# CRISPR Gene Editing in the Kidney

*Nelly M. Cruz and Benjamin S. Freedman*

CRISPR is a nuclease guidance system that enables rapid and efficient gene editing of specific DNA sequences within genomes. We review applications of CRISPR for the study and treatment of kidney disease. CRISPR enables functional experiments in cell lines and model organisms to validate candidate genes arising from genetic studies. CRISPR has furthermore been used to establish the first models of genetic disease in human kidney organoids derived from pluripotent stem cells. These gene-edited organoids are providing new insight into the cellular mechanisms of polycystic kidney disease and nephrotic syndrome. CRISPR-engineered cell therapies are currently in clinical trials for cancers and immunologic syndromes, an approach that may be applicable to inflammatory conditions such as lupus nephritis. Use of CRISPR in large domestic species such as pigs raises the possibility of farming kidneys for transplantation to alleviate the shortage of donor organs. However, significant challenges remain, including how to effectively deliver CRISPR to kidneys and how to control gene editing events within the genome. Thorough testing of CRISPR in preclinical models will be critical to the safe and efficacious translation of this powerful young technology into therapies.

*Note from Editors: This article was commissioned to celebrate the selection of CRIPSR-Cas9 as a finalist in NephMadness 2017. NephMadness is an educational project styled as a tournament in which key concepts in nephrology "compete" to determine which deserves to be crowned the most notable recent advance in the field.*

## The Discovery of CRISPR Gene Editing

In 2012, two landmark articles described an enzyme that could be "programmed" with a customizable RNA input to cleave specific DNA sequences of interest.[1,2] The versatility and ease of use of this system suggested that it could be useful for a variety of gene editing and gene targeting applications.[1,2] The technique was rapidly shown to work in mammalian (including human) cells, raising the possibility of using it for gene therapy in the clinic.[3,4]

This gene editing system was called CRISPR (clustered regularly interspaced short palindromic repeats; see Box 1 for expansion and definitions of other key terms), after the bacterial DNA sequences that originally led to its discovery.[5-9] As shown in Figure 1A, it has 2 main components: a CRISPR-associated system (Cas) endonuclease and a guide RNA (gRNA). The gRNA combines an invariant "scaffold" sequence that binds to Cas and a variable "spacer" sequence that provides sequence specificity. The spacer is approximately 20 nucleotides in length and terminates immediately upstream of a characteristic NGG sequence called the protospacer adjacent motif. Because there are 4 possible nucleotides for each position in the gRNA, the spacer provides up to $4^{20}$ possible sequence combinations (>1 trillion). This greatly exceeds the number of base pairs in any known genome, providing sufficient sequence specificity to identify unique sites.

Cas forms a ternary complex with the gRNA and a genomic DNA sequence complementary to the spacer and introduces a double-stranded break at that site. A commonly used Cas nuclease, Cas9, typically cuts both strands of DNA between the third and fourth nucleotides upstream of the protospacer adjacent motif.[1,2] There are then 2 main ways that DNA can be repaired by the cell. The first is nonhomologous end joining (NHEJ), an error-prone process involving trimming of the severed DNA followed by direct ligation. NHEJ occurs throughout the cell cycle and is the primary pathway by which CRISPR-induced lesions are repaired. NHEJ typically leads to indel (insertion or deletion) mutations in the original sequence, which can disrupt open reading frames (Fig 1B).

The second repair mechanism is homology-directed repair (HDR), in which DNA is corrected based on a template from undamaged DNA of similar sequence. This template DNA may be supplied by the experimentalist or alternatively may reside elsewhere in the genome, for instance, in an undamaged sister chromatid following DNA replication.[3,4,10] In the presence of such a template, CRISPR can be used to engineer specific mutations in the genome through the HDR pathway, which provides a mechanism for more accurate repair of DNA than NHEJ (Fig 1C). In certain cell types, rates of both NHEJ and HDR can be moderately enhanced by synchronizing the cells at either the replication or division stages of the cell cycle.[11] However, HDR remains inefficient compared to NHEJ, which predominates even in the presence of template DNA.[3,4,11]

The discovery of CRISPR opens new avenues for our ability to modify genomes. This has important ramifications for genetic disease and bioengineering applications. CRISPR builds on previous gene editing technologies, including homologous recombination, zinc-finger nucleases, TALEN (transcription activator-like effector nucleases), and AAV (adeno-associated virus).[12-16] No gene editing technology is 100% specific, and all have the potential to introduce off-target mutations. Also, CRISPR is not necessarily more efficient than some of these earlier systems. However, CRISPR has significant advantages over

**Box 1.** Definitions of Key Terms

- Adeno-associated virus (AAV): a small virus that infects human cells and can be used as a vector for gene therapy
- Chimeric antigen receptors (CAR) T cells (CAR-T): T lymphocytes with receptors that have been engineered to recognize specific antigens
- CRISPR (clustered regularly interspaced short palindromic repeats): a gene editing system combining a programmable nuclease with a customizable input RNA; can also refer to the bacterial DNA sequences that led to the discovery of this system
- CRISPR-associated system (Cas): a family of nucleases used for CRISPR gene editing
- Gene editing: introduction of targeted mutations into a specific sequence of DNA within the genome
- Guide RNA (gRNA): a short RNA sequence that directs Cas to complementary sites within the genome
- Indel: mutations featuring the insertion or deletion of base pairs, commonly introduced during DNA repair processes
- Interspecies blastocyst complementation (IBC): establishment of a genetic niche within a host embryo to grow a tissue from another species
- Kidney organoids: multicellular units in vitro containing podocytes, proximal tubules, and distal tubules in nephron-like patterns
- Pluripotent stem cells (PSCs): cells at an early embryonic stage that can give rise to the entire body

other systems in terms of cost and ease of use.[17] This has led to widespread adoption of CRISPR in research laboratories and, more recently, in clinical trials. We discuss applications of CRISPR relevant to the study and treatment of kidney disease.

