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PERSPECTIVE

Spotlight on Genetic Kidney Diseases: A Call for Drug Delivery and Nanomedicine Solutions

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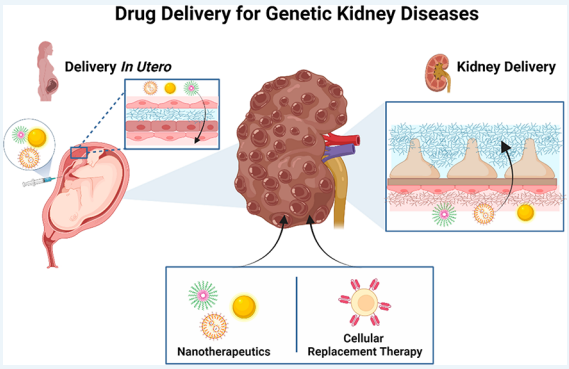
ABSTRACT: **Nanoparticles as drug delivery carriers have benefited diseases, including cancer, since the 1990s, and more recently, their promise to quickly and efficiently be mobilized to fight against global diseases such as in the COVID-19 pandemic have been proven. Despite these success stories, there are limited nanomedicine efforts for chronic kidney diseases (CKDs), which affect 844 million people worldwide and can be linked to a variety of genetic kidney diseases. In this Perspective, we provide a brief overview of the clinical status of genetic kidney diseases, background on kidney physiology and a summary of nanoparticle design that enable kidney access and targeting, and emerging technological strategies that can be applied for genetic kidney diseases, including rare and congenital kidney diseases. Finally, we conclude by discussing gaps in knowledge remaining in both genetic kidney diseases and kidney nanomedicine and collective efforts that are needed to bring together stakeholders from diverse expertise and industries to enable the development of the most relevant drug delivery strategies that can make an impact in the clinic.**



KEYWORDS: *genetic kidney disease, congenital disease, nanomedicine, cell therapy, drug delivery, rare diseases*

KIDNEY PHYSIOLOGY AND CHRONIC KIDNEY DISEASES

The kidneys are vital organs that filter blood and remove waste from the body and help maintain fluid, pH, and ionic balance. Blood circulates through the kidneys 20−60 times a day, and the kidneys filter approximately 200 L of blood daily. When the kidneys fail to function and are unable to remove excess waste from the body, survival can be maintained for only a few days to a week without medical intervention.

Each of our two kidneys consists of approximately one million filtration units called nephrons, which contain (1) a network of capillaries called the glomerulus that acts as a filter and molecular sieve and (2) a tubule which returns and reabsorbs necessary water, salts, or ions back into systemic circulation (Figure 1). When blood enters the glomerulus through the afferent arterioles, the hydrostatic pressure in the glomerulus forces metabolic waste and excess fluid to pass through the glomerular filtration barrier (GFB), which enables filtration. Filtered blood, which includes red blood cells and nonfilterable components like albumin, return into systemic circulation through the efferent arterioles, which give rise to a second set of capillary beds, including the peritubular capillaries and the vasa recta that surround the nephron. The filtrate which crosses the GFB is passed into the proximal tubules and peritubular capillaries for reabsorption of fluid and ions back into blood. The remaining fluid and metabolite waste products, drugs, urea, and excess acids and salts that are not reabsorbed are removed from the peritubular capillaries and secreted into the filtrate to become urine, which is excreted out of the body through the collecting ducts and the bladder.

Unfortunately, 37 million adults in the US and 844 million people worldwide have chronic kidney disease (CKD). CKD includes abnormalities in kidney structure or a declining function of the kidneys over the course of months to years and has emerged as one of the most prevalent causes of death in recent years. In the US, approximately 800,000 patients have

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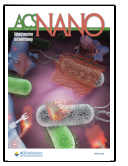

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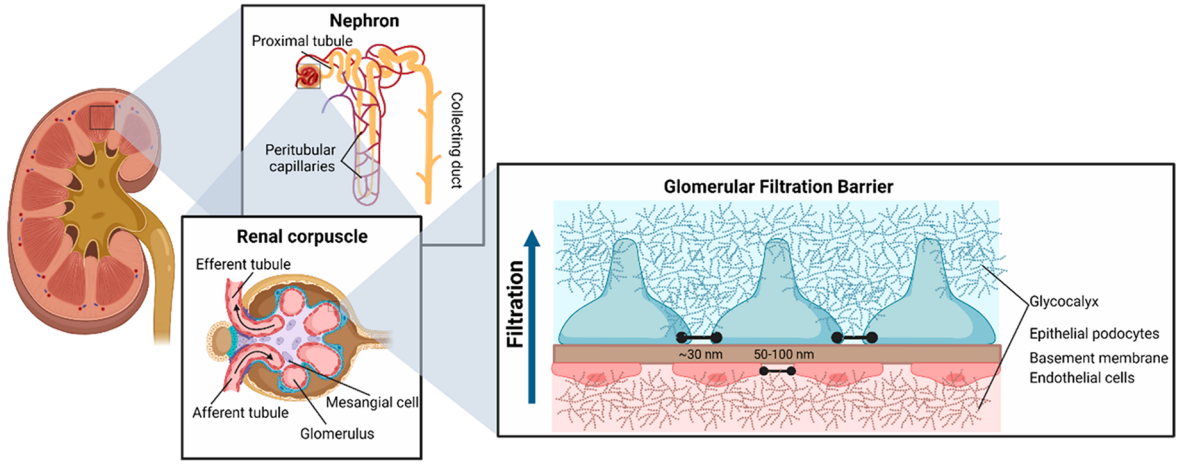

Figure 1. Anatomy of the kidney. The kidney contains millions of basic filtration units called nephrons that are comprised of a renal corpuscle, a proximal tubule innervated with peritubular capillaries, and a collecting duct for reabsorption into systemic circulation. The renal corpuscle contains a network of capillaries named the glomerulus housed within a sac-like structure called the Bowman's capsule. Associated with the glomerulus is the glomerular filtration barrier consisting of a fenestrated endothelial cell layer, a collagenous matrix called the basement membrane, epithelial podocytes, and a glycocalyx layer rich in proteoglycans which acts as a sieve to biomacromolecules and nanoparticles.

