models demonstrate changes in immune and epithelial cell interactions that are consistent with the disease states they represent (5, 8, 9). Beyond determining localization and expression changes of tubule segment-specific markers and other genes of interest, spatially resolved transcriptomics can reveal how communication within tubule microenvironments responds to genetic variation and disease. The pairing of the spatially resolved transcriptomics pipeline with sc/snRNAseq and other sequencing modalities, such as assay for transposase-accessible chromatin with sequencing (ATAC-seq), will build a multi-dimensional picture of how the kidney responds to health and disease states, leading us closer to defining mechanisms of kidney physiology. ■

Eryn E. Dixon, PhD, is a Postdoctoral Research Scholar with the Washington University School of Medicine in St. Louis, MO.

The author reports no conflicts of interest.

References

1. Eng C-HL, et al. Transcriptome-scale super-resolved imaging in tissues by RNA seqFISH+. *Nature* 2019; 568:235–239. doi: 10.1038/s41586-019-1049-y
2. Rudman-Melnick V, et al. Single-cell profiling of AKI in a murine model reveals novel transcriptional signatures, profibrotic phenotype, and epithelial-to-stromal crosstalk. *J Am Soc Nephrol* 2020; 31:2793–2814. doi: 10.1681/ASN.2020010052
3. Ståhl PL, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 2016; 353:78–82. doi: 10.1126/science.aaf2403
4. 10X Genomics. Visium Spatial Gene Expression. Map the whole transcriptome within the tissue context. 2022. https://www.10xgenomics.com/products/spatial-gene-expression
5. Dixon EE, et al. Spatially resolved transcriptomic analysis of acute kidney injury in a female murine model. *J Am Soc Nephrol* 2022; 33:279–289. doi: 10.1681/ASN.2021081150
6. Kirita Y, et al. Cell profiling of mouse acute kidney injury reveals conserved cellular responses to injury. *Proc Natl Acad Sci USA* 2020; 117:15874–15883. doi: 10.1073/pnas.2005477117
7. Park J, et al. Single cell-transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. *Science* 2018; 360:758–763. doi: 10.1126/science.aar2131
8. Melo Ferreira R, et al. Integration of spatial and single-cell transcriptomics localizes epithelial cell-immune cross-talk in kidney injury. *JCI Insight* 2021; 6:e147703. doi: 10.1172/jci.insight.147703
9. Janosevic D, et al. The orchestrated cellular and molecular responses of the kidney to endotoxin define a precise sepsis timeline. *Elife* 2021; 10:e62270. doi: 10.7554/eLife.62270

Pancreatic Islet Cell Transplantation: Are We Getting Closer?

By Collin Jordan and Xunrong Luo

For the greater part of the 20th century, exogenous insulin administration and whole organ pancreatic transplantation served as the predominant therapeutic interventions for patients with type 1 diabetes mellitus. The success of the Edmonton group in achieving insulin independence in the early 2000s via islet cell transplantation in a cohort of patients with autoimmune diabetes led to renewed optimism that this treatment could serve as an alternative to solid organ transplantation (1). However, 16 years later, a shortage of donor pancreatic islet cells remains a major challenge in increasing the scale of human allogeneic islet transplantation.

Various novel approaches to alleviate the donor shortage have been studied; however, a promising treatment offered by Vertex Pharmaceuticals seeks to eliminate this obstacle. In collaboration with the lab of Douglas Melton, Xander University Professor at Harvard and an Investigator of the Howard Hughes Medical Institute, Vertex has generated VX-880, an investigational stem cell-derived, fully differentiated pancreatic islet cell replacement therapy for patients with type 1 diabetes (2).

Typically, in whole islet transplantation, purified islets are infused via the hepatic portal vein, upon which they migrate to and engraft in the sinusoids of the liver. For Vertex's clinical trial, the technical mechanism of transplantation remains the same; however, unlike whole islet transplantation, VX-880 is selectively a beta cell therapy. Thus far, it has not been confirmed that beta cells engraft in the same spatial orientation of the liver as do whole islets, given that they are magnitudes smaller in size. Despite this, we know that transplanted beta cells do produce and secrete insulin into the blood immediately following transplantation (Figure 1).