## Studying Kidney Disease With CRISPR

A significant proportion of kidney disease is genetic, with up to 15% of all cases deriving directly from a Mendelian mutation and many more involving more complex inheritance patterns.[18] For instance, polycystic kidney disease (PKD), which is predominantly caused by hereditary mutations, is the primary diagnosis in ∼10% of patients requiring renal replacement therapy.[19] The influence of genetics in kidney disease is partially masked by the high incidences of hypertension and diabetes, which together account for up to 75% of kidney failure, but primarily affect other organ systems.[18] Genetic causes therefore contribute disproportionately to cases of kidney disease associated with primary defects in the kidneys themselves.

Although many of the genes that cause kidney disease have been identified, functional experiments are required to validate candidate genes arising from genetic studies and determine how mutations cause disease at the cellular and tissue level. In addition, many genes involved in kidney disease remain unknown. CRISPR enables researchers to perform targeted experiments to address these questions. The general approach has been to knock out candidate genes

and inspect the mutants for phenotypic differences compared with isogenic (ie, having a uniform genetic background) controls that were not modified by CRISPR. Sequencing of DNA amplified from the target region, followed by protein analysis (using immunoblot and immunofluorescence) to confirm the absence of the gene product, are the standard methods used to verify gene knockout.

CRISPR carries a risk for inadvertent edits to DNA sequences that are similar but not identical to the gRNA. To minimize the likelihood of these "off-target" effects, gRNAs can be selected based on computational algorithms that maximize sequence specificity.[3,4] Minimizing the duration and concentration of the genome's exposure to Cas9 is one way to reduce the risk for accumulated mutations. "Nickase" mutants of Cas9 that require 2 gRNAs (on opposite sides of the target) to efficiently cleave DNA can also provide an additional measure of specificity.[20] However, this approach is more complicated than single-site CRISPR, may be less efficient, and increases the number of off-target sites that need to be considered. To verify that the appropriate modifications have been made, whole-genome sequencing would ideally be performed for every CRISPR product,[21,22] although this may not be economically feasible or efficiently capture certain types of mutations such as copy number variations and large deletions. At a minimum, to reduce the possibility of off-target effects, multiple separate knockouts created by different gRNAs are typically compared for each genotype and phenotype, because these would not be expected to produce the same off-target effects.[23] When CRISPR is being used to generate clonal cell lines, it is similarly important to isolate and characterize multiple clones for each genotype to reduce the possibility of clonal idiosyncrasies.

Following this approach, CRISPR has been applied to kidney epithelial cell lines to model features of tubular physiology and disease. For instance, knockout of multidrug resistance protein 1 was observed to reduce efflux of transporter substrates in Madin-Darby canine kidney cells, whereas knockout of a tight junction scaffolding protein was shown to increase paracellular flux.[24,25] In mouse inner medullary collecting duct cells, knockout of A-kinase anchoring protein 220 was found to result in actin organization defects and buildup of aquaporin 2 at the apical plasma membrane, corresponding to reduced urine-diluting capacity in animal models.[26] In human renal cortical tubular epithelial cells, knockout of GANAB, a candidate gene for PKD, was observed to result in failed trafficking of polycystin proteins to primary cilia, which is associated with PKD.[27]

More complex models of disease are possible with human pluripotent stem cells (hPSCs), a cell type that includes embryonic stem cells derived from embryos and induced pluripotent stem cells reprogrammed from somatic cells.[28,29] hPSCs have emerged as a valuable system for studying mechanisms of kidney disease in vitro.[23,30-33] Early work established a cohort of hPSCs from patients with PKD, including both autosomal dominant and autosomal recessive forms of the disease.[30] hPSCs with
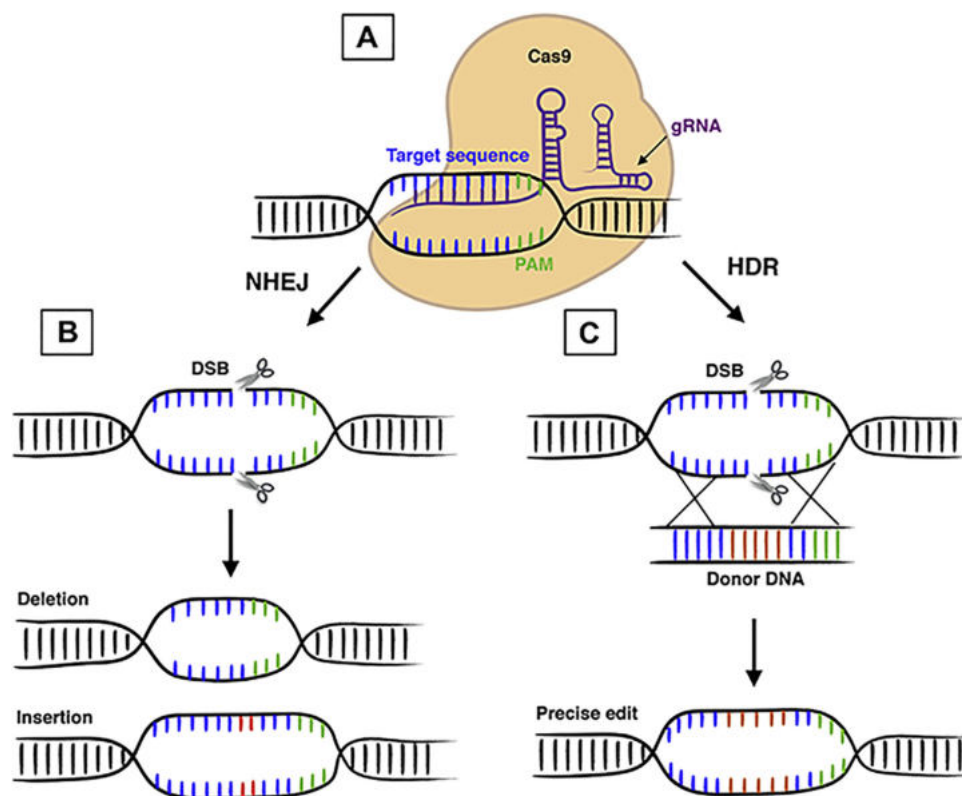
**Figure 1.** Schematic of the CRISPR system for gene editing. (A) A short guide RNA sequence (gRNA, purple) pairs with complementary DNA upstream of a 5′ NGG protospacer adjacent motif (PAM) motif (blue). This pairing directs the Cas9 nuclease to the target DNA, where it creates a double-stranded break (DSB). (B) In the absence of a repair template, the DSB is repaired by nonhomologous end joining (NHEJ), an error-prone process often resulting in indel mutations at the site of repair. (C) Alternatively, a donor template DNA can be provided to create precise edits by the homology-directed repair (HDR) pathway through homologous recombination.