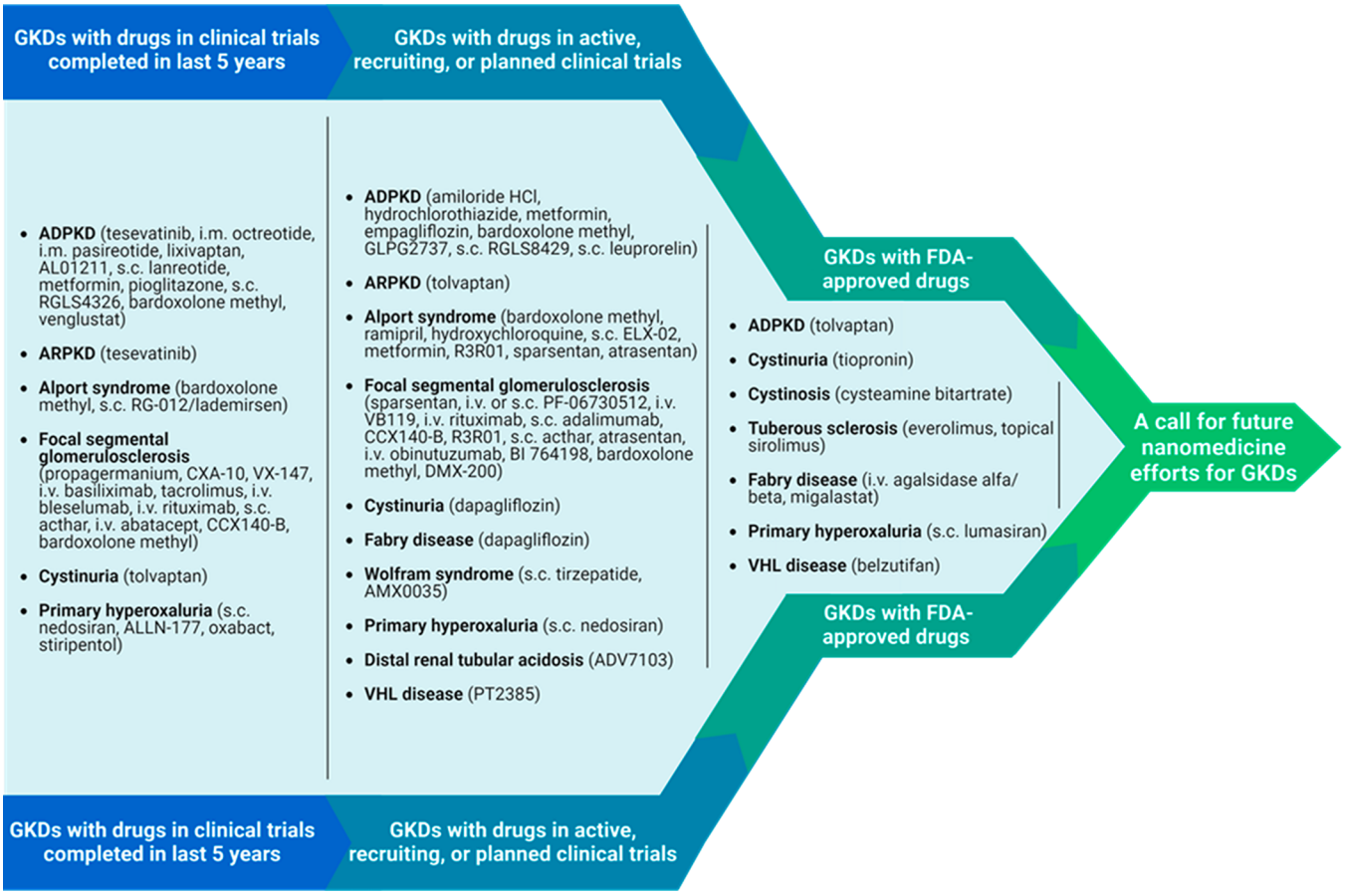

Figure 2. The current clinical landscape of genetic kidney diseases (GKDs) has no nanomedicine efforts. Clinical research has led to the FDA approval of drugs for several GKDs, while symptom management through dietary changes, ACE inhibitors, and angiotensin receptor blockers remain the mainstay treatments for most GKDs. Despite the clinical success of nanomedicine in cancer therapy and as vaccines against COVID-19, a nanotherapeutic tailored for GKDs has yet to be developed. The route of administration is oral unless otherwise stated.

been diagnosed with end-stage kidney disease (ESKD),[1] which represents the final stage of CKD and a lack of kidney function and results in the need for a regular course of dialysis or kidney transplantation to sustain life.

GENETIC KIDNEY DISEASES

With the advances of next-generation sequencing and the development of relevant animal models, approximately 500 monogenic causes have been linked to CKD and up to 20−30% of pediatric and adult CKD have been attributed to genetic

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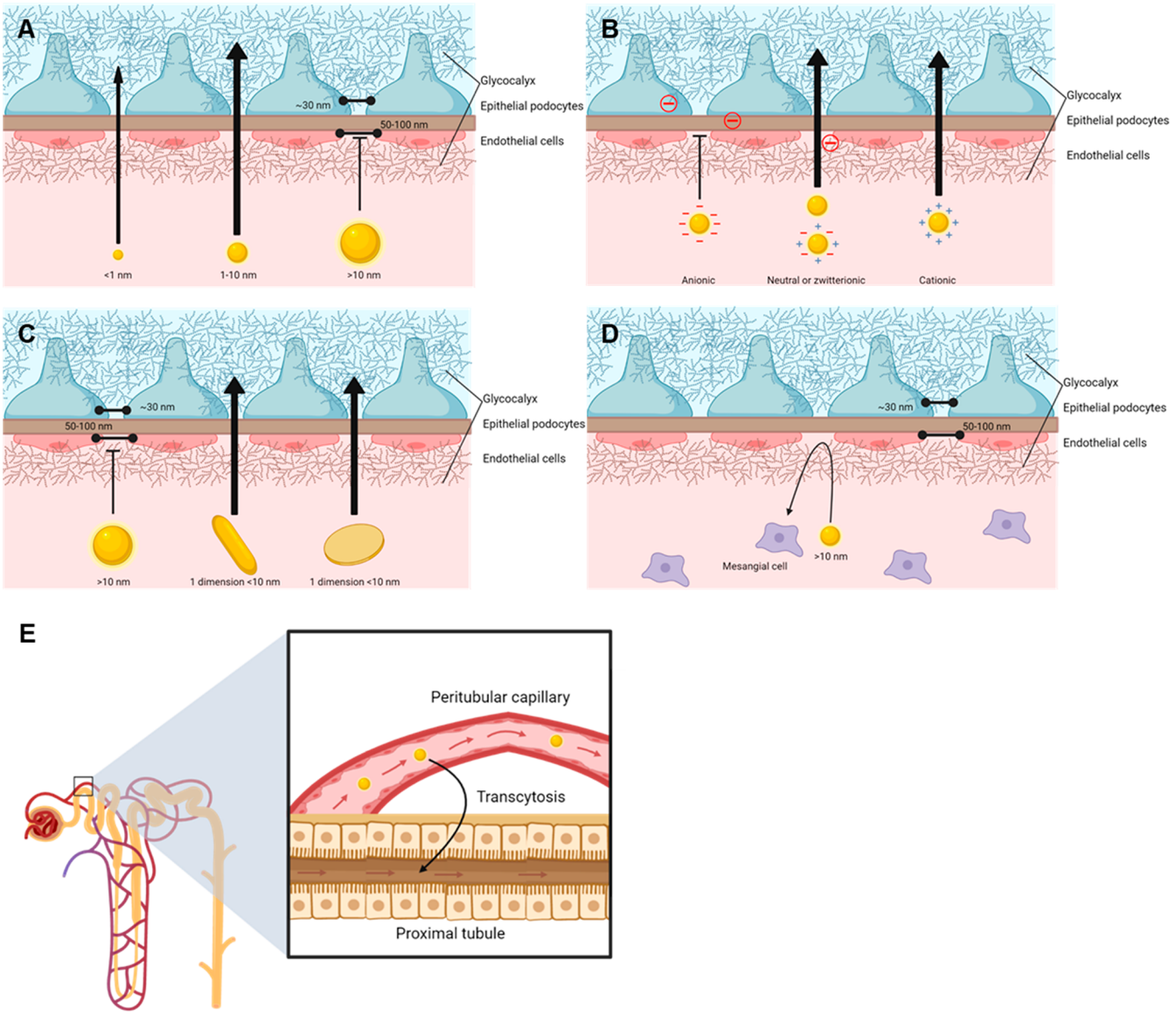

