Experimental models of acute kidney injury for translational research

Neil A. Hukriede[1], Danielle E. Soranno[2], Veronika Sander[3], Tayla Perreau[3], Michelle C. Starr[4], Peter S. T. Yuen[5], Leah J. Siskind[6], Michael P. Hutchens[7], Alan J. Davidson[3], David M. Burmeister[8], Sarah Faubel[9] and Mark P. de Caestecker[10]✉

Abstract | Preclinical models of human disease provide powerful tools for therapeutic discovery but have limitations. This problem is especially apparent in the field of acute kidney injury (AKI), in which clinical trial failures have been attributed to inaccurate modelling performed largely in rodents. Multidisciplinary efforts such as the Kidney Precision Medicine Project are now starting to identify molecular subtypes of human AKI. In addition, over the past decade, there have been developments in human pluripotent stem cell-derived kidney organoids as well as zebrafish, rodent and large animal models of AKI. These organoid and AKI models are being deployed at different stages of preclinical therapeutic development. However, the traditionally siloed, preclinical investigator-driven approaches that have been used to evaluate AKI therapeutics to date rarely account for the limitations of the model systems used and have given rise to false expectations of clinical efficacy in patients with different AKI pathophysiologies. To address this problem, there is a need to develop more flexible and integrated approaches, involving teams of investigators with expertise in a range of different model systems, working closely with clinical investigators, to develop robust preclinical evidence to support more focused interventions in patients with AKI.

Acute kidney injury (AKI) complicates 10–15% of hospitalizations and is independently associated with increased mortality[1]. AKI is currently defined using the 2012 Kidney Disease Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine of ≥0.3 mg/dl (26.5 μmol/l) within 48 h or an increase in serum creatinine to ≥1.5 times baseline within 7 days or urine volume <0.5 ml/kg/h for 6 h[2]. Surviving patients often have delayed recovery of kidney function, classified as acute kidney disease (glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 or a decrease in GFR by ≥35% over baseline or an increase in serum creatinine by >50% over baseline or an elevated marker of kidney function for <3 months), or incomplete recovery with chronic kidney disease (CKD; GFR < 60 ml/min/1.73 m^2 or elevated marker of kidney damage for >3 months)[3].

AKI is a highly heterogeneous condition with respect to aetiology (for example, sepsis, obstruction or nephrotoxins), pathophysiology and kidney outcomes (for example, glomerular disease, interstitial disease or tubular injury)[4]. The development and adoption of a standardized definition of AKI has helped to provide uniformity to the inclusion criteria used in clinical trials. However, a major limitation of the KDIGO definition is that AKI is defined without respect to aetiology. Clinically, the most common cause of AKI is acute tubular injury (ATI), formerly known as acute tubular necrosis. ATI has diverse aetiologies, including nephrotoxins, reduced renal blood flow leading to ischaemia, muscle injury leading to rhabdomyolysis, major trauma including surgery, and cardiopulmonary bypass[4,5]. Sepsis-associated AKI (SA-AKI) is also considered to be a form of ATI, although the histological appearance and pathophysiology are distinct from other causes of ATI[6].

Pre-existing conditions, including a history of previous AKI, prematurity, old age, CKD and diabetes, increase the risk of AKI and likely influence disease pathobiology and responses to treatment. The role of sex as a biological variable in AKI is unclear. Although the KDIGO Clinical Practice Guidelines lists female sex as a risk factor for AKI, a large meta-analysis reported that male sex is associated with an increased risk of hospital-associated AKI[7]. Clinical investigations have also demonstrated that CKD progression occurs more slowly in women than in men[8]. Responses to AKI vary between individuals; some patients with similar injuries have complete recovery, whereas others have delayed or incomplete recovery[3].