heterozygous mutations in *PKD1*, encoding polycystin 1, were found to exhibit reduced localization of its binding partner polycystin 2 to primary cilia. This defect was observed in undifferentiated hPSCs and differentiated somatic epithelial cells and liver hepatoblasts.[30]

Subsequently, protocols have been developed to differentiate hPSCs into kidney organoids, multicellular units containing podocytes, proximal tubules, and distal tubules in nephron-like arrangements in vitro.[31,34-36] One study described not only differentiation of kidney organoids, but also the application of CRISPR in organoids to model genetic kidney disease.[31] CRISPR was used because hPSCs from different patients showed significant variability in their capacity to differentiate into kidney organoids, owing to heterogeneities in cell source and genetic background.[32] This is a common issue with hPSCs, which can overshadow and confound efforts to accurately model disease.[23,37,38] To avoid this problem of genetic heterogeneity among patients, CRISPR was used to generate series of mutant hPSCs that were otherwise isogenic.[31]

Using this approach, the first genetic models of disease in human kidney organoids were established (Table 1).[31-33] To model PKD, CRISPR was applied to hPSCs to introduce

loss-of-function mutations in either *PKD1* or *PKD2*.[31] The mutations did not affect the ability of hPSCs to differentiate into kidney organoids, and initially no differences were observed.[31,32] However, shortly after differentiation, a low but detectable percentage of organoids with PKD mutations formed cysts in vitro, which were not observed in control organoids of identical genetic background.[31] These findings suggested that PKD cyst formation was a cell-intrinsic process that can be reconstituted in vitro.[31]

Subsequent experiments in this system provided new insight into the molecular function of *PKD1* and *PKD2*.[32] Time-lapse imaging of PKD organoids revealed that cysts formed from whole tubular structures that partially detached from the culture dish.[32] When PKD organoids were cultured in low-attachment plates, they formed cysts at a much higher rate than in the previous adherent culture system, growing to diameters of ∼1 cm over several months in culture (Fig 2).[32] The background rate of cyst formation in isogenic control organoids remained very low compared with CRISPR-mutant organoids, demonstrating that enhanced cystogenesis in the mutants was disease specific.[32] This background rate likely reflected the

**Table 1.** Organoid Models of Kidney Disease Generated With CRISPR

| Disease | Genotype[a] | Phenotype | Cell Type | Reference |
|---|---|---|---|---|
| PKD | $PKD1^{-/-}$ or $PKD2^{-/-}$ | ↓ ciliary polycystin 2 | Tubule | 32 |
| PKD | $PKD1^{-/-}$ or $PKD2^{-/-}$ | ↑ cystogenesis | Tubule | 31, 32 |
| PKD | $PKD1^{-/-}$ or $PKD2^{-/-}$ | ↓ ECM compaction | Tubule | 32 |
| PKD | $PKD2^{-/-}$ | ↓ polycystin 1 levels | Tubule | 32 |
| Nephrotic syndrome | $PODXL^{-/-}$ | ↓ junctional migration | Podocyte | 31, 33 |
| Nephrotic syndrome | $PODXL^{-/-}$ | ↓ cell-cell spacing | Podocyte | 31, 33 |
| Nephrotic syndrome | $PODXL^{-/-}$ | ↓ microvillus formation | Podocyte | 33 |

Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; ECM, extracellular matrix; PKD, polycystic kidney disease.
[a]Indicates the gene(s) knocked.

strong tendency of polarized epithelial cells to form cysts in three-dimensional cultures, even in the absence of PKD mutations. For example, undifferentiated hPSCs rapidly form spheroid cysts in 3D culture conditions, at a rate completely independent of PKD mutations.[31] In contrast, PKD mutations had a dramatic effect promoting cystogenesis in kidney organoids derived from these same hPSCs.[31,32] Cysts expressed markers of both proximal and distal tubules and exhibited increased signatures of growth and proliferation, similar to PKD tissues.[32] PKD organoids further displayed a defect in their ability to interact with collagen droplets and compact them to a smaller size.[32] Collectively, these experiments revealed a critical role for microenvironment in early PKD cystogenesis.