Figure 3. Nanoparticle considerations for kidney targeting. Physicochemical properties such as size, charge, and shape of nanoparticles have been reported to impact passive kidney targeting. (A) Nanoparticles around 10 nm or less in diameter have been reported to cross the GFB. Recent reports have also indicated that ultrasmall (<1 nm) particles may be retained by the glycocalyx, impairing their ability to cross the GFB.[18] (B) Surface charge has also been reported as a physical property impacting GFB filtration, as anionic particles are repulsed by the negatively charged GFB, while neutral, zwitterionic, and cationic particles are able to pass. (C) Nanoparticle shape can also be precisely engineered to achieve GFB filtration, as particles of larger surface areas or volumes that maintain one dimension below the 10 nm threshold are sufficient to enable GFB filtration.[26] Alternative strategies that enable kidney accumulation aim to avoid GFB filtration through size effects, which can increase uptake in (D) renal mesangial cells through phagocytosis and (E) proximal tubule cells following transcytosis through the peritubular capillaries.

causes.[2−6] Genetic kidney diseases encompass a broad range of inherited kidney diseases and include single-gene, monogenic disorders as well as polygenic diseases, which typically manifest in adulthood, have weak genotype−phenotype correlation, and are influenced by environmental factors. X-linked, mitochondrial, and epigenetic causes are also represented in genetic kidney diseases, but this Perspective will focus primarily on monogenic, or Mendelian, kidney disease.

The most prevalent genetic kidney disease is autosomal dominant polycystic kidney disease (ADPKD), which affects 600,000 and 12.5 million people in the US and worldwide, respectively. ADPKD is caused by mutations in the *PKD1* or *PKD2* genes and is characterized by progressive kidney cyst growth and enlargement of both kidneys.[7] Autosomal dominant diseases represent disorders where a mutation in one allele is sufficient to cause disease phenotype. In contrast, autosomal recessive (AR) diseases receive an abnormal allele from each parent and are passed on by two carriers. Thus, carriers of the autosomal recessive version of polycystic kidney disease (ARPKD), or those that have only one copy of an abnormal *PKHD1* or *DZIP1L* gene and thus do not manifest a disease phenotype, can be unaware that they are carriers until pregnancy.[8] Additional genetic kidney diseases include Alport syndrome, amyloidosis, focal segmental glomerulosclerosis (FSGS), cystinosis, nephropathic cystinuria, and a group of

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congenital disorders described as congenital anomalies of the kidney and urinary tract (CAKUT).[6,9]

While ADPKD is the most common cause of ESKD among genetic kidney diseases, ARPKD is a rare disease, or a disease that affects less than 200,000 people as defined by the FDA.[10] Although ARPKD patients progress to ESKD at varying ages, the most common disease phenotype is severe and found *in utero* by ultrasound during pregnancy and can result in death in 20−30% of newborns within 48 h after birth. Additionally, unlike ADPKD, which received its first FDA-approved therapy, tolvaptan, in 2018 to slow cyst formation, ARPKD has no cure or therapeutic options, similar to 95% of the 7,000 rare diseases that have been identified to date. Nonetheless, the fact that only one drug exists which only recently received FDA approval for ADPKD which affects 12.5 million globally suggests that kidney diseases require much more research and therapeutic efforts than previously given.

Despite FDA approval, orally taken drugs such as tolvaptan aimed to target kidney diseases do not actually accumulate to a high degree in the kidneys and have limitations. Only a fraction of the initial dose reaches the target organ; thus, the therapeutic benefits are often modest at best.[11,12] Simultaneously, off-target, adverse side effects can lead to drug discontinuation by patients and initiation of the Risk Evaluation and Mitigation Strategy (REMS) program by the FDA to survey each patient for whether the risk of taking a new therapy outweighs its benefits. Thus, research efforts in targeted drug delivery and nanomedicine which have greatly benefited other diseases, including cancer and more recently the COVID-19 pandemic, have the potential to advance therapeutic outcomes in genetic kidney diseases as well. However, no nanomedicines are currently under clinical testing or available for patients with a genetic kidney disease (Figure 2).

Before tolvaptan was approved for ADPKD, it was utilized to treat hyponatremia, or low sodium in blood, often found in patients with heart failure. Although gene therapy would be ideal to directly treat the underlying cause of a genetic disease, many therapeutic approaches for genetic kidney diseases have often applied already FDA-approved small-molecule drugs that are known to target an aberrant pathway that is also present in the genetic kidney disease to partially inhibit disease progression, like in the case for tolvaptan. Additionally, noncurative treatments to control symptoms and secondary consequences of the disease remain to be another main strategy for managing genetic kidney diseases. As such, drug discovery efforts and new therapeutic and delivery strategies that can directly target and address both efficacy and safety are needed for patients with genetic kidney diseases.

NANOMEDICINE FOR KIDNEY DISEASES

The first generation of nanoparticles was developed to increase the water solubility of hydrophobic drugs to enable higher therapeutic doses and has been used clinically for decades.[13] Since then, nanomedicine has made advances in drug delivery by tuning physical characteristics, including particle size and charge, as well as incorporating ligands for active targeting of biomarkers specifically expressed in diseased tissues. Although kidney nanomedicine is still in its infancy compared to other fields such as cancer nanomedicine, a growing body of literature has identified several design criteria, namely particle size, charge, and shape, for maximal kidney accumulation which have been detailed at length in recent reviews[14−16] (Figure 3). These design criteria stem from the distinct physiological barriers within the kidney, such as the trilayered GFB.

Passive Targeting. The GFB consists of three key components, each serving as a barrier and design criterion for developing nanoparticles for renal accumulation (Figure 1).[17,18] The first barrier is comprised of glomerular endothelial cells with fenestrations ranging from 50 to 100 nm scattered throughout the endothelium.[14] The intermediate layer is the glomerular basement membrane, a protein-rich matrix of collagen, laminin, and proteoglycans sporting pores less than 10 nm in diameter in a healthy nephron.[15] The third and final barrier consists of epithelial podocytes characterized by foot processes that form specialized cell−cell junctions called slit diaphragms that restrict the movement of molecules larger than 40 nm in diameter.[19] In addition, the endothelial cells and epithelial podocytes of the GFB are coated in a layer of proteoglycans called the glycocalyx that can entrap nanoparticles and restrict their passage. Due to these physiological barriers, particles that are less than 10 nm in diameter have generally been reported to cross the GFB most efficiently.[20] Although many sub-10 nm particles have been reported to be rapidly cleared through GFB filtration and subsequently have little renal accumulation, ultrasmall nanoparticles near the subnanometer range have been reported to be retained in the kidneys over extended periods of time (>24 h).[21,22] A proposed mechanism for the retention of ultrasmall particles proposed by Du et al. postulates that the GFB functions as a band-pass filter in which optimal filtration occurs within a size range between ∼1 nm and ∼6 nm.[18] Their studies show that at the subnanometer range, the glycocalyx functions similarly to the porous bed of a size-exclusion column, in which larger particles are excluded from the matrix entirely and pass unencumbered, while smaller particles are retained within the glycocalyx. As a result of the barrier function of the GFB, nanoparticle size represents an important design criterion for renal targeting (Figure 3A).