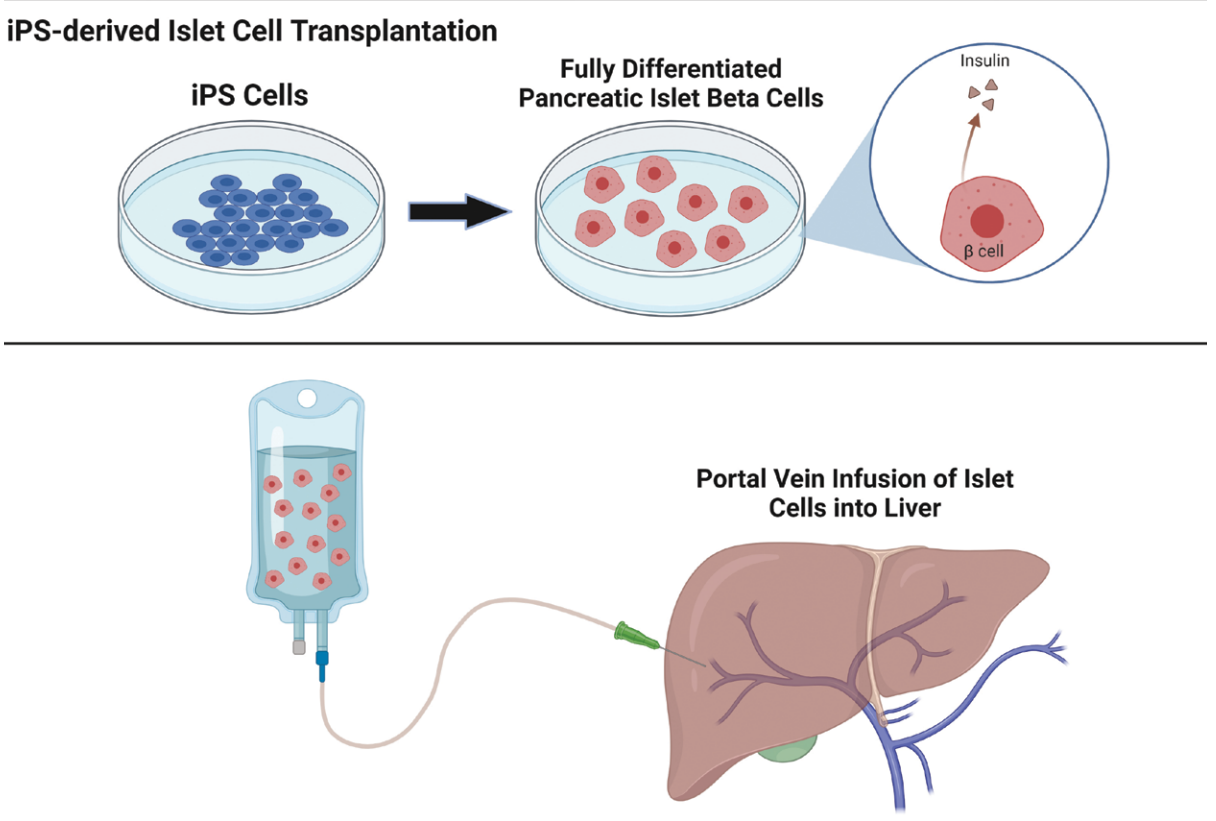
A game-changing therapy, VX-880 has the potential to completely obviate the need for an organ donor. In March 2021, the collaborators initiated a phase 1/2 clinical trial to evaluate the safety, tolerability, and efficacy of VX-880 (3). Seven months later, Vertex issued a press release to announce positive day 90 data for the first patient recruited to the phase 1/2 trial. Brian Shelton, the patient in question, whose identity was revealed in a feature in *The New York Times*, had lived with autoimmune diabetes for nearly 50 years before receiving a single infusion of VX-880 at one-half the target dose (4). In conjunction with immunosuppression therapy, Shelton demonstrated successful engraftment and restoration of insulin production. Furthermore, rapid improvement in fasting and stimulated C-peptide, glycemic control, and HbA1c were observed, along with a 91% decrease in daily exogenous insulin administration. These results are certainly encouraging for the continued progression of the VX-880 clinical studies, although what makes them truly striking is that they were achieved at one-half the target dose.

Perhaps the most transformative advancement to extrapolate from these early results is the potential for a rapid expansion of the donor islet pool readily available to patients with type 1 diabetes. Without the need for a deceased donor, stem cell-derived VX-880 offers new hope for the large-scale feasibility of clinical islet transplantation. However, beyond that, our ultimate goal should be to deliver islet cell transplantation without the need for a robust immunosuppression regimen, thus mitigating the risk for opportunistic infections among recipients. Whether this comes from Vertex's already-developed encapsulated islets or through another mechanism, such as gene-editing technology or immunoprotective implantation devices, remains to be seen.

Regardless, future trials involving VX-880 must explore the attrition of these stem cell-derived islets after transplantation. Do the cells functionally decline over time? Will a patient potentially need multiple infusions to remain euglycemic? If so, would the multiple infusions precipitate a donor-specific alloimmune response, subsequently driving

Continued on page 22 >

Figure 1.



iPS, induced pluripotent stem cell.