The goal of disease modeling with CRISPR is not merely to reproduce phenotypes of known disease genes, but also to help identify new ones. PODXL, encoding podocalyxin, is highly expressed in podocytes and is a candidate gene for focal segmental glomerulosclerosis.[39] Mutations in PODXL are very rare, so there is a need for functional studies in model systems. Podocytes are highly specialized postmitotic cells that grow poorly in primary cultures[40] but differentiate efficiently in kidney organoids to the capillary loop stage.[33] When PODXL was disrupted using CRISPR, organoid podocytes were seen to exhibit defects in their ability to segregate junctions basally.[31] Ultrastructural analysis revealed a striking absence of lateral microvilli in podocytes in which PODXL had been knocked out, which correlated with a failure to establish empty spaces between cells.[33] This suggested a mechanism for the basal migration of junctional complexes in podocytes at the capillary loop stage of glomerular maturation.[33] Findings in human organoids were further validated in a mouse genetic model of podocalyxin deficiency, in which the animals die postnatally of kidney failure.[33] Subsequent to these studies, a case study described a human patient with biallelic loss-of-function mutations in PODXL who was affected by congenital nephrotic syndrome.[41] Thus, gene editing with CRISPR in human organoids, together with the mouse model, correctly predicted a kidney disease phenotype.

Use of CRISPR is not limited to cell culture. Injection of Cas and gRNA into zygotes can produce gene-edited animals. This method has been used to generate "knock-in" mice, in which a sequence encoding a modified protein is introduced into the genome. For example, a modified version of the developmental kidney marker encoded by Osr1 was used to analyze its co-expression with Wt1 in nascent nephrons.[42] A knock-in mouse encoding an allele of Lamb2, associated in humans with delayed-onset Pierson syndrome and proteinuria, has also been generated using CRISPR and was found to sensitize these mice to mutations that cause Alport syndrome.[43] Furthermore, in mice and miniature pigs, co-injection of multiple gRNAs can be used to knock out multiple genes simultaneously.[3,44,45] CRISPR may soon be used to study kidney disease in large animal species, which may better mimic humans.

CRISPR also has many possible applications beyond single-gene editing. Libraries of CRISPR gRNA, targeting much of the genome, can be applied to collections of cells



**Figure 2.** CRISPR establishes a human organoid model of PKD. Photographs of (right) $PKD2^{-/-}$ human kidney organoids and (left) isogenic control organoids. Adapted from Cruz et al[32]; original images are © 2017 Springer Nature.
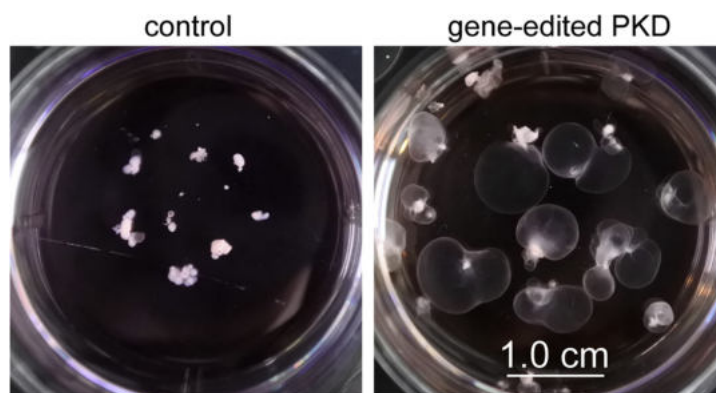
in vitro, which can then be selected for a phenotype of interest.[46] Using such an approach, it may be possible to identify novel disease gene candidates, which can be further validated in human cohorts. The case of *PODXL*, in which the organoid phenotype preceded the patient case study, suggests that CRISPR may have such predictive power for kidney disease genes.[33] However, there are also certain limitations to CRISPR's predictive power. For instance, studies performed in a single genetic background ignore the effect of modifier genes, which can affect the rate of disease progression. To address such complex effects, it may be useful to perform phenotyping studies in hPSCs from patients of different genetic backgrounds, using CRISPR as necessary to confirm the effects of possible modifiers.[47,48] In addition to editing genes, modified versions of CRISPR can also be used to silence or activate specific genes without changing their sequence.[49,50] Such creative repurposing of CRISPR technology promises to introduce a diverse suite of new research tools.

## Gene Therapy With CRISPR

Gene therapy is an attractive approach in principle for treating many genetic diseases, but has been challenging to implement in the clinic due to low editing efficiency and concerns over potential side effects. CRISPR can be used to target and repair specific disease-causing mutations with higher efficiency and easier implementation than traditional gene therapy methods. This method is being used broadly as a research tool to correct disease in animal models.

In a recent study of mice with transplanted human immune cells, CRISPR was used to block replication of human immunodeficiency virus (HIV) by excising the integrated virus from DNA; the method also worked for acutely infected mice.[51] In mice and rats, CRISPR has also been used to treat an array of genetic diseases in vivo, such as Duchenne muscular dystrophy,[52-56] Huntington disease,[57] and retinal degenerative diseases.[58-61] For the latter, local delivery and electroporation in the neonatal retina prevented retinal degeneration in mice, providing proof of concept for the use of CRISPR for gene therapy.[58]

AAV is a small virus that is a preferred method of delivery for gene therapy and has shown substantial promise in preclinical studies. AAV is believed to be relatively safe and can infect a variety of cell types, including nondividing cells.[16] Recent studies are using AAV vectors for local and systemic delivery of the Cas9 protein and single gRNAs for CRISPR-mediated gene therapy in animal models. This approach has been used for gene correction in the postmitotic retina[60,61] in rats and mice and to target dystrophin mutations (which cause Duchenne muscular dystrophy) in mice.[52-56] Moreover, in a mouse model of Huntington disease, inactivation of the mutant huntingtin allele was achieved by local administration of AAV vectors to the striatum of affected animals.[57]

Current clinical trials for CRISPR gene editing are all at early stages. These initial trials focus on the use of CRISPR as an alternative approach for immunotherapies, a field that has progressed substantially in recent years and has been successful in developing new drugs for treating certain cancers. These new trials are based on approaches that have already shown promise in the past, but incorporate CRISPR as the method of gene editing with the goal of examining safety and efficacy (Table 2).