In addition to particle size, the surface charge of nanoparticles has been found to influence their renal uptake and filtration. Due to the presence of anionic proteoglycans and molecules like heparin sulfate in each of its layers, the GFB is strongly negatively charged and negatively charged particles are reported to be repulsed by the GFB while positively charged particles are capable of passing through the GFB with relative ease. For example, Liang et al. reported that quantum dots ranging from 3.5 to 6.0 nm with positive surface charges could be found in urine 4 h post-injection, while similarly sized anionic quantum dots could not.[23] Moreover, the cationic particles had 5-fold increased kidney retention compared to the anionic particles after 24 h (15% ID vs 3% ID). Additional studies have reported that particles with neutral or zwitterionic surfaces may increase kidney uptake, owing to their ability to resist surface opsonization with anionic serum proteins such as albumin and subsequent protein corona formation, and thereby hindering an increase in nanoparticle size and negative surface charge.[24] In addition to general kidney accumulation, particle charge has also been found to influence the region of the kidney in which the particle is internalized. For example, anionic quantum dots were reported to be repulsed by the GFB and subsequently taken up by the surrounding mesangial cells.[23] On the other hand, Zuckerman et al. reported the rapid accumulation and disassembly of cationic 60−100 nm siRNA nanoparticles in the anionic regions of the glomerular basement membrane within 30 min of injection.[25] Thus, surface charge represents

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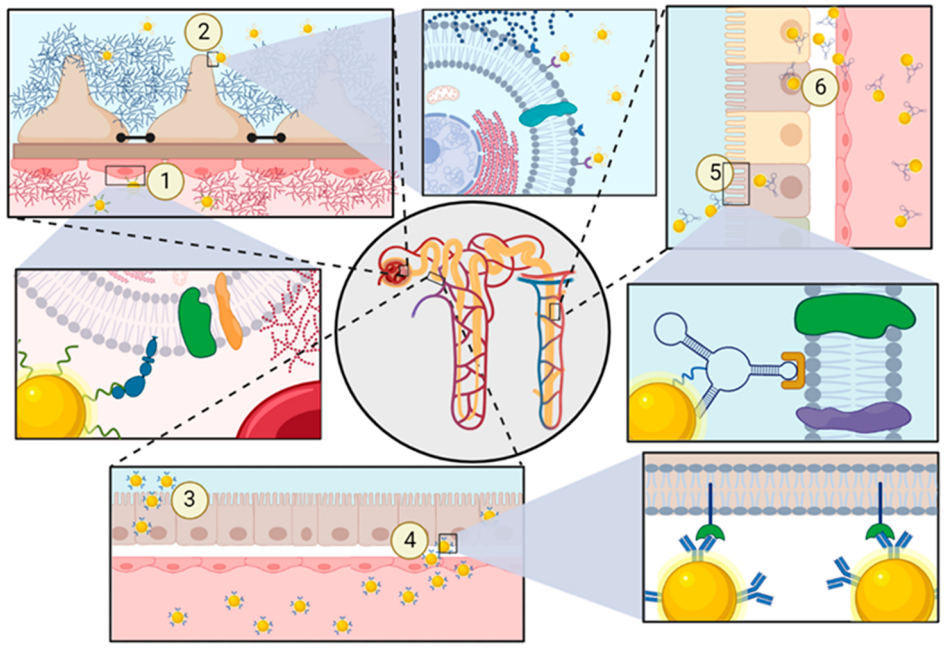

Figure 4. Active targeting of nanoparticles within the nephron. Active targeting of distinct regions of the nephron is a strategy currently being explored to treat kidney related diseases. This approach aims to increase the efficacy of the treatment by directing the therapeutic agent to the targeted area, while also reducing systemic exposure and side effects. Active targeting can be achieved through methods that include conjugation of the drug with a targeting moiety (e.g., peptides, aptamers, antibodies) that binds to specific receptors expressed by renal cells such as (1) glomerular endothelial cells, (2) podocytes, (3) the apical region of renal proximal tubular epithelial cells, (4) the basolateral region of renal proximal tubular epithelial cells, (5) the apical region of renal cortical collecting duct cells, and (6) the basolateral region of renal cortical collecting duct cells.

another design criterion that should inform nanoparticle design for kidney targeting (Figure 3B).

Beyond size and surface charge, nanoparticle shape can also be considered when designing nanoparticles for the kidney (Figure 3C). Recent reports have shown that keeping at least one dimension of a nanoparticle as sub-10 nm retains its ability to filter through the GFB, allowing the use of rod-like or cylindrical nanoparticles with near-micrometer lengths.[26] Moreover, non-spherical particles reportedly have significantly longer circulation half-times compared to their spherical counterparts, attributed to inhibition in the ability of phagocytic cells like macrophages to recognize and clear away high aspect ratio particles, and this increased circulation can enable higher kidney uptake over time.[27] Proposed mechanisms for the decreased uptake by phagocytic cells include the decreased induction of membrane curvature of high aspect particles, limiting endocytosis. Another proposed mechanism is that when one end of a rod-like or cylindrical nanoparticle interacts with a phagocyte, the other end is subjected to increased shear stress due to its high aspect ratio, brushing the particle off the cell surface before uptake is completed.

Despite the growing number of studies correlating how the physicochemical properties of nanoparticles affect their biodistribution and renal uptake, few have investigated nanoparticles within animal models of kidney disease in which the selective permeability of the GFB and kidney physiology is compromised. Similar to what has been observed in the blood brain barrier of patients with brain tumors, the GFB becomes leaky under diseased conditions, which may potentially alter the physiological barriers that govern nanoparticle uptake into the kidney. For example, Liu et al. reported that the renal accumulation of 20 and 100 nm polystyrene nanoparticles increased 2-fold in a mouse model of glomerular disease compared to healthy mice.[28] One hypothesis behind the leaky GFB stems from a decrease in contractility of injured podocytes, which limits the compression of the glomerular basement membrane, widens its porous structure, and allows larger particle passage. The characterization of renal accumulation of nanoparticles in animal models of kidney disease represents a largely unexplored vein of research that has the potential to advance our understanding and design of drug delivery strategies in the context of kidney disease.