✉*e-mail:* *mark.de.caestecker@vumc.org*

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Key points

- Human induced pluripotent stem cell-derived kidney organoid models of toxin-induced acute kidney injury (AKI) are amenable to high-throughput drug discovery and may provide insight into inter-individual variations in responses to therapeutic interventions.
- Zebrafish models of toxin-induced AKI can be used for high-throughput, rapid therapeutic discovery before translation into mammalian systems.
- Ischaemic, cardiac, toxin and sepsis-associated rodent models of AKI can be used to reflect diverse pathophysiologies in human AKI, validate therapeutic targets using genetic studies and explore distant organ effects of AKI.
- Large animal models provide opportunities to more closely model human AKI pathophysiology and pharmacology, with increasingly complex, layered models of injury.
- The discovery of molecular subtypes of human AKI will drive the development of focused preclinical therapeutic strategies to target defined AKI pathophysiologies.
- We recommend multidisciplinary, bench-to-bedside approaches to the development and design of preclinical research pipelines using multiple models and species to optimize the potential for translation of findings into therapies for human AKI.

Current treatment options for AKI are limited to supportive care. No intervention has been definitively shown to prevent AKI or to accelerate kidney function recovery after AKI[3]. In part, this lack of success stems from weaknesses in clinical study design, but there is also concern that preclinical models fail to appropriately recapitulate human AKI[9]. In this Review, we summarize the state-of-the art of model development for AKI due to tubular injury, focusing on the translatability of discoveries using human kidney organoids, zebrafish, rodent and large animal models (LAMs). We describe the advantages, limitations and challenges of these model systems and the approaches that are being used to optimize these models for translation into humans. Finally, we make a series of recommendations for investigators interested in preclinical therapeutic development for AKI.

Kidney organoids

Human kidney organoids, small masses of kidney tissue grown in vitro from pluripotent stem cells (PSCs), are a relatively new addition to the AKI toolbox (BOX 1). Organoids derived from induced PSCs (iPSCs) from different individuals incorporate human genetic heterogeneity, enabling analysis of inter-individual variations in AKI responses. Organoids also enable data from animal models of AKI to be validated in a human context without the need for scarce patient kidney tissue samples. In addition, because of their small size and genetic malleability, PSC-derived organoids provide a platform for high-throughput therapeutic discovery (for example, using organoids generated from fluorescent reporter PSCs)[10].

Methods of generating kidney organoids from human PSCs rely on self-organizing programmes of kidney development that are initiated by the small molecule Wnt activator CHIR99021 (REF.[10]). Subsequent differentiation is induced by various factors and media supplements. Despite differences in cost, complexity and scalability between protocols, the resulting organoids are similar in tissue complexity and maturity. The structures generated resemble nascent nephrons containing glomeruli at the capillary loop stage of development. These structures are interconnected distally but lack a collecting duct tree. Additional cell types in kidney organoids include interstitial cells, endothelial cells and several non-renal cell types[11–18]. Protocols in which nephron and ductal progenitor cells are generated separately and then combined generate more complex structures in which collecting duct morphogenesis and nephrogenesis occur together[19–21].

Toxin-induced injury

To date, AKI modelling in organoids has been restricted to toxin-induced injury, mainly focused on gentamicin and cisplatin (TABLE 1). Early observations suggested that these drugs induce proximal tubule epithelial cell (PTEC) injury in kidney organoids[14,17,22–24]. However, a comprehensive analysis revealed that high-dose cisplatin induces cell death throughout the organoid, mainly targeting rapidly dividing interstitial cells[25]. More selective PTEC injury is seen when cisplatin is administered to organoids in repeated low doses, suggesting that this regimen is a better model for cisplatin-induced AKI[25]. A study that profiled differentially expressed genes in cisplatin-treated organoids identified upregulation of three clinical AKI biomarkers (*KIM1*, *CLU*, *CST3*) and some emerging biomarkers (*MMP9*, *CYR61*/*CCN1*, *CHI3L1*, *SPP1*)[25], suggesting that organoids can be used to study core AKI injury responses. Inflammatory and pro-fibrotic genes were also upregulated, raising the possibility that organoids could be used to test immune modulators and anti-fibrotic agents. For example, incubation of kidney organoids with interleukin (IL)-1β-induced organoid hypertrophy, proximal tubule injury and fibrogenesis; these effects were abrogated by incubation with the BET bromodomain inhibitor JQ1, which was found to inhibit the ability of IL-1β to convert kidney organoid stromal cells into activated myofibroblasts[26].