In all but one of these studies, cells are genetically modified ex vivo, which is both safer and more efficacious than delivering CRISPR to a specific tissue. Cells are obtained from the patient, genetically modified, and then returned to his or her body, overcoming the challenges of immunocompatibility with donor cells and allowing for screening of cells that were successfully modified before transplantation. Many of the trials use CRISPR to modify the patient's T lymphocytes to express chimeric antigen receptors (CAR-Ts) that specifically recognize the cancer being treated. For the kidney, such a trial is currently planned for the treatment of metastatic renal carcinoma (Table 2). Because the major vehicle for such therapies is a T cell with immunomodulatory functions, such a cell therapy approach might also be useful in modulating other types of kidney diseases with a strong immune component, such as lupus nephritis or immunoglobulin A nephropathy.

At the moment, only one phase 1 study is scheduled for editing the cells inside the body. The study aims to examine the safety and efficacy of CRISPR to treat cervical cancer caused by the human papillomavirus by using a gel to locally administer DNA that expresses CRISPR-Cas9. Direct delivery of CRISPR for gene therapy could be suitable for monogenic kidney diseases without a major autoimmune component, such as autosomal dominant PKD and Alport syndrome. However, gene editing in solid organs faces the challenge of effective delivery to specific cells or tissues in the body. Preclinical studies in mice suggest that AAV can be used to efficiently target CRISPR to solid organs to rescue phenotypes.[62] Notably, kidney epithelial cells are a primary site of uptake for oligonucleotide reabsorption, which may make the kidney amenable to systemic administration of DNA and RNA therapeutics.[63]

Alternatively, CRISPR can be applied to genetically modify human embryos at the single-cell stage, which would affect all daughter cells. Initial studies using microinjection of CRISPR messenger RNA into human zygotes produced genetically modified embryos, but revealed off-target mutations and mosaic embryos containing both modified and unmodified cells.[64-66] A recent study describing correction of a mutation that causes hypertrophic cardiomyopathy in human embryos suggested higher efficiency with no evidence of off-target effects or mosaicism.[10] This was attributed to the double strand break being repaired by interhomolog repair, using the maternal wild-type allele as a template, rather than the more common HDR mechanism that uses a donor

**Table 2.** Clinical Trials Using CRISPR for Gene Editing

| Study Title | Condition | Study Phase | Intervention | Sponsors/Collaborators | Status |
|---|---|---|---|---|---|
| Safety of transplantation of CRISPR CCR5-modified CD34$^+$ cells in HIV-infected patients with hematological malignances | HIV-1 infection | 1 | CCR5 gene modification | Affiliated Hospital to Academy of Military Medical Sciences, Peking University & Capital Medical University | Recruiting |
| A safety and efficacy study of TALEN and CRISPR/Cas9 in the treatment of HPV-related cervical intraepithelial neoplasia | HPV-related malignant neoplasm | 1 | Disruption of HPV16 and HPV18 E6/E7 DNA | First Affiliated Hospital, Sun Yat-Sen University & Jingchu University of Technology | Not yet recruiting |
| A study evaluating UCART019 in patients with relapsed or refractory CD19$^+$ leukemia and lymphoma | B-cell leukemia & B-cell lymphoma | 1/2 | Universal CRISPR/Cas9 gene-editing of CAR-T cells targeting CD19 (UCART019) | Chinese PLA General Hospital | Recruiting |
| PD-1 knockout engineered T cells for advanced esophageal cancer | Esophageal cancer | 2 | PD-1 knockout T cells | Hangzhou Cancer Hospital & Anhui Kedgene Biotech Co, Ltd | Recruiting |
| PD-1 knockout engineered T cells for muscle-invasive bladder cancer | Invasive bladder cancer stage IV | 1 | PD-1 knockout T cells | Peking University & Cell Biotech Co, Ltd | Not yet recruiting |
| PD-1 knockout engineered T cells for castration-resistant prostate cancer | Hormone-refractory prostate cancer | 1 | PD-1 knockout T cells | Peking University & Cell Biotech Co, Ltd | Not yet recruiting |
| PD-1 knockout engineered T cells for metastatic renal cell carcinoma | Metastatic renal cell carcinoma | 1 | PD-1 knockout T cells | Peking University & Cell Biotech Co, Ltd | Not yet recruiting |
| PD-1 knockout engineered T cells for metastatic non–small cell lung cancer | Metastatic non–small cell lung cancer | I | PD-1 knockout T cells | Sichuan University & Chengdu MedGenCell, Co, Ltd | Recruiting |
| PD-1 knockout EBV-CTLs for advanced stage Epstein-Barr virus–associated malignancies | Stage IV gastric carcinoma, nasopharyngeal carcinoma, T-cell lymphoma, adult Hodgkin lymphoma, diffuse large B-cell lymphoma | 1/2 | PD-1 knockout EBV-CTL cells | Yang Yang & The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School | Recruiting |
| NY-ESO-1–redirected CRISPR (T cell receptor endogenous and PD-1) edited T cells (NYCE T cells) | Multiple myeloma, melanoma, synovial sarcoma, myxoid/round cell liposarcoma | I | T cells engineered to express NY-ESO-1[a] T-cell receptor and gene edited to eliminate endogenous T-cell receptor and PD-1 | University of Pennsylvania | Not yet recruiting |
| A feasibility and safety study of universal dual specificity CD19 and CD20 or CD22 CAR-T cell immunotherapy for relapsed or refractory leukemia and lymphoma | B-cell leukemia, B-cell lymphoma | 1/2 | Universal CRISPR-Cas9 gene editing of CAR-T cells targeting CD19 and CD20 or CD22 | Chinese PLA General Hospital | Recruiting |