While some nanoparticles have been designed to pass through the GFB for kidney access, another kidney targeting strategy is to design nanoparticles to avoid glomerular filtration altogether and accumulate in the kidneys through other mechanisms (Figure 3D,E). For example, the anionic charge of the quantum dots in Liang et al.'s study restricted nanoparticle interaction with the GFB, which enabled a strategy to target the kidney mesangium.[23] Targeting kidney mesangial cells was also evaluated by Choi et al., who reported that gold nanoparticles with hydrodynamic diameters of ~80 nm are too large to cross the GFB and thus can be available to be phagocytosed by the surrounding mesangial cells.[29] Following a similar approach, Williams et al. reported the accumulation of mesoscale (350–400 nm) PLGA nanoparticles in the kidneys for up to 7 days.[30,31] Subsequent imaging and histology studies revealed that renal targeting was achieved largely through basolateral accumulation in the proximal tubule epithelial cells following transcytosis by the endothelial cells lining the peritubular capillaries.

Active Targeting. In addition to designing the nanoparticle physicochemical properties to enable passive targeting and general access to the kidney, nanoparticles can be functionalized with targeting ligands to enable active targeting and specificity to kidney components such as renal tubule cells (Figure 4). Three commonly used targeting moieties in kidney nanomedicine

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include peptide ligands, antibodies, and aptamers which can allow for improved drug delivery for the treatment of specific kidney diseases.

Peptide ligands are short amino acid chains (<50 amino acids) that are customizable and compatible with a variety of linkers and can exploit the high specificity between the peptide sequence and complementary target proteins residing on the surface of the target cell. Additionally, peptide functionalization on the surface of the nanoparticle can minimize dramatic changes in final nanoparticle size due to its small molecular weight, which can benefit nanoparticles that are small enough to passage through the GFB. To that end, peptide amphiphile micelles that are on the order of 10−20 nm have been decorated with the megalin-targeting peptide $(KKEEE)_3K$ and proposed as a strategy to target early stages of PKD in which cystogenesis occurs in the proximal tubule.[22,32] To target later stages of PKD which affects the cortical collecting duct (CCD), the CCD-targeting peptide ELRGDMAAL can be functionalized onto nanoparticles and can also be used to treat other CCD disorders, including pseudohypoaldosteronism type 1.[33−35] To target the glomerulus, the peptide sequence CLPVASC identified by Pasqualini et al. through phage display may assist in targeted drug delivery for glomerular diseases, including glomerulitis.[36] A comprehensive review of peptides as targeting moieties for improved kidney drug delivery can be found by Wang et al.[37]

Like peptides, antibodies offer the advantage of target specificity but are typically much larger than peptide ligands, averaging 150 kDa (vs <10 kDa for peptides). This significant difference in size limits antibody-nanoparticle formulations to access the kidneys apically through the GFB but can be designed to reach tubule cells via the basolateral side through the peritubular capillaries. To that end, Giesecke et al. identified an antibody that selectively binds to the basolateral surface of intercalated cells in the CCD, which has the potential to be incorporated into nanoparticles for improved drug and gene delivery for disorders affecting the CCD such as Liddle's syndrome.[38,39] Along with improved drug delivery, antibody-coated nanoparticles can be utilized for the diagnosis of renal diseases as reported by Rubio-Navarro et al., in which iron oxide nanoparticles displaying an antibody against CD163 were developed to detect M2-macrophages in rhabdomyolysis-induced acute kidney injury using magnetic resonance imaging.[40]

In addition to peptides and antibodies, aptamers have been explored for kidney targeting and can be conjugated onto the surface of many nanostructures. Ranches et al. used systematic evolution of ligands by exponential enrichment (SELEX) to identify DNA aptamers that selectively bind to renal proximal tubular epithelial cells (RPTEC) and successfully identified candidates that displayed higher binding to RPTECs compared to a scrambled aptamer control.[41] Similarly, Wang et al. used SELEX to identify a DNA aptamer that exhibited binding to clear cell renal cell carcinoma cells (ccRCC) but not healthy renal cells.[42] Although the use of aptamers for renal targeting *in vivo* has yet to be fully explored, studies utilizing aptamers as targeting moieties for other cancer applications *in vivo* point to their potential.[43]

CONGENITAL KIDNEY DISEASES

Unlike diseases such as ADPKD which primarily affect adults, congenital kidney diseases are present at birth and transpire during kidney development, largely affecting infants and children, for which there are limited therapeutic options. Thus, even among genetic kidney diseases, therapeutic strategies for congenital kidney disease represent an area of immense need. Fetal kidney development begins at week five of gestation, and the kidneys start functioning and producing urine which then drains into amniotic fluid between weeks 13 and 20.[44] Kidney nephrogenesis continues until week 34, as nephron progenitor cells terminally differentiate into the kidney epithelium. Because nephrogenesis occurs during fetal development, congenital anomalies of the kidney and urinary tract (CAKUT), including aplasia (kidney absence), hypoplasia, or dysplasia, also begin manifesting at this time. In hypoplasia, or congenitally small kidneys, nephron function is maintained but in dysplasia, incorrect cellular differentiation and impaired renal function are found in addition to decreased kidney size.[45,46] Kidneys may also migrate during embryogenesis and form in an abnormal position along the urinary tract known as embryonic migration. Downstream of the kidney and the ureter, the lower urinary tract (LUT) includes the bladder and urethra. Abnormalities of the LUT include physical or functional urinary tract obstruction. Together, these anomalies prevent proper function of the kidney and urinary tract.

CAKUT are currently associated with over 40 known genes, and monogenic mutations can cause preterm birth, low birth weight, and even fetal loss.[47] However, it is estimated that about 100 genes are associated with monogenic causes of CAKUT but have yet to be characterized.[47] CAKUT are further categorized based on clinical presentation. For example, Prune Belly Syndrome (PBS) is defined by poor bladder emptying and is associated with high risk for CKD development, but the exact genetic cause remains unknown. ARPKD presents as oversized cyst-covered kidneys which inhibit kidney and lung development. Alport syndrome (AS) manifests in high levels of hematuria and proteinuria resulting from a type IV collagen mutation and often progresses to ESKD by adolescence or early adulthood.[48] Gaps in knowledge of CAKUT presentation and genetic causes pose a challenge to clinical intervention and developing therapeutic strategies.

Currently, there is no FDA-approved treatment that targets the root genetic causes of CAKUT. A potential time frame for intervention may be during pregnancy to correct the genetic defect of the developing fetus. However, testing new therapies and conducting clinical trials in pregnant populations is difficult, as recruitment is low, and the risk of fetal harm classifies this population as "vulnerable", which influences Institutional Regulatory Boards to employ higher levels of scrutiny.[49] Because CAKUT are devastating disorders and represent a largely unmet clinical need, extensive research efforts are needed toward the development of therapies for CAKUT.

