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CRISPR Gene Editing in the Kidney

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Abstract

CRISPR is a nuclease guidance system that enables rapid and efficient gene editing of specific DNA sequences within genomes. Here, 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 immunological 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 transplant, to alleviate the shortage of donor organs. Significant challenges remain, however, including how to effectively deliver CRISPR to kidneys, and how to control gene editing events within the genome. Thorough testing of CRISPR in pre-clinical models will be critical to the safe and efficacious translation of this powerful young technology into therapies.

Keywords

CRISPR; gene editing; genome; organoids; polycystic kidney disease (PKD); nephrotic syndrome; human pluripotent stem cells (hPSC); chimeric antigen receptor T-cells (CART); gene therapy; animal model; in vitro culture; gene knockout; review

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The discovery of CRISPR gene editing

In 2012, two landmark papers 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 (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 two 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 twenty nucleotides in length and terminates immediately upstream of a characteristic NGG sequence called the protospacer adjacent motif (PAM). As there are four possible nucleotides for each position in the gRNA, the spacer provides up to 4²⁰ possible sequence combinations (over one 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 (DSB) at that site. A commonly used Cas nuclease, Cas9, typically cuts both strands of DNA between the third and fourth nucleotides upstream of the PAM ^{1, 2}. There are then two main ways that DNA can be repaired by the cell. The first is non-homologous 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 (Figure 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 (Figure 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 upon 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. Nor is CRISPR necessarily more efficient than some of these earlier systems. CRISPR, however, has significant advantages over 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. Here, we will 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, which is predominantly caused by hereditary mutations, is the primary diagnosis in approximately 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 to 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 to 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 (via immunoblot and immunofluorescence) to confirm absence of the gene product, are the standard methods used to verify gene knockout.

CRISPR carries a risk of 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 of accumulated mutations. 'Nickase' mutants of Cas9 that require two 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 gRNA are typically compared for each genotype and phenotype, as 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 the 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 models²⁶. In human renal cortical tubular epithelial cells, knockout of *GANAB*, a candidate gene for polycystic kidney disease (PKD), was observed to result in failed trafficking of polycystin proteins to primary cilia, which is associated with PKD²⁷.

More complex models of disease are possible with human pluripotent stem cells (hPSC), a cell type that includes embryonic stem cells derived from embryos as well as induced pluripotent stem cells reprogrammed from somatic cells^{28, 29}. hPSC have emerged as a valuable system for studying mechanisms of kidney disease *in vitro*^{23, 30-33}. Early work established a cohort of hPSC from patients with PKD, including both autosomal dominant and autosomal recessive forms of the disease³⁰. hPSC with 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 hPSC, as well as in differentiated somatic epithelial cells and liver hepatoblasts³⁰.

Subsequently, protocols have been developed to differentiate hPSC 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 using CRISPR to have them model genetic kidney disease³¹. CRISPR was employed because hPSC from different patients showed significant variability in their capacity to differentiate into kidney organoids, owing to heterogeneities in cell source and genetic background³². This is a common issue with hPSC, which can overshadow and confound efforts to accurately model disease^{23, 37, 38}. To avoid this problem of genetic heterogeneity among patients, CRISPR was utilized to generate series of mutant hPSC that were otherwise isogenic³¹.