Use of kidney organoids to model AKI has several limitations. Their fetal stage of maturation is of concern for modelling drug-induced AKI as many solute transporters required for drug uptake are only expressed later in development[25,27]. Efforts to improve the conversion of PSCs into missing lineages and to develop more complex co-culture protocols are expected to increase the cellular diversity and maturation of kidney organoids,

Author addresses

[1]Department of Developmental Biology, University of Pittsburgh, Pittsburgh, USA.
[2]Department of Paediatrics, University of Colorado, Aurora, USA.
[3]Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand.
[4]Department of Paediatrics, Indiana University School of Medicine, Indianapolis, USA.
[5]Renal Diagnostics and Therapeutic Unit, National Institute of Diabetes, Digestive and Kidney Disease, NIH, Bethesda, USA.
[6]Department of Pharmacology and Toxicology, University of Louisville Medical School, Louisville, USA.
[7]Department of Anaesthesiology and Perioperative Medicine, Oregon Health and Science University, Portland, USA.
[8]Department of Medicine, Uniformed Services University of Health Sciences, Bethesda, USA.
[9]Department of Medicine, University of Colorado, Aurora, USA.
[10]Department of Medicine, Vanderbilt University Medical Center, Nashville, USA.

Box 1 | **Human kidney organoids for AKI modelling**

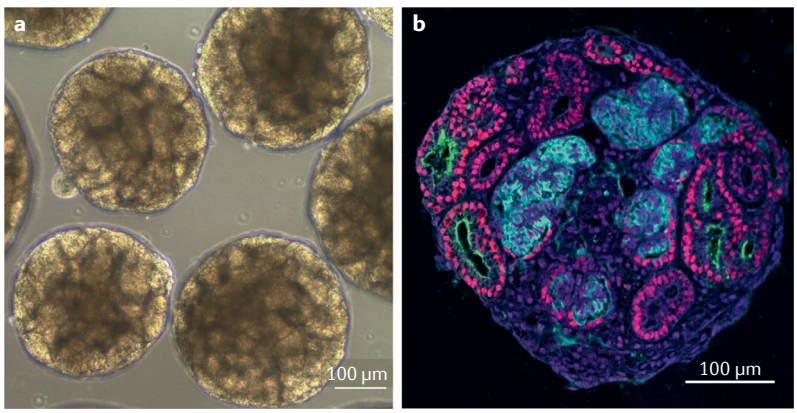
Kidney organoids generated in vitro from human induced pluripotent stem cells have been used to model toxin-induced acute kidney injury (AKI). The figure shows a brightfield image of kidney organoids (part **a**) and immunohistochemical staining of a sectioned kidney organoid (part **b**). In part **b**, the tubules are labelled in red (HNF1B), proximal tubules in green (apical staining with *Lotus tetragonolobus* lectin) and podocytes in cyan (podocalyxin). The nuclear counterstain (dark blue) is Hoechst. Kidney organoids have several advantages and disadvantages for modelling AKI.