Gene and Protein Fetal Delivery via Vitelline Vein and Intraamniotic Injection. Potential strategies to treat genetic diseases include knockout of defective genes or delivery of functioning genes, RNA, and protein.[50] For gene and protein delivery, drug carriers such as nanoparticles can protect biological cargo from degradation as well as enable specific delivery to a target site, thus limiting off-target effects.[51] Nanoparticle-mediated gene, protein, and drug delivery to the fetus can be accomplished via injection into amniotic fluid or into the vitelline vein, which drains into the fetal circulatory system from the yolk sac, or umbilical vein. Vitelline vein or umbilical vein and intraamniotic injection methods are direct administration routes for high nanoparticle fetal biodistribution, as interaction with the placental barrier and maternal circulatory system is bypassed. Additionally, designing nanoparticles to

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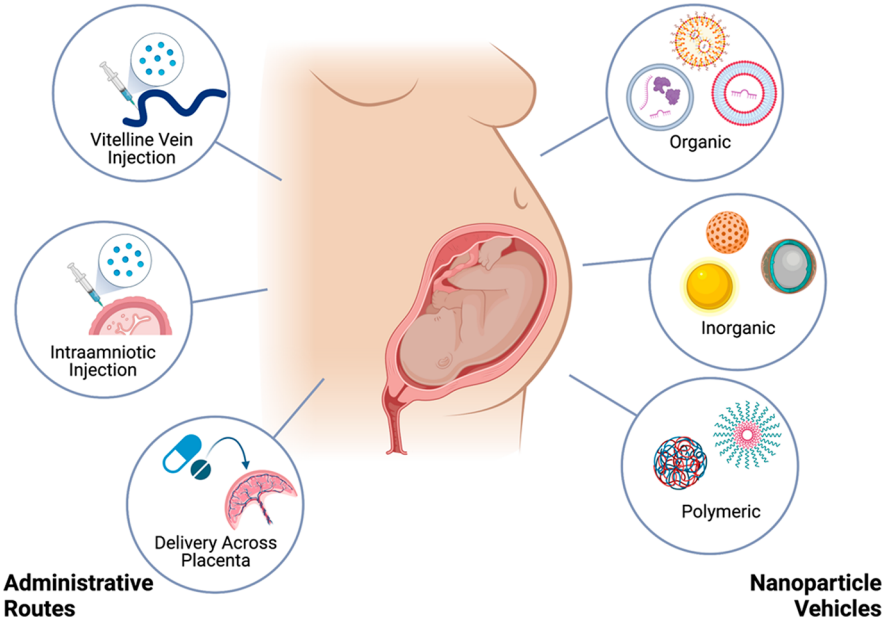

Figure 5. Fetal delivery of nanomedicine. Strategies for *in utero* delivery include injection via the vitelline vein or directly into the amniotic fluid. Engineering nanoparticles that transport across the placental barrier can enable delivery through maternal systemic circulation. Nanocarriers for drug delivery that have been recently reported for congenital diseases and fetal delivery include liposomes, extracellular vesicles, lipid nanoparticles, gold nanoparticles, polystyrene nanoparticles, and polymeric micelles.

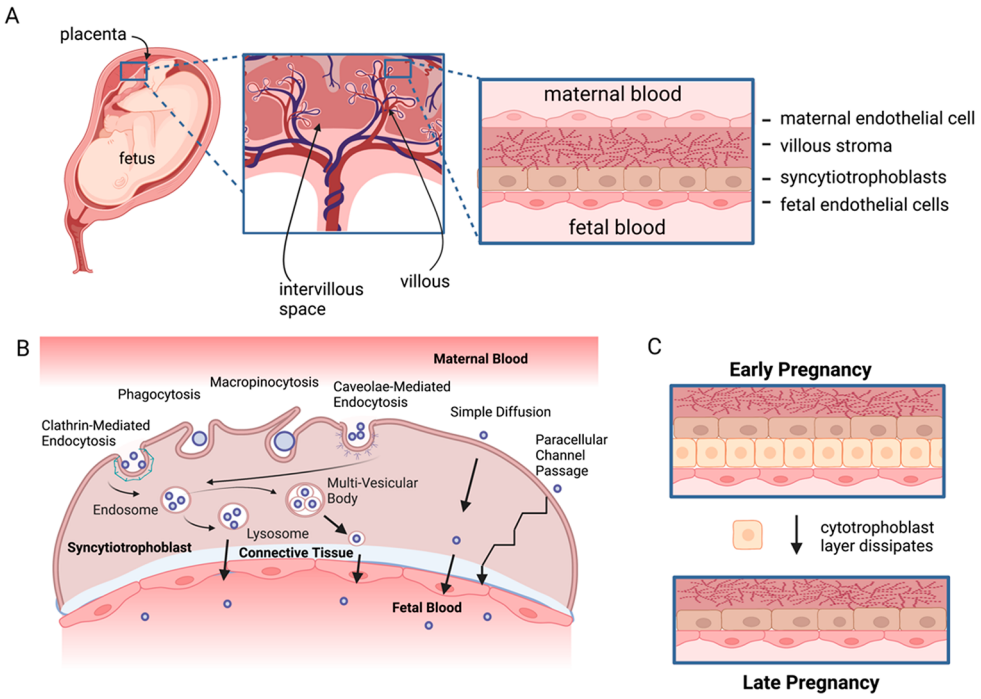

Figure 6. Overview of the human placental barrier. (A) Anatomy of the human placenta and the interface between the intervillous space and the villous space that enable maternal to fetal delivery. (B) Key pathways for nanoparticle delivery from maternal to fetal circulation. Cell barriers include syncytiotrophoblast, villous connective tissue, and fetal endothelial cells. Small nanoparticles (<25 mm in diameter) can cross the placenta via paracellular passage or via simple diffusion. Nanoparticles can cross the placental barrier via clathrin-mediated endocytosis, phagocytosis, micropinocytosis, or caveolae-mediated endocytosis. Transcytosis through the syncytiotrophoblast is mediated by endosomes, lysosomes, and multivesicular bodies. (C) Physiological changes of the placental cell structures between early and late pregnancy. Notably, the cytotrophoblast layer dissipates.

cross the placental barrier can unlock the possibility of using administration routes that are more accessible upon maternal administration (Figure 5).[52]

In murine models, the vitelline vein drains into the fetal hepatic system via the portal vein for delivery to the liver, similar to what has been observed in humans.[53] From the liver, the inferior vena cava delivers blood to the heart for circulation. The renal arteries then deliver blood from the heart to the kidneys. For intraamniotic injection, cargo can interact with tissues that directly interface with amniotic fluid such as the skin and eyes, or

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with tissues such as the lungs and gastrointestinal (GI) tract upon amniotic fluid ingestion by the fetus. From the GI tract, the portal vein delivers blood to the liver and thus, follows a pathway to the kidneys similar to that of vitelline vein injection. However, intraamniotic fluid injection is subject to dilution due to the volume of the amniotic cavity compared to fetal blood, which should be considered during dosing.

Depreux et al. utilized intraamniotic delivery of antisense oligonucleotides (ASOs) to downregulate metastasis-associated lung adenocarcinoma transcript 1 (MALAT1).[54] When injected into pregnant mice *in utero* via intraamniotic injection, a 3-fold reduction in MALAT1 was found 1 week after birth in pups that were treated with ASOs during gestation. Therefore, gene delivery upon intraamniotic injection may be a promising route to alter gene expression during development which can be sustained after birth. To investigate treatment for hypohidrotic ectodermal dysplasia, a congenital disease wherein ectodysplasin protein dysfunction causes malformation of the skin, glands and teeth, Hermes et al. delivered functional ectodysplasin *in utero*.[55] Via intraamniotic injection, they delivered ectodysplasin fused to immunoglobulin G (IgG) and found a single injection in pregnant mice led to complete correction of disease phenotype in the offspring.

