. By 2040, it is predicted to become the fifth most common cause of years of life lost. Unfortunately, CKD is irreversible and progressive; patients who advance to end-stage renal disease require dialysis or renal transplantation, which negatively impacts quality of life and uses 2-4% of annual healthcare budgets in high-income countries (2).

Inherited kidney disorders, like PKD, represent a large number of conditions that can lead to chronic kidney disease or kidney failure. Currently, around 100 genetic kidney disorders have been found, the majority of which have no curative therapies available (1). Instead, treatments focus on preserving kidney function and delaying disease progression (1).

As the field of gene therapies expands, RNA-based therapies could offer a new approach to treating kidney disease. While several RNA-based oligonucleotides are approved for targeting the liver, those directed toward the kidney have not advanced as far. However, preclinical studies using RNA-based therapies to treat kidney conditions are underway.

The kidney: a historically complex organ for genetic medicines

Oligonucleotide therapies have become promising tools for the treatment of diseases. However, the significant obstacle of directing them to specific organs remains. The kidney is a complex, highly vascularized organ that's been difficult for oligonucleotides to specifically target (2). Following administration, most oligonucleotides rapidly distribute in the bloodstream and accumulate in the liver, spleen, and kidneys (2). The kidneys account for up to 20% of the oligonucleotide concentration and are reabsorbed through receptors like megalin. A significant advantage of targeting the kidneys with oligonucleotides is that the therapies are swiftly cleared from the circulation through renal filtration, favoring the kidney's biodistribution over other organs (2). However, a high concentration in the kidneys can also lead to adverse effects like ASO-induced toxicity, which must be considered when designing therapies for the kidneys (2). Optimized delivery platforms can help minimize toxicity and increase the drug's efficiency.

A delivery method proven to be highly effective at delivering different types of oligonucleotides to various cell types is ligand conjugation. A ligand is any molecule or atom that irreversibly binds to a receiving protein molecule, also known as a receptor, and can be divided into five categories: peptides, antibodies, nucleic acid aptamers, carbohydrates, and small molecules (1). When these ligands are linked to an oligonucleotide, they facilitate delivery to cells with the corresponding receptor and allow precise delivery to desired cell types, which minimizes drug exposure to other parts of the body, improves the drug's safety, and reduces the amount of drug needed to treat the disease.

RNA-BASED STRATEGIES FOR KIDNEY DELIVERY

Promisingly, research is underway to develop systems that safely and effectively deliver siRNA therapeutics to the kidney, as well as studies examining how several other RNA-based therapies may succeed in targeting the kidney.

Short interference RNAs (siRNA) are short, double-stranded RNAs that can silence genes by targeting and degrading mRNA in a sequence-specific manner. Currently, there are approved siRNA-based therapies that target the liver via the chemically conjugated N-acetylgalactosamine (GalNAc) ligand, which effectively binds to asialoglycoprotein receptor (ASGPR), a protein on the surface of liver cells (2). While siRNA delivery to the liver has been solved, siRNA therapies generally have had significant delivery barriers due to its negative charge, high molecular weight, poor membrane permeability, and the instability of unmodified siRNA in the bloodstream (3).

One of the earliest studies targeting the kidney used siRNA to improve glomerularsclerosis in a mouse model of glomerulonephritis — a group of kidney diseases that damage the tiny kidney filters called glomeruli — by modulating the transforming growth factor beta (TGF) pathway by silencing *Mapk1* (2). Other studies have evaluated the use of siRNA-based drugs in alleviating the extent of acute kidney injury (AKI), an unavoidable side effect of numerous medical treatments and surgical procedures that deprive the kidney of oxygen (2). For example, a study aiming to reduce the effects of AKI showed promising results in a mouse model that evaluated the potential of 53 different siRNA targets, primarily related to apoptosis, inflammation, and immune rejection pathways after ischemia-reperfusion caused by transplantation. This approach is still being studied, and the mouse model has shown promising results (2).