Abbreviations and definitions: CAR-T, chimeric antigen receptor T-cells; Cas, CRISPR-associated system; CCR5, C-C chemokine receptor type 5; CD, cluster of differentiation; CRISPR, clustered regularly interspaced short palindromic repeats; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; PD-1, programmed cell death protein 1; TALEN, transcription activator-like effector nucleases; PLA, People's Liberation Army.
[a]NY-ESO-1 is a human tumor antigen.
Source: ClinicalTrials.gov.

template.[10] However, further studies are needed to rule out events that could result in false positives, such as large deletions, as suggested in a recent commentary.[67]

Use of CRISPR in human zygotes raises ethical concerns about the potential misuse of this method to design babies with certain attributes. Similar to any other therapy, benefits of using CRISPR in human embryos should outweigh the risks before the technique is used. In this regard, it is unclear what the advantage of gene editing embryos would be over the established method of preimplantation genetic diagnosis (PGD) that is currently used in the clinic. PGD refers to the screening of specific genetic defects in embryos during in vitro fertilization.[68] PGD is safer than gene editing, the risks of which are not yet fully understood. However, there may be rare cases in which PGD may not be an option, and CRISPR may provide an alternative. For instance, CRISPR may allow the repair of an autosomal recessive mutation in a baby when both parents are affected by the disease.

## CRISPR in Organ Transplantation

An exciting potential use of CRISPR in nephrology is to expand our available sources of kidneys for transplantation. For decades, scientists have explored the possibility of transplanting organs from other species (xenotransplantation). The pig has emerged as the primary candidate species due to its abundance, domestication, and similar organ structure to humans.[69,70] However, a problem is that pig cells express a variety of antigens that provoke an extreme (hyperacute) rejection response from the human immune system.

CRISPR is currently being used in the pig in an effort to mitigate this rejection barrier. In general, this approach begins by subjecting pig cells to CRISPR-Cas gene editing. Nuclei from these cells are subsequently purified and transferred into enucleated pig eggs to create diploid embryos. Implantation of these embryos into surrogate sows results in the birth of CRISPR-modified piglets. Although this approach is laborious, it allows researchers to select for multigene editing events in the pig genome.

Using this CRISPR-based approach, 3 porcine surface antigens that provoke hyperacute rejection have been knocked out in pigs, resulting in diminished immunoreactivity of pig blood cells with human antibodies.[71] CRISPR has similarly been used to generate pigs with knockout mutations in the 3 class I major histocompatibility complex genes in pigs, the porcine equivalent of the human leukocyte antigen (HLA) system.[72] In combination with nonconventional immunosuppression regimens that block the costimulatory complement cascade, ablation of such immunoreactive gene products is suggested to increase survival of pig kidneys in nonhuman primate transplant recipients from days to months.[73,74] CRISPR has furthermore been used to disrupt multiple gene sequences in pigs required for porcine endogenous retrovirus (PERV) activity, resulting in animals in which no traces of PERV could be detected.[75] Because transmission of PERV to humans is a potential risk of xenotransplantation and there are many copies of PERV in the pig genome, these endogenous retroviruses present a challenge for conventional gene editing approaches. If combined with genetic modifications to promote immunocompatibility, this approach could produce a pig transplant donor with the highest safety profile yet.

Despite these advances, it may prove difficult for xenotransplant kidneys to achieve sufficient tolerance in humans to compete with the gold standard of human allograft. However, what if it were possible to grow a human kidney in a pig? CRISPR is being used to test this scenario, using a technique called interspecies blastocyst complementation (IBC). In IBC, pluripotent stem cells of a donor species are injected into a host blastocyst of another species that has been genetically engineered to lack a specific tissue. The donor cells fill the niche of the missing tissue in the host embryo, creating a chimera. IBC was first successfully demonstrated in a pancreas-knockout mouse strain implanted with rat cells, resulting in the growth of rat pancreas inside a mouse host.[76] Use of CRISPR to create the niche (CRISPR-IBC) has enabled the expansion of this method to other organs, including the heart and eye.[77]

Although to date, use of CRISPR-IBC has been limited to rodents, it is likely that this technique could be used in other species amenable to CRISPR and same-species blastocyst complementation, such as pigs.[71,72,75,78] In the absence of IBC, implantation of hPSCs into pig blastocysts results in a very low but detectable contribution of human cells to the resultant embryos.[77] IBC is therefore likely to be required to grow human tissues in other species.

Significant technical and conceptual challenges remain before CRISPR-IBC can be used to generate a pure human kidney.[79] However, in theory, hPSCs could be directly derived from a human patient, implanted into a CRISPR-IBC pig embryo, and used to grow a pig containing kidneys grown from that patient's cells, likely obviating the need for immunosuppression. As such, a kidney may be very expensive to produce, and an alternative would be to generate CRISPR-IBC kidneys in pigs using hPSCs that have been gene-edited to disrupt the pathways of immune rejection. Such "universal" hPSCs are predicted to have sustained immunotolerance compared with allografts.[80] Such an approach would enable farming of humanized pigs for "off-the-shelf" organ transplantation. Thus, while challenging, CRISPR approaches would offer significant advantages in both supply and immunocompatibility of transplanted organs.

## Conclusion and Outlook

CRISPR is a powerful tool for research and potentially gene therapy and transplantation for many organs, including the kidneys. Many challenges remain, including how to properly deliver CRISPR to specific organs to correct mutations and how to genetically design chimeras containing human organs suitable for transplantation. For the kidneys,

it is vital that studies of humans be predicated on strong preclinical data identifying effective strategies that complement or improve on existing therapies. Such an approach will establish a firm foundation for safe and efficacious translation of this powerful technology to human patients.