Advantages

- Human cell-derived system
- 3D structures composed of multiple cell types
- Unlimited supply at low cost
- Recapitulation of injury response
- Application to compound testing and biomarker discovery

Disadvantages

- Resembles fetal kidney tissue, with gene expression that is not representative of adult kidney
- Short lifespan before nephron degeneration
- Poor vascularization, lack of perfusion
- Lack of glomerular filtration and tubular flow
- Lack of non-renal cell populations, for example, immune cells



enhancing their value as the foundation for experimental AKI models. In addition, kidney organoids are poorly vascularized and have no tubular filtrate or flow, which limits modelling of drug-induced AKI as uptake of filtered nephrotoxins by PTECs drives injury[28]. Kidney organoids can be vascularized by transplantation under the kidney capsules of rodents or into chick embryos[29–32]. Culturing organoids embedded in extracellular matrix and exposed to flow also improves vascularization and nephron maturation[13]. We anticipate that the application of this technology to kidney organoids will likely see functional coupling to bioengineered vascular channels in the future[33]. As many nephrotoxins are filtered by the glomerulus and then become concentrated in the proximal tubule, we expect these more advanced protocols to better model the tubular injury seen in AKI. Moreover, such flow conditions may enhance the maturation of organoids[13] and therefore create conditions under which new therapeutics and targets can be compared in developmentally immature versus mature organoids to enable identification of therapies that might be effective during fetal development and/or early postnatal life.

Zebrafish models

Fish models of AKI were originally developed in goldfish[34,35] and subsequently extended to rainbow trout, catfish, tilapia and zebrafish[36]. Zebrafish models of AKI have shown promise for studying molecular mechanisms of AKI and for high-content therapeutic screens. Zebrafish larvae have a functional pronephric kidney that consists of two bilateral nephrons, whereas the terminal kidney of adult zebrafish is mesonephric. Both kidney types contain segments analogous to those seen in mammals; however, larval zebrafish only have two proximal tubule segments and lack the thin limb components of the loop of Henle[37]. Another major difference between fish and mammalian kidneys is that unlike mammals, which have a fixed nephron endowment, adult zebrafish continue to generate new nephrons as the animal increases in size[38]. Studies in zebrafish that used gentamicin to induce nephron damage identified the cellular niche that drives new nephron formation, termed neo-nephrogenesis, in adult fish[39,40]. AKI studies in larval zebrafish are particularly useful for high-content therapeutic and genetic screening because of their short development time, ease with which they can be bred, small size, facility of genetic manipulations and transparency, which enables fluorescence-based screening[41].

Toxin-induced injury

Studies of AKI in larval zebrafish show that gentamicin causes histological and functional changes consistent with mammalian aminoglycoside toxicity[42,43] as well as expression of the PTEC injury marker, Kim-1 (REFS[44,45]). The small size of zebrafish larvae makes them useful for screening in 96-well plate formats, as they can be soaked in compounds with a small amount of dimethyl sulfoxide[46]. This simple exposure method enables a broad diversity of compounds to be utilized in zebrafish assays without the need for in-depth in vivo pharmacological studies. Larval nephrotoxin models have been used in screens to identify therapeutic candidates that subsequently showed efficacy in mouse AKI[42,47,48].

Sepsis

Sepsis models of AKI have also been developed in zebrafish. An early study showed that injection of live *Pseudomonas aeruginosa* into zebrafish embryos resulted in lethal infection, which responded to antibiotics, and a systemic immune response[49]. Subsequently, *Edwardsiella tarda* was used to model SA-AKI[50]. Injection of *E. tarda* into the circulation of zebrafish larvae resulted in renal dysfunction with >50% mortality and pericardial oedema. Expression of the AKI markers insulin-like growth factor-binding protein-7, tissue inhibitor of metalloproteinases 2 and Kim-1 were increased in the kidney tubules of septic fish. These studies indicate that zebrafish can be used as an SA-AKI model to study host–pathogen interactions, immune responses and potential therapies[48].