A 2024 study published in Chinese Chemical Letters used a delivery system that chemically conjugated p53 siRNA to renal tubular cell-targeting peptides (3). Results showed that the peptide-siRNA conjugate could specifically enter renal tubular epithelial cells and effectively silence the targeted p53 gene in

cisplatin-induced acute kidney injury (AKI) mice and reduce kidney damage (3). This therapy presents a potential new way of treating AKI.

Small activating RNAs (saRNA) are double-stranded RNAs that have a biologically opposite function of siRNAs, and instead of silencing genes, they enhance gene transcription. Therapeutically, saRNA has been tested in rats using an in vivo model of kidney crystal formation and resulted in significantly increased expression of Trpv5, a key protein for calcium transport and reabsorption in the kidney and was, therefore, able to promote calcium reabsorption and reduce crystal formation and kidney stones in rats (2).

MicroRNA (miRNA) are a class of single-stranded non-coding RNAs that regulate gene expression and protein production. Anti-miRNA oligonucleotides (AMOs), a type of antisense oligonucleotide (ASO), have successfully limited injury and kidney fibrosis in two mouse models of acute kidney infection (2). Additionally, a mouse model of diabetic nephropathy demonstrated improvement of renal fibrosis by an AMO (2).

"However, translation of preclinical findings is sometimes complicated, as a deep understanding of the miRNA regulatory networks underlying the disease is needed," states a 2021 paper in Biomedicines. "Additionally, most miRNAs are regulated in a cell-type or organ-specific manner; thus, the possibility of off-target and undesired effects in unrelated organs is high. This problem could explain why few investigations using miRNA-based therapies move toward the clinical stage" (2).

Currently, there are two AMOs in clinical trials for the treatment of kidney diseases. First, RGLS4326 is an ASO designed to target the kidney and inhibit the function of the miRNA-17 (miR-17) family of miRNAs in autosomal dominant polycystic kidney disease (2). The drug is currently in Phase 1 clinical trials in the United States. Second, RG012 is an oligonucleotide designed to inhibit miR-21 for treating Alport's syndrome and is currently in a Phase 2 clinical trial (2).

Antisense Oligonucleotides have been studied in numerous mouse models of kidney conditions. Within the kidney, ASOs are filtered freely by the glomerulus and reabsorbed by proximal tubule epithelial cells, making antisense technology an appealing method for the potential treatment of renal disease. One of the first studies used an ASO-gapmer targeted connective tissue growth factor (Ctgf), which is involved in the pathology of diabetic kidney disease, a chronic condition that happens when the kidneys of someone with diabetes gradually lose function. The study successfully demonstrated the inhibition of Ctgf expression in a mouse model (2). Another study showed the effectiveness of using an ASO-gapmer to target the renin-angiotensin system upregulated in PKD; by inhibiting angiotensinogen, the study found a significant decrease in proinflammatory cytokines, interstitial fibrosis, and cyst volume density in two PKD mouse models (2).

In a limited number of studies, ASOs have also been studied for their potential to target the regulation of renal tumor development and metastasis in both in vitro and in vivo renal cell carcinoma models. One study examined the anti-tumor efficacy of an ASO directed against vascular endothelial growth factor (VEGF), a key player in tumor angiogenesis. The study showed reduced VEGF expression, impaired cell proliferation and migration in a renal cell carcinoma cell line, and slower tumor growth in mice bearing RCC xenografts following treatment with the ASO directed against VEGF. These studies indicate that new therapeutic tools based on ASOs are feasible for treating renal diseases (2).

Novel ligands used for targeting the kidney

Recently, Judo Bio has discovered a way to create and deliver oligonucleotide therapies to the kidney via its proprietary ligand-siRNA conjugates that allow receptor-mediated uptake of oligonucleotides to specific kidney cell types, resulting in gene silencing of disease-modifying target genes.