Pancreatic Islet Cell Transplantation

Continued from page 21

sensitization of the recipient and thus, making future infusions progressively less effective? These are questions that must be addressed if we are to proclaim VX-880 a landscape-changing cell therapy for autoimmune diabetes. Until then, it will be interesting to follow the continued progression of this trial to see if these results can be generalized to a larger cohort of patients. ■

Collin Jordan, BS, is with the Division of Nephrology, Department of Medicine, Duke University School of Medicine, and Xunrong Luo, MD, PhD, is with the Division of Nephrology, Department of Medicine, and the Duke Transplant Center, Duke University School of Medicine, Durham, NC.

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest, in the subject matter or materials discussed in this manuscript.

References

1. Shapiro AMJ, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; 355:1318–1330. doi: 10.1056/NEJMoa061267
2. Vertex Pharmaceuticals. Vertex announces positive day 90 data for the first patient in the phase 1/2 clinical trial dosed with VX-880, a novel investigational stem cell-derived therapy for the treatment of type 1 diabetes. October 18, 2021. https://investors.vrtx.com/news-releases/news-release-details/vertex-announces-positive-day-90-data-first-patient-phase-12
3. ClinicalTrials.gov. A safety, tolerability, and efficacy study of VX-880 in participants with type 1 diabetes. First posted: March 8, 2021; Last update posted: February 17, 2022. Identifier: NCT04786262. https://clinicaltrials.gov/ct2/show/NCT04786262
4. Kolata, G. A cure for type 1 diabetes? For one man, it seems to have worked. *The New York Times* November 27, 2021. https://www.nytimes.com/2021/11/27/health/diabetes-cure-stem-cells.html

Ferroptosis, an Emerging Therapeutic Target for Acute and Chronic Kidney Diseases

By Koki Abe and Tomokazu Souma

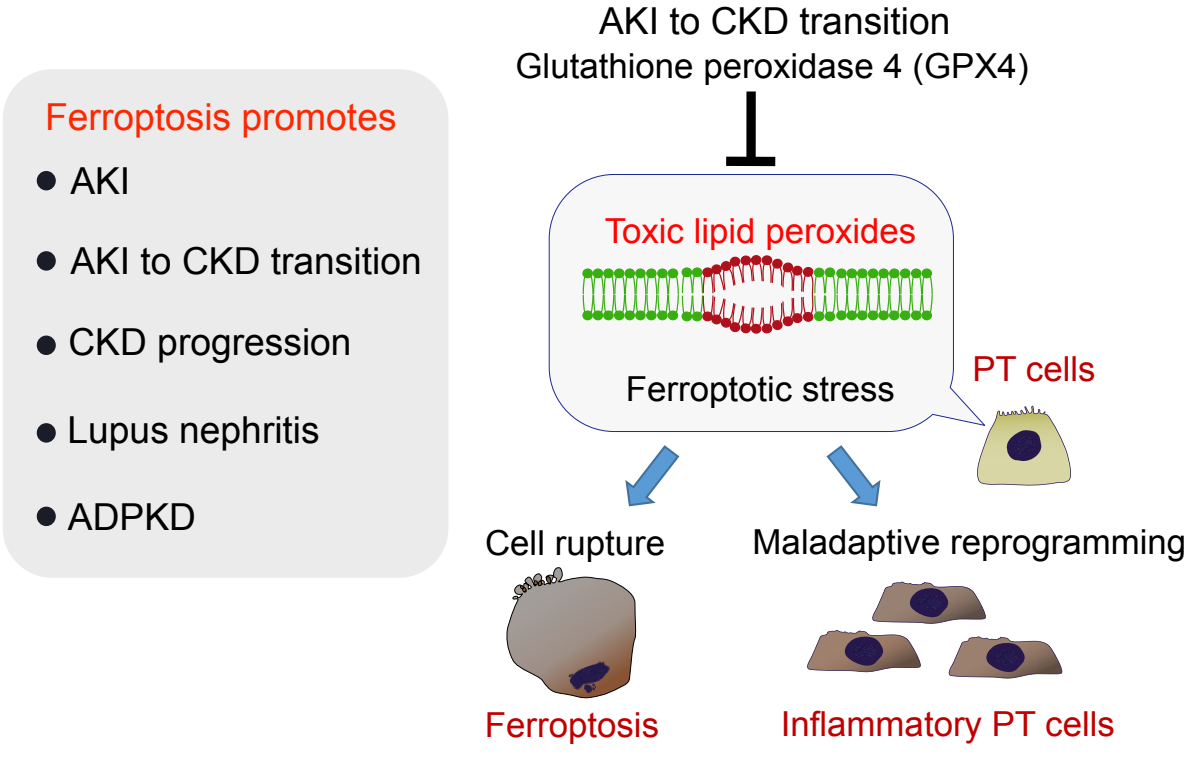
Cell death is a fundamental biological process underlying normal development, homeostasis, and diseases. Regulated cell death is defined as a molecularly controlled cell death that can be modulated (either promoting or preventing) by specific interventions (1). Although apoptosis has been the focus of interest regarding research on regulated cell death and has been historically considered a major cell death pathway in kidney disease processes, there are surprisingly many other ways cells end their lives in a molecularly regulated manner, such as necroptosis, pyroptosis, ferroptosis, and others. Among them, ferroptosis is attracting attention as a critical contributor and a potential novel therapeutic target for many common pathologic states, such as acute and chronic kidney diseases, cardiovascular diseases, neurodegeneration, stroke, chemotherapy-resistant cancers, and more (1, 2).