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 hPSC to introduce loss-of-function mutations in either *PKD1* or *PKD2*³¹. The mutations did not affect the ability of hPSC to differentiate into kidney organoids, and initially no differences were observed^{31, 32}. Shortly after differentiation, however, a low but detectable percentage of organoids with PKD mutations formed cysts *in vitro*, which were not observed in control organoids of identical genetic background³¹. 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 dish³². 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 (Figure 2)³². The background rate of cyst formation in isogenic control organoids remained very low compared to CRISPR-mutant organoids, demonstrating that enhanced cystogenesis in the mutants was disease-specific³². This background rate likely reflected the strong tendency of polarized epithelial cells to form cysts in three-dimensional cultures, even in the absence of PKD mutations. For example, undifferentiated hPSC rapidly form spheroid cysts under certain culture conditions, at the rate completely independent of PKD mutations³¹. In contrast, PKD mutations have a dramatic effect promoting cystogenesis in kidney organoids derived from these same hPSC^{31, 32}. Cysts express markers of both proximal and distal tubules, and exhibit increased signatures of growth and proliferation, similar to PKD tissues³². PKD organoids further display a defect in their ability to interact with collagen droplets and compact them to a smaller size³². 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 glomerulosclerosis³⁹. Mutations in *PODXL* are very rare, so there is a need for functional studies in model systems. Podocytes are highly specialized, post-mitotic cells that grow poorly in primary cultures⁴⁰, but differentiate efficiently in kidney organoids to the capillary loop stage³³. When *PODXL* was disrupted using CRISPR, organoid podocytes were seen to exhibit defects in their ability to segregate junctions basally³¹. 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 cells³³. This suggested a mechanism for the basal migration of junctional complexes in podocytes at the capillary loop stage of glomerular maturation³³. Findings in human organoids were further validated in a mouse genetic model of podocalyxin deficiency, as these animals die postnatally of kidney failure³³. 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 syndrome⁴¹. Thus, gene editing with CRISPR in human organoids, together with the mouse model, correctly predicted a kidney disease phenotype.

The 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 utilized to generate “knock-in” mice, in which a sequence encoding a modified protein is introduced. For example, a version of the developmental kidney marker encoded by *Osr1* that contained a tag to facilitate subsequent assays was used to analyze *Osr1* co-expression with *Wt1* in nascent nephrons⁴². 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's syndrome⁴³. Furthermore, in mice and miniature pigs, co-injection of multiple gRNA can be used to knock out multiple genes simultaneously^{3, 44, 45}. CRISPR may soon be utilized 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 *in vitro*, which can then be selected for a phenotype of interest⁴⁶. Using such an approach, it

may be possible to identify novel disease gene candidates, which can be further validated in human cohorts. Indeed, 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 genes³³. 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 hPSC 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-Cas can also be employed 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 transplanted with human immune cells, CRISPR was utilized to block replication of HIV by excising the integrated virus from DNA; the method also worked for acutely infected mice⁵¹. In mice and rats, CRISPR has also been used to correct an array of genetic diseases *in vivo*, such as Duchenne muscular dystrophy⁵²⁻⁵⁶, Huntington's disease⁵⁷ and retinal degenerative diseases⁵⁸⁻⁶¹. 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 therapy⁵⁸.

AAV is a small virus that is the preferred method of delivery for gene therapy and has shown the most promise in pre-clinical studies. AAV is believed to be relatively safe and can infect a variety of cell types, including non-dividing cells¹⁶. 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 is the approach that was used for gene correction in the post-mitotic retina^{60, 61} in rats and mice, and to target dystrophin mutations (which causes Duchenne muscular dystrophy) in mice⁵²⁻⁵⁶. Moreover, in a mouse model of Huntington's disease, inactivation of the mutant huntingtin allele was achieved by local administration of AAV vectors to the striatum of affected animals⁵⁷.

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 utilize CRISPR to modify the patient's T-lymphocytes to express chimeric antigen receptors (CAR-T) 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). As 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 IgA 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 (HPV), by using a gel to locally administer DNA that expresses CRISPR-Cas9 designed to disrupt the viral DNA. Direct delivery of CRISPR for gene therapy could be suitable for monogenic kidney diseases without a major autoimmune component, such as ADPKD and Alport syndrome. Gene editing in solid organs, however, faces the challenge of effective delivery to specific cells or tissues in the body. Pre-clinical studies in mice suggest that AAV can be used to efficiently target CRISPR to solid organs, alleviate phenotyping⁶². 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 therapeutics⁶³.

Alternatively, CRISPR-Cas9 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-Cas9 mRNA into human zygotes produced genetically modified embryos, but revealed off-target mutations and mosaic embryos containing both modified and unmodified cells⁶⁴⁻⁶⁶. 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 mosaicism¹⁰. This was attributed to the double strand break being repaired by inter-homolog repair, using the maternal wild-type allele as a template, rather than the more common HDR mechanism that uses a donor template¹⁰. However, further studies are needed to rule out events that could result in false positives, such as large deletions, as suggested in a recent commentary⁶⁷.