To evaluate and compare the biodistribution of nanoparticles upon *in utero* delivery via vitelline vein vs. intraamniotic injection, Ricciardi et al. administered PLGA nanoparticles for gene therapy.[56,57] The authors found that delivery through the vitelline vein resulted in fetal liver accumulation, whereas intraamniotic injection resulted in accumulation in the fetal lungs and GI tract. Less than <0.000002% off-target gene delivery was found, suggesting that nanoparticle-encapsulated gene delivery can be a safe route for site-specific genetic editing in the fetus. Additionally, Riley et al. generated a panel of PLGA nanoparticles for delivery via the vitelline vein in murine models.[53] One formulation, B-4, was composed of a 14-carbon-long alkyl tail on an ionizable lipid and polyamide core and showed fetal kidney uptake, suggesting that nanoparticles can be specifically designed for fetal kidney targeting via fetal intravenous delivery, although further investigation is needed to elucidate the mechanisms that dictated fetal kidney targeting.

Nanoparticle Delivery Across the Placental Barrier. Alternatively, nanoparticle delivery can be achieved via crossing the placental barrier. Biomolecules such as IgG and glucocorticoids that naturally cross the placental barrier from maternal circulation to fetal circulation can provide insight into engineering nanoparticles for fetal delivery via maternal circulation.[58] The placental barrier that is an interface of nutrient and waste exchange between the mother and the fetus is composed of a syncytiotrophoblast layer, a cytotrophoblast layer (which becomes thinner and eventually disappears later in pregnancy), villous connective tissue, and the fetal endothelium (Figure 6A,C). These layers also represent barriers to fetal drug delivery and can inform nanoparticle design. Placental transport can be paracellular via simple diffusion or through flexural channels or transcellular via active transport (Figure 6B). Drugs with low molecular weight (<500 Da) and charge close to neutral charge can cross the placental barrier via diffusion.[58] Characterization of the placental barrier in a rodent model by Kertschanska et al. revealed that syncytiotrophoblast paracellular channels are between 15 and 25 nm.[59] Thus, nanoparticles less than 25 nm in diameter may cross the placenta passively via paracellular transport or flexural channels.[60]

Active transport across the placenta is achieved through transcytosis and is mediated by the placental syncytiotrophoblast cells. Nanoparticles can be first uptaken into the cell by clathrin-mediated endocytosis, caveolae-mediated endocytosis, phagocytosis, or micropinocytosis.[58] Then, exocytosis can occur by endosomal escape, lysosomal-mediated secretion, and multivesicular body secretion by the syncytiotrophoblast cells for transport across villous connective tissue into the fetal endothelial cells and fetal blood.[60] For active transport, Yamashita et al. found that the cutoff size for nanoparticle delivery to the placenta is ∼300 nm when studied using silica and titanium dioxide nanoparticles injected in murine models via the tail vein.[61,62] Charge did not seem to affect delivery, as particles of −76 and −13 mV of similar size were both delivered across the placenta.

Active targeting by nanoparticles can also enhance transport across the placental barrier; however, few placental receptors and targets have been identified or studied. Of those identified, FcRn are well-characterized receptors present on syncytiotrophoblast cells that facilitate transport of large molecules such as IgG across the placenta. Tse et al. functionalized chitosan nanoparticles with IgG and tested them through a transwell model of the placental barrier consisting of placental epithelial cells.[63] IgG-functionalized nanoparticles showed 2.8 times higher delivery across the placental model than control nanoparticles without disturbing barrier integrity. Alternatively, King et al. utilized surface ligand homology between the placenta and solid tumors to develop nanoparticles that target alpha-V integrin by decorating liposomes with iRGD.[64] Delivery of insulin-like growth factor via iRGD liposomes increased fetal weight in growth-restricted murine models while maternal spleen and kidney weights were unchanged, suggesting the safety of these nanoparticles. Additionally, liposomes functionalized with chondroitin sulfate A (CSA) targeting peptides showed enhanced delivery of drugs to the placenta.[65] While free drugs remained on the maternal side upon intravenous administration in pregnant mice, delivery of drug-loaded CSA-liposomes enabled efficient accumulation in placentas and led to a downstream effect in the fetus. These examples highlight the potential of active targeting nanomedicine strategies to specifically target and cross the placental barrier for fetal drug delivery.

Alternatives to synthetic nanoparticles include extracellular vesicles (EVs), which are naturally produced nanoparticles that carry proteins, DNA, and RNA and promote cell-to-cell communication.[66] Upon secretion from their parent cell, EVs enter the extracellular space and can enter circulation to reach and deliver cargo to tissues far from their origin. EVs are released by the cell either through direct blebbing of the cell membrane or via an endosomal pathway and are characterized by size as small (50−200 nm) or large (>200 nm) EVs. To target and cross the placental barrier, Zhang et al. engineered small EVs produced from human embryonic kidney cells and functionalized EVs with rabies virus glycoprotein that enables cell entry via clathrin-mediated endocytosis for fetal siRNA delivery to protect against Zika-virus fetal transmission during pregnancy and associated microcephaly.[67,68] They showed that EVs can transport across the placenta and that surface modification can direct organ-specific delivery in the fetus, in this case to the brain. Building on this success of fetal delivery of EVs, investigation of engineering EVs for fetal kidney targeting is warranted.

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CELL THERAPIES FOR GENETIC KIDNEY DISEASES

The resulting mutations in genetic and congenital diseases result in defective cell and tissue function; hence, in addition to nanomedicine, cell therapy approaches aimed at tissue regeneration can be another effective therapeutic strategy that should be further explored for genetic kidney diseases.[69] Early studies indicated the promise of bone marrow derived stem cells for AS, which is characterized by an absence of glomerular basement membrane (GBM) due to a defect in GBM-associated collagen IV genes.[70,71] Bone marrow derived progenitor cells have been used to differentiate into resident podocytes or mesangial cells in the glomeruli and partially replenish collagen in the GBM.[70] However, this treatment modality has not yet made it to the clinic. More recently, additional sources of stem cells have been explored. For example, human fetal chorionic stem cells (CSCs) were reported to be effective in treating a mouse model of AS by homing to the glomeruli and differentiating into podocytes.[72] As a result, CSC treatment decreased renal fibrosis and reduced inflammation, leading to a delay in AS progression. Additionally, amniotic fluid stem cells (ASFCs) have also been reported as an effective cell therapy for treating AS.[73] Their mechanism of function, however, was paracrine signaling rather than differentiation into resident kidney cells: EVs secreted by ASFCs reduced excess VEGF and hence reduced kidney fibrosis and overall disease progression.[74] Thus, this study points to the potential of using cells to target multiple pathways involved in a genetic kidney disease.