Although larval and adult zebrafish have been invaluable for studying AKI events and therapeutic interventions, several limitations must be considered. The zebrafish larval kidney comprises two nephrons, making

Table 1 | **Nephrotoxicity studies using human iPSC-derived 3D kidney models**

Study (year)	Drug(s)	Injury readout	Results
Human iPSC-derived kidney organoids			
Takasato et al. (2015)[17]	Cisplatin (5, 20 and 100 μM)	Apoptosis (C-CASP3)	CASP3 colocalized with LTL$^+$CDH1$^+$ tubule cells with 5 μM and 20 μM cisplatin; global cell death with 100 μM cisplatin
Morizane et al. (2015)[14]	Cisplatin (5 and 50 μM); gentamicin (5×10^{-4}, 5×10^{-2} and 5 mg/ml)	KIM1 protein and gene (*HAVCR1*) expression; DNA damage (γH2AX)	γH2AX colocalized with LTL$^+$ tubule cells with 5 μM cisplatin; global cell death with 50 μM cisplatin
Freedman et al. (2015)[12]	Cisplatin (50 μM); gentamicin (5 mM)	KIM1 expression	KIM1 colocalized with LTL$^+$ tubule cells
Hale et al. (2018)[254]	Doxorubicin (0.1, 0.5, 1 and 5 μM) treatment of glomeruli isolated from organoids generated from a MAFB-BFP2 podocyte reporter iPSC line	Glomerular diameter, apoptosis (C-CASP3), BFP2 intensity	Dose-dependent reduction of BFP2 intensity (reflecting podocyte injury), decreased glomerular diameter, induction of apoptosis
Lemos et al. (2018)[26]	IL-1β (500 nM) with or without JQ1	Organoid diameter, protein expression of KIM1, p21, collagen I and fibrogenic markers (*COL1A1*, *FN1*, *ACTA2*)	Upregulation of KIM1, collagen I and p21 in LTL$^+$ proximal tubule cells; upregulation of fibrogenic markers; PDGFRβ$^+$ stromal cell activation and fibrosis via stabilization of MYC; co-treatment with JQ1 was protective
Czerniecki et al. (2018)[23]	Cisplatin (16, 50 and 150 μM)	Cell viability luminescence assay; KIM1 protein	KIM1 colocalized with LTL$^+$ tubules
Kumar et al. (2019)[255]	Doxorubicin (2.5 and 5 μg/ml)	Cell death (TUNEL); gene expression of tubule (*CUBN*, *CDH6*, *CDH1*) and podocyte (*NPHS1*, *SYNPO*, *LAMA5*) markers	Colocalization of TUNEL and NPHS1 indicating podocyte death; reduced expression of podocyte marker genes
Digby et al. (2020)[25]	Single high-dose cisplatin (25 and 50 μM); repeated low-dose cisplatin (4 × 5 μM)	*CXCL8* expression, KIM1 protein and gene (*HAVCR1*) expression, DNA damage (γH2AX) and cell death (TUNEL)	γH2AX colocalized predominantly with MEIS1/2/3$^+$ stromal cells with the single high-dose regimen; more pronounced colocalization of γH2AX with LTL$^+$ tubules with repeated low-dose regimen; low susceptibility of proximal tubules to cisplatin-induced injury coincided with low expression of the cisplatin transporter *SLC22A2*
Lawlor et al. (2021)[256]	Doxorubicin (0.3–10 μM) and aminoglycoside antibiotics (1.5 μg/ml to 25 mg/ml of amikacin, tobramycin, gentamycin, neomycin or streptomycin)	CASP3 and MAFB protein; injury and podocyte marker expression (*HAVCR1*, *BAX*, *PODXL*, *NPHS1*); cell viability (cellular ATP content)	Concentration-dependent effects on cell viability with all drugs, upregulation of injury markers and downregulation of podocyte markers with doxorubicin
Human iPSC-derived 3D multicellular kidney cultures			
Bajaj et al. (2018)[22]	Gentamicin (0.016–10 mg/ml); citrinin (0.096–60 μM); cisplatin (0.098–25 μM); rifampicin (0.48–300 μM); puromycin (0.098–25 μM; doxorubicin (0.048–30 μM)	*HAVCR1*, *HMOX1*, *NPHS1* and *WT1* gene expression	Dose-dependent induction of *HAVCR1* and *HMOX1* with gentamicin, citrinin, cisplatin and rifampicin; upregulation of *NPHS1* and *WT1* with puromycin and doxorubicin
Human iPSC-derived kidney organoids implanted into chick embryos			
Garreta et al. (2019)[30]	Intravenous injection of cisplatin	KIM1 and CASP3 protein	KIM1 and CASP3 colocalized with LTL$^+$ tubules

ACTA2, actin-α2, smooth muscle; ATP, adenosine triphosphate; BAX, BCL2-associated X; BFP2, blue fluorescent protein 2; CASP3, caspase 3; CDH1, cadherin 1; CDH6, cadherin 6; CDKN1A/p21, cyclin dependent kinase inhibitor 1A; COL1A1, collagen type 1 α1 chain; CUBN, cubilin; CXCL8, C-X-C motif chemokine ligand 8; FN1, fibronectin 1; HAVCR1, hepatitis A virus cellular receptor 1; HMOX1, haem oxygenase 1; IL-1β, interleukin-1β; iPSC, induced pluripotent stem cell; KIM1, kidney injury molecule 1; LAMA5, laminin subunit-α5; LTL, *Lotus tetragonolobus* lectin; MAFB, MAF BZIP transcription factor B; MEIS, Meis homeobox 1/2/3; MYC, MYC proto-oncogene, BHLH transcription factor; NPHS1, nephrin; PDGFRβ, platelet-derived growth factor receptor-β; PODXL, podocalyxin like; SLC22A2, solute carrier family 22 member 2; SYNPO, synaptopodin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; WT1, Wilms tumour 1; γH2AX, γH2A.X variant histone.

the larvae vulnerable to death if the injury is severe. In addition, both larval and adult zebrafish lack the fibrotic response that is seen in mammals. Finally, because the neo-nephrogenesis response to injury in adult zebrafish is robust and does not occur in mammals, long-term regenerative responses following AKI in adult zebrafish might mask any effects of treatments that have been designed to promote nephron recovery in humans.

Rodent models

A variety of rat and mouse models of AKI have been developed to study pathophysiological mechanisms and therapeutics. Global and tissue-specific knockout and fluorescence labelling studies in genetically modified mice have had a critical role in the assessment of cellular and molecular targets for therapeutic intervention in various diseases[51]. Studies in rodent models led to the development of angiotensin-converting enzyme

inhibitors as effective therapeutics for CKD[52]. However, successful translation of results from rodent models to patients with AKI has not been achieved, leading to concern that these models do not recapitulate the pathophysiology of human AKI[9].

Ischaemia–reperfusion injury

In rodents, kidney ischaemia–reperfusion injury (IRI) is induced by surgical clamping, usually of the renal pedicles in mice and renal arteries in rats. This approach provides a defined and controllable AKI, with dominant injury to the PTECs in the S3 segment of the nephron, which is the most metabolically active segment[53]. Kidney IRI is used to model the effects of reduced renal blood flow after cardiac bypass, kidney transplantation or nephron-sparing surgery as well as in the setting of hypotension[9]. Although the severity of injury seen on histological analysis following kidney IRI in rodents is rarely seen in human AKI[54], comparisons of mouse kidneys following IRI with human transplanted kidneys with AKI[55,56] show conservation of molecular responses between species.

Several critical variables influence the severity of AKI after kidney IRI in rats and mice. These include renal pedicle clamp times and clamp pressure; body temperature, which is affected by body temperature regulators (for example, rectal probe-regulated temperature controllers, water baths or electrically heated surgical platforms) as well as changes in ambient room temperature; the hydration status of the animal; and the surgical skills of the operator[57–60]. In addition, the use of inhalational anaesthesia, including halothane and isoflurane, compared with phenobarbital and ketamine–xylazine, reduces the severity of AKI after IRI via a mechanism that is not yet clear[61].

The severity of injury after bilateral IRI in mice is limited by mortality with long renal pedicle clamp times, such that long-term studies looking at effects on CKD