The term "ferroptosis" was coined in 2012 to describe a distinct form of cell death caused by the pathologic accumulation of toxic lipid peroxides (i.e., oxidized lipids) in an iron-dependent manner (1–3). Accumulation of toxic lipid peroxides worsens the redox status of the cell membrane (ferroptotic stress) and causes plasma membrane damage and subsequent cellular rupture (Figure 1). The glutathione/glutathione peroxidase 4 (GPX4) defense pathway prevents this pathologic consequence by detoxifying toxic lipid peroxides. Ferroptosis is particularly relevant to kidney diseases because the kidney is one of the organs most vulnerable to dysregulation and insufficient activity of GPX4 (4). This was highlighted by examining mice with complete absence (through genetic deletion) of the *Gpx4* gene. Mice without the *Gpx4* gene had massive albuminuria, kidney tubular epithelial cell death, and subsequent mortality just a few weeks after inducing the gene deletion (4). Moreover, recent human studies suggest the potential involvement of the ferroptotic process in acute kidney injury (AKI) and chronic kidney disease (CKD), highlighting its clinical significance (5–8).

The first investigations of the ferroptotic process in nephrology examined its role in AKI. Ferroptosis inhibitors have been shown to diminish the severity of AKI in multiple preclinical (animal) AKI models such as ischemia-reperfusion injury and folic acid-induced nephropathy (1, 9). Our study further identified how the ferroptotic process involves maladaptive repair after AKI using single-cell transcriptomics, a revolutionizing tool to decipher complex biological and pathological processes at single-cell resolution (10). After ischemic and toxic injuries, proximal tubular cells of the kidney alter their cellular state significantly and acquire a proinflammatory state. They also revert to a more primitive state called dedifferentiation (10–14). The accumulation of these inflammatory proximal tubular cells appears to promote kidney inflammation and a maladaptive repair process (10–14). The ferroptotic process uniquely contributes to this dynamic alteration of the proximal tubule cell state (10). Our group found that ferroptotic stress promotes the accumulation of these inflammatory proximal tubule cells inside the severely damaged kidneys, in addition to triggering ferroptotic death of these cells (Figure 1). Our results, using a mouse model, suggest that inhibiting the ferroptotic process holds the potential to disrupt the AKI to CKD transition.

Although most of the currently available data are derived from animal models, emerging evidence supports the pathogenic role of ferroptosis in multiple forms of CKD. By detailed and integrated analyses of genome-wide association studies on kidney function with multiple human transcriptomic and epigenomic datasets, two genes (*DPEP1* and *CHMP1A*) were identified as potential causal genes of CKD progression (6). This mechanistic study using animal models found that these two genes control cellular ferroptosis sensitivity of proximal tubule cells by regulating cellular iron homeostasis. Ferroptosis is also linked with autosomal dominant polycystic kidney diseases (15). Surprisingly, pharmacological induction of the ferroptotic process increased the cyst growth in *Pkd1* null mice by triggering cellular proliferation of cyst-lining epithelial cells in addition to inducing cell death. Conversely, pharmacological inhibition of ferroptosis reduced the cyst size. Clinical and experimental data also show ferroptosis of the neutrophil promotes the pathogenesis of lupus nephritis in mice and likely in humans (7). These data collectively support that ferroptosis inhibition represents an attractive therapeutic strategy to prevent multiple forms of CKD. We also need to be aware that a therapeutic strategy that enhances ferroptosis gains significant attention to treat chemotherapy-resistant cancers, as ferroptosis is identified as a targetable vulnerability of therapy-resistant cancers (16). Therefore, we may see increased AKI incidence due to enhanced tubular toxicity in cancer patients treated with the

Figure 1. Ferroptosis underlies multiple forms of acute and chronic kidney diseases



Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; ADPKD, autosomal dominant polycystic kidney disease; PT cells, proximal tubular cells.