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 pre-implantation genetic diagnosis (PGD) that is currently used in the clinic. PGD refers to the screening of specific genetic defects in embryos during *in vitro* fertilization⁶⁸. PGD is safer than gene editing, the risks of which are not yet fully understood. There may, however, 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 where the both parents are affected by the disease.

Using 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}. A problem, however, 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 birth of CRISPR-modified piglets. Although this approach is laborious, it allows researchers to select for multi-gene editing events in the pig genome.

Using this CRISPR-based approach, three 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 utilized to generate pigs with knockout mutations in the three class I major histocompatibility complex genes in pigs – the porcine equivalent of the human leukocyte antigen (HLA) system ⁷². In combination with non-conventional immunosuppression regimens that block the costimulatory complement cascade, ablation of such immunoreactive gene products is suggested to increase survival of pig kidneys in non-human primate transplant recipients from days to months ^{73, 74}. CRISPR has furthermore been utilized 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. Since transmission of PERV to humans is a potential risk of xenotransplantation, and as 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. But 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 pancreas-knockout mice, 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 use of CRISPR-IBC has so far 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 hPSC into

pig blastocysts results in 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. In theory, however, hPSC could be directly derived from a human patient, implanted into a CRISPR-IBC pig embryo, and used to grow a pig containing that kidneys grown from that patient's cells, likely obviating the need for immunosuppression. As such a kidney may be very expensive to produce, an alternative would be to generate CRISPR-IBC kidneys in pigs using hPSC that have been gene-edited to disrupt the pathways of immune rejection. Such 'universal' hPSC are predicted to have sustained immunotolerance, compared to 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 human studies be predicated on strong pre-clinical data identifying effective strategies that complement or improve upon existing therapies. Such an approach will establish a firm foundation for safe and efficacious translation of this powerful technology to human patients.

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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

Figure 1.

Schematic of the CRISPR system for gene editing. (A) A short RNA sequence (gRNA, purple) pairs with complementary DNA upstream of a 5' NGG 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 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 HDR pathway through homologous recombination.

Figure 2. CRISPR establishes a human organoid model of PKD. Photographs of *PKD2*^{-/-} human kidney organoids (right) and isogenic control organoids (left). Adapted from Cruz et al³²; original images are © 2017, Springer Nature.

Table 1
Organoid models of kidney disease generated with CRISPR

disease	Genotype*	phenotype	cell type	reference
PKD	<i>PKD1</i> ^{-/-} or <i>PKD2</i> ^{-/-}	↓ ciliary polycystin 2	tubule	32
PKD	<i>PKD1</i> ^{-/-} or <i>PKD2</i> ^{-/-}	↑ cystogenesis	tubule	31,32
PKD	<i>PKD1</i> ^{-/-} or <i>PKD2</i> ^{-/-}	↓ ECM compaction	tubule	32
PKD	<i>PKD2</i> ^{-/-}	↓ polycystin 1 levels	tubule	32
nephrotic syndrome	<i>PODXL</i> ^{-/-}	↓ junctional migration	podocyte	31,33
nephrotic syndrome	<i>PODXL</i> ^{-/-}	↓ cell-cell spacing	podocyte	31,33
nephrotic syndrome	<i>PODXL</i> ^{-/-}	↓ microvillus formation	podocyte	33

* indicates the gene(s) knocked

PKD, polycystic kidney disease; ECM, extracellular matrix; CRISPR, clustered regularly interspaced short palindromic repeats

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 subjects with hematological malignancies	HIV-1 infection	I	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	I	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	I/II	Universal CRISPR Cas9 gene-editing CAR-T Cells targeting CD19 (UCART019)	Chinese PLA General Hospital	Recruiting
PD-1 knockout engineered T cells for advanced esophageal cancer	Esophageal cancer	II	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	I	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	I	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	I	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	I/II	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 *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	I/II	Universal CRISPR-Cas9 gene editing of CAR-T cells targeting CD19 and CD20 or CD22	Chinese PLA General Hospital	Recruiting

Source: clinicaltrials.gov.

* NY-ESO-1 is a human tumor antigen.

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