In more advanced studies, bone marrow derived mesenchymal stromal cells (BMMSCs) have been explored for treating PKD, with a phase I trial already proving BMMSC infusion to be safe in ADPKD patients (NCT04115345). Additionally, renal autologous cell therapy (REACT) has demonstrated positive clinical outcomes in ADPKD.[75] Unlike stem cell therapy, REACT uses a sorted population of the patient's autologous selected renal tubular cells (SRC) and ongoing trials investigating REACT include NCT04115345, NCT05018416, and NCT02836574. Early findings from NCT02836574 indicated that SRCs can recreate neo-kidney-like tissue at the kidney site, and the therapy led to a decline in eGFR over a 1 year period.[76]

While regenerative cell therapies have shown promise, targeting other pathways involved in disease progression may be required. For example, T-regs have been identified as dominant infiltrating cells in CKD and autoimmune kidney disease and developing cell-based therapies related to the T-reg axis may provide a strategy to enable protection against kidney injury.[77,78] In summary, these advances in nanoparticle delivery and cell therapy for the treatment of genetic and congenital kidney diseases showcase the potential of safe and effective strategies for early intervention and restoration of impaired kidney function.

CHALLENGES AND A CALL TO ACTION

Many genetic kidney diseases lead to CKD, which continues to be a leading cause of morbidity and mortality worldwide. As mentioned, patients with ESKD currently require renal replacement therapy in the form of dialysis or transplantation. However, kidney transplantation is a nonideal solution, as waitlists include ~100,000 patients with average wait times of 6 years.[79] Notably, infants with ESKD must undergo dialysis for the first two years of life, as transplantation cannot occur until a weight of about 10 kg, posing additional comorbidities and burdens such as neurocognitive delay, infectious complications, and the frequent need for hospitalizations.[80,81] As such, therapeutic strategies to deter disease progression before reaching ESKD is of immense need.

To achieve this, therapies should be directed to the diseased site, but therapeutic delivery to the kidneys continues to be a challenge, and as presented in this Perspective, efforts to develop kidney-targeting vehicles have been limited. Part of the challenge is that there is still a lack of understanding regarding nanoparticle interaction with the kidneys; thus, the design criteria for developing nanoparticles to reach the kidneys remains to be fully elucidated. For example, while earlier work in adult animal models showed inorganic nanoparticles of <6−8 nm passage through the GFB passage and access the kidneys,[82] these particles were reported to be mostly cleared out through urine and were unsuitable for sustained drug delivery applications. More recently, our lab has shown that organic micelle-based nanoparticles of 12−15 nm can pass through the GFB as well. However, in contrast to earlier reports on small gold nanoparticles and quantum dots, surface modification of the micelles with targeting ligands and zwitterionic moieties enabled retention (vs clearance) in the kidneys. Nonetheless, others have demonstrated that mesoporous nanoparticles of 350−400 nm can also significantly accumulate in the kidneys.[30] Thus, how nanoparticle physiochemical properties facilitate kidney bioavailability and access via the GFB (apically) or through systemic circulation and the peritubular capillaries (basolaterally) remains to be teased out and future studies identifying the ideal design criteria for nanoparticles in a kidney disease-specific manner will be needed. Additionally, as the constituents of the protein corona on the nanoparticle surface can dictate nanoparticle fate, how the protein corona facilitates or hinders kidney access, influences the mechanism of nanoparticle entry into the nephron, and impacts therapeutic outcomes are yet additional aspects that will require further study.

More broadly, nanoparticles designed for kidney applications range in size, charge, and material makeup which is often specific to each individual lab and many times a trial-and-error process that is labor intensive. Thus, developing systematic nanoformulation protocols and standardized reporting metrics to enable and leverage machine learning approaches has the potential to accelerate clinical development of nanomedicine for genetic kidney diseases.[83] These advances have already begun to be applied for understanding how the lipid constituents in lipid nanoparticles (LNPs) affect organ-specific targeting for mRNA delivery[84] and in developing computationally guided design of nanoparticles for cancer drug delivery, which provide an initial blueprint for kidney nanomedicine.[85]

As validated through the success of mRNA-LNP vaccine technology, the adoption of nucleic acid based nanoparticle therapies into the clinic has been proven feasible, which advances possibilities for treating the underlying genetic cause of hereditary kidney diseases. Even before COVID vaccines, siRNA encapsulated and delivered by LNPs (Onpattro) was FDA approved for the treatment of polyneuropathies resulting from hereditary disease transthyretin-mediated amyloidosis (hATTR amyloidosis).[86] Onpattro was approved in 2018 and inhibits transthyretin (TTR) protein synthesis in the liver. Beyond short RNAs, mRNA-LNPs are now currently under clinical investigation as protein replacement therapies for genetic diseases, including ornithine transcarbamylase deficiency (NCT03767270), methylmalonic acidaemia

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(NCT03810690), and propionic acidaemia (NCT04159103).[87] Thus, toxicity and safety profiles as well as infrastructure including good manufacturing practices and global distribution procedures already implemented for such LNPs can be leveraged for developing nucleic acid based nanomedicine for genetic kidney diseases.

Despite the potential, genetic kidney diseases including PKD and AS and many rare diseases lack a full understanding of genotype−phenotype correlation, disease etiology, and pathogenesis, and as a result, genetic kidney diseases have an absence of relevant biomarkers, targets, and animal models for testing and developing effective drug delivery strategies, which poses a "chicken and an egg" problem. For instance, for reasons unknown, *PKHD1* mutations that drive ARPKD in humans do not cause cystogenesis in mice. However, recent work by Yang et al. has shown that animal models with *Cys1* mutations phenocopy ARPKD and later demonstrate *CYS1* mutations to also exist in the human condition.[88] With relevant animal models still on the horizon, the field can leverage available organoid models of genetic kidney disease[89−91] to elucidate disease mechanism and enable drug discovery and identification of effective combination therapies in a high-throughput manner.[92] Approaches using organ chip technology can potentially fast track therapeutic options for patients of need, especially as recent regulatory changes enable FDA approval of drugs without the need for animal testing.[93]

Related to the knowledge gap present in genetic kidney disease etiology, another challenge that still remains in renal drug delivery is that, in general, ligands that are available for targeting specific kidney cell types are limited and few. For instance, although a handful of peptides that target the proximal tubule, distal tubule, cortical collecting duct, glomerulus, and podocytes have been identified, their validation within a disease indication is limited.[37,94,95] Thus, methods such as *in vivo* phage display, aptamer discovery, and molecular dynamics and *in silico* approaches that can establish ligands and demonstrate specificity and high affinity to specific renal cell types or even immune cells that participate in the disease[96] are needed. By doing so, these ligands can be conjugated to drugs or decorated onto nanoparticles to be tailored for diseases where specific cell types are of particular concern. Although antibodies have also been proposed for renal application, how efficiently antibodies can reach the kidneys is another area that is yet to be determined.

For fields such as kidney nanomedicine with rapidly growing knowledge and convergence of multiple disciplines, collective and comprehensive research efforts and sharing of knowledge and data through activities that bring together basic scientists, clinicians, patients, government agencies, industry, and engineers are needed such that the most up-to-date basic research and clinical results can be integrated and enable the development of relevant therapeutic strategies. Recent examples including PKD Connect, PKD Research Resource Consortium (PKD RCC) Annual Symposium, and American Society of Nephrology (ASN) Kidney Week "Emerging Therapeutic Approaches in AKI (Acute Kidney Injury)" session, where nanomedicine fundamentals, pharmacology, drug discovery, disease biology, *in vitro* modeling, omics, patient advocacy, and clinical observations can converge will lead to open dialogue among the diverse stakeholders toward successful solutions that can benefit this patient population.

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Notes

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