The Cambridge, Massachusetts-based biotech uses a platform called STRIKE (Selectively Targeting RNA Into KidnEy) to discover and develop ligand-siRNA conjugates that "harness the natural, endogenous process of receptor-mediated endocytosis to deliver oligonucleotide therapeutics selectively to specific kidney cell populations."

The approach is based on the concept of using kidney recycling receptors to encourage siRNA uptake and activity in specific kidney cell populations. Starting with the Megalin receptor family, which is highly expressed on proximal tubular epithelial cells (PTEC), its rapid internalization, slow degradation, and high recycling ability make it an optimal entry point for intracellular delivery of a ligand-siRNA conjugate.

After binding to the megalin, the ligand-siRNA is internalized into PTEC and released into the cytoplasm for intracellular processing; the siRNA then degrades mRNA, thereby reducing specific solute carrier (SLC) proteins and decreasing solute concentration.

According to Judo Bio, inhibiting the function of SLC proteins in the proximal tubule has been clinically shown to manage uncontrollable solute levels in diseases, allowing Judo to create medicines explicitly directed to the kidney, which could potentially treat conditions including inborn errors of metabolism, gout, hypertension, type 2 diabetes, chronic heart failure, nephrolithiasis, and other endocrine disorders. Additionally, it may be able to help with polycystic kidney disease as data suggests the megalin family of receptors are expressed on podocytes and cysts in PKD. Judo Bio said it has finished its preclinical studies and will continue to advance its research to clinical studies and further develop its STRIKE platform.

Using ASOs to target *APOL1*

Another genetic cause of kidney disease is a mutation in the *APOL1* gene, which is responsible for creating a protein for the body's immune system. While everyone has two copies of the gene, a mutation in both increases the risk of developing early-onset kidney disease. People of West African descent are at an increased risk of developing end-stage renal disease, and an *APOL1* mutation may be the cause of this risk.

According to a 2023 paper published by the International Society of Nephrology, ASOs designed to alleviate the effect of the *APOL1* mutation are being studied and state that, in theory, inhibiting APOL1 toxicity should help with the adverse effects of kidney diseases. In *APOL1* G1-transgenic mice, subcutaneous administration of an ASO provided dose-dependent reductions in kidney and liver *APOL1* mRNA and prevented dose-dependent interferon-induced proteinuria. Data from a Phase 1 trial has demonstrated safety, tolerability, and proof of mechanism in healthy participants. However, caution is warranted until the impact of *APOL1* expression in other systems is better understood (5).

"There are still other unresolved questions in developing *APOL1* therapeutics. There is uncertainty regarding the impact of circulating APOL1 on the kidneys and consequently a lack of clarity regarding the

relative efficacy of reducing systemic APOL1 levels versus inhibiting the function of mutant APOL1 protein," the paper states (5).

CRISPR: a genetic therapy that could help with kidney disease

While it's not an oligonucleotide therapy, the CRISPR-Cas9 system has great therapeutic potential for renal diseases that result from genetic mutations (2). However, the technique still has limitations in effectively delivering to specific cells or tissues and is still in the development of novel in vitro and in vivo models of renal disease (2). The technology could also expand available sources of kidneys for transplantation by modifying kidneys from other species. Genetic causes account for around 70% of the pediatric and 10% of the adult patients that receive kidney replacement therapy (1). Currently, a transplant from another species to a human would lead to an extreme human immune response and organ rejection. However, recently a pig kidney was gene-edited by CRISPR-Cas9 and successfully transplanted into a man with chronic kidney disease, marking the first-of-its-kind transplant.

The future of gene therapies for kidney diseases

Developments in the treatment field could have a significant positive effect on global health (2), and research into how oligonucleotide therapies can be delivered to the kidneys shows promise. Companies are pioneering techniques, and as research continues, these advancements may not only improve the management of conditions like polycystic kidney disease but also pave the way for new, targeted therapeutic options that could ultimately transform patient outcomes in the realm of genetic kidney disorders.

References

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