## References

1. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc Natl Acad Sci U S A.* 2012;109:E2579-E2586.
2. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science.* 2012;337: 816-818.
3. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science.* 2013;339:819-823.
4. Mali P, Yang L, Esvelt KM, et al. RNA-guided human genome engineering via Cas9. *Science.* 2013;339:823-826.
5. Jansen R, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol Microbiol.* 2002;43:1565-1575.
6. Mojica FJ, Diez-Villasenor C, Garcia-Martinez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol.* 2005;60:174-182.
7. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology.* 2005;151:653-663.
8. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science.* 2007;315:1709-1712.
9. Brouns SJ, Jore MM, Lundgren M, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science.* 2008;321: 960-964.
10. Ma H, Marti-Gutierrez N, Park SW, et al. Correction of a pathogenic gene mutation in human embryos. *Nature.* 2017;548:413-419.
11. Lin S, Staahl BT, Alla RK, Doudna JA. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *Elife.* 2014;3:e04766.
12. Smithies O, Gregg RG, Boggs SS, Koralewski MA, Kucherlapati RS. Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. *Nature.* 1985;317:230-234.
13. Thomas KR, Folger KR, Capecchi MR. High frequency targeting of genes to specific sites in the mammalian genome. *Cell.* 1986;44:419-428.
14. Liu Q, Segal DJ, Ghiara JB, Barbas CF 3rd. Design of polydactyl zinc-finger proteins for unique addressing within complex genomes. *Proc Natl Acad Sci U S A.* 1997;94:5525-5530.
15. Cermak T, Doyle EL, Christian M, et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res.* 2011;39: e82.
16. Russell DW, Hirata RK. Human gene targeting by viral vectors. *Nat Genet.* 1998;18:325-330.
17. Miyagi A, Lu A, Humphreys BD. Gene editing: powerful new tools for nephrology research and therapy. *J Am Soc Nephrol.* 2016;27:2940-2947.
18. McKnight AJ, Currie D, Maxwell AP. Unravelling the genetic basis of renal diseases; from single gene to multifactorial disorders. *J Pathol.* 2010;220:198-216.
19. Gilg J, Methven S, Casula A, Castledine C. UK Renal Registry 19th Annual Report: Chapter 1 UK RRT adult incidence in 2015: national and centre-specific analyses. *Nephron.* 2017;137(suppl 1):11-44.
20. Ran FA, Hsu PD, Lin CY, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell.* 2013;154:1380-1389.
21. Veres A, Gosis BS, Ding Q, et al. Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing. *Cell Stem Cell.* 2014;15:27-30.
22. Smith C, Gore A, Yan W, et al. Whole-genome sequencing analysis reveals high specificity of CRISPR/Cas9 and TALEN-based genome editing in human iPSCs. *Cell Stem Cell.* 2014;15:12-13.
23. Freedman BS. Modeling kidney disease with iPS cells. *Biomark Insights.* 2015;10:153-169.
24. Simoff I, Karlgren M, Backlund M, et al. Complete knockout of endogenous Mdr1 (Abcb1) in MDCK cells by CRISPR-Cas9. *J Pharm Sci.* 2016;105:1017-1021.
25. Van Itallie CM, Tietgens AJ, Krystofiak E, Kachar B, Anderson JM. A complex of ZO-1 and the BAR-domain protein TOCA-1 regulates actin assembly at the tight junction. *Mol Biol Cell.* 2015;26:2769-2787.
26. Whiting JL, Ogier L, Forbush KA, et al. AKAP220 manages apical actin networks that coordinate aquaporin-2 location and renal water reabsorption. *Proc Natl Acad Sci U S A.* 2016;113: E4328-E4337.
27. Porath B, Gainullin VG, Cornec-Le Gall E, et al. Mutations in GANAB, encoding the glucosidase iialpha subunit, cause

autosomal-dominant polycystic kidney and liver disease. *Am J Hum Genet*. 2016;98:1193-1207.

28. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282:1145-1147.

29. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861-872.

30. Freedman BS, Lam AQ, Sundsbak JL, et al. Reduced ciliary polycystin-2 in induced pluripotent stem cells from polycystic kidney disease patients with PKD1 mutations. *J Am Soc Nephrol*. 2013;24:1571-1586.

31. Freedman BS, Brooks CR, Lam AQ, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun*. 2015;6:8715.

32. Cruz NM, Song X, Czerniecki SM, et al. Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. *Nature Materials*. 2017;16:1112-1119.

33. Kim YK, Refaeli I, Brooks CR, et al. Gene-edited human kidney organoids reveal mechanisms of disease in podocyte development. *Stem Cells*. 2017;35:2366-2378.

34. Taguchi A, Kaku Y, Ohmori T, et al. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. *Cell Stem Cell*. 2014;14:53-67.

35. Takasato M, Er PX, Chiu HS, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature*. 2015;526:564-568.

36. Morizane R, Lam AQ, Freedman BS, Kishi S, Valerius MT, Bonventre JV. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat Biotechnol*. 2015;33:1193-1200.

37. Bock C, Kiskinis E, Verstappen G, et al. Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell*. 2011;144:439-452.

38. Boulting GL, Kiskinis E, Croft GF, et al. A functionally characterized test set of human induced pluripotent stem cells. *Nat Biotechnol*. 2011;29:279-286.

39. Barua M, Shieh E, Schlondorff J, Genovese G, Kaplan BS, Pollak MR. Exome sequencing and in vitro studies identified podocalyxin as a candidate gene for focal and segmental glomerulosclerosis. *Kidney Int*. 2014;85:124-133.

40. Shankland SJ, Freedman BS, Pippin JW. Can podocytes be regenerated in adults? *Curr Opin Nephrol Hypertens*. 2017;26:154-164.

41. Kang HG, Lee M, Lee KB, et al. Loss of podocalyxin causes a novel syndromic type of congenital nephrotic syndrome. *Exp Mol Med*. 2017;49:e414.

42. Xu J, Liu H, Chai OH, Lan Y, Jiang R. Osr1 interacts synergistically with wt1 to regulate kidney organogenesis. *PLoS One*. 2016;11:e0159597.

43. Funk SD, Bayer RH, Malone AF, McKee KK, Yurchenco PD, Miner JH. Pathogenicity of a human laminin beta2 mutation revealed in models of Alport syndrome. *J Am Soc Nephrol*. 2017;29:949-960.

44. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell*. 2013;153:910-918.

45. Wang X, Cao C, Huang J, et al. One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system. *Sci Rep*. 2016;6:20620.

46. Shalem O, Sanjana NE, Hartenian E, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*. 2014;343:84-87.

47. Zeltner N, Fattahi F, Dubois NC, et al. Capturing the biology of disease severity in a PSC-based model of familial dysautonomia. *Nat Med*. 2016;22:1421-1427.

48. Pashos EE, Park Y, Wang X, et al. Large, diverse population cohorts of hiPSCs and derived hepatocyte-like cells reveal functional genetic variation at blood lipid-associated loci. *Cell Stem Cell*. 2017;20:558-570.e510.

49. Konermann S, Brigham MD, Trevino AE, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*. 2015;517:583-588.

50. Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*. 2013;152:1173-1183.

51. Yin C, Zhang T, Qu X, et al. In vivo excision of HIV-1 provirus by saCas9 and multiplex single-guide RNAs in animal models. *Mol Ther*. 2017;25:1168-1186.

52. Bengtsson NE, Hall JK, Odom GL, et al. Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. *Nat Commun*. 2017;8:14454.

53. El Refaey M, Xu L, Gao Y, et al. In vivo genome editing restores dystrophin expression and cardiac function in dystrophic mice. *Circ Res*. 2017;121:923-929.

54. Long C, Amoasii L, Mireault AA, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science*. 2016;351:400-403.

55. Nelson CE, Hakim CH, Ousterout DG, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science*. 2016;351:403-407.

56. Tabebordbar M, Zhu K, Cheng JKW, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science*. 2016;351:407-411.

57. Yang S, Chang R, Yang H, et al. CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. *J Clin Invest*. 2017;127:2719-2724.

58. Bakondi B, Lv W, Lu B, et al. In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. *Mol Ther*. 2016;24:556-563.

59. Latella MC, Di Salvo MT, Cocchiarella F, et al. In vivo editing of the human mutant rhodopsin gene by electroporation of plasmid-based CRISPR/Cas9 in the mouse retina. *Mol Ther Nucleic Acids*. 2016;5:e389.

60. Suzuki K, Tsunekawa Y, Hernandez-Benitez R, et al. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. *Nature*. 2016;540:144-149.

61. Yu W, Mookherjee S, Chaitankar V, et al. Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice. *Nat Commun*. 2017;8:14716.

62. Liao HK, Hatanaka F, Araoka T, et al. In vivo target gene activation via CRISPR/Cas9-mediated trans-epigenetic modulation. *Cell*. 2017;171:1495-1507.e1415.

63. Molitoris BA, Dagher PC, Sandoval RM, et al. siRNA targeted to p53 attenuates ischemic and cisplatin-induced acute kidney injury. *J Am Soc Nephrol*. 2009;20:1754-1764.

64. Kang X, He W, Huang Y, et al. Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. *J Assist Reprod Genet*. 2016;33:581-588.

65. Liang P, Xu Y, Zhang X, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein Cell*. 2015;6:363-372.

66. Tang L, Zeng Y, Du H, et al. CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. *Mol Genet Genomics*. 2017;292:525-533.

67. Egli D, Zuccaro MV, Kosicki M, Church GM, Bradley A, Jasin M. Inter-homolog repair in fertilized human eggs? *Bioarxiv.* 2017;181255:1-5.

68. Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. *Nat Rev Genet.* 2016;17:643-656.

69. Wijkstrom M, Iwase H, Paris W, Hara H, Ezzelarab M, Cooper DK. Renal xenotransplantation: experimental progress and clinical prospects. *Kidney Int.* 2017;91:790-796.

70. Cowan PJ, Tector AJ. The resurgence of xenotransplantation. *Am J Transplant.* 2017;17:2531-2536.

71. Estrada JL, Martens G, Li P, et al. Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/beta4GalNT2 genes. *Xenotransplantation.* 2015;22:194-202.

72. Reyes LM, Estrada JL, Wang ZY, et al. Creating class I MHC-null pigs using guide RNA and the Cas9 endonuclease. *J Immunol.* 2014;193:5751-5757.

73. Higginbotham L, Mathews D, Breeden CA, et al. Pre-transplant antibody screening and anti-CD154 costimulation blockade promote long-term xenograft survival in a pig-to-primate kidney transplant model. *Xenotransplantation.* 2015;22:221-230.

74. Iwase H, Liu H, Wijkstrom M, et al. Pig kidney graft survival in a baboon for 136 days: longest life-supporting organ graft survival to date. *Xenotransplantation.* 2015;22:302-309.

75. Niu D, Wei HJ, Lin L, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science.* 2017;357:1303-1307.

76. Kobayashi T, Yamaguchi T, Hamanaka S, et al. Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. *Cell.* 2010;142:787-799.

77. Wu J, Platero-Luengo A, Sakurai M, et al. Interspecies chimerism with mammalian pluripotent stem cells. *Cell.* 2017;168:473-486.e415.

78. Matsunari H, Nagashima H, Watanabe M, et al. Blastocyst complementation generates exogenic pancreas in vivo in apancreatic cloned pigs. *Proc Natl Acad Sci U S A.* 2013;110:4557-4562.

79. Freedman BS. Hopes and difficulties for blastocyst complementation. *Nephron.* 2018;138:42-47.

80. Gornalusse GG, Hirata RK, Funk SE, et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat Biotechnol.* 2017;35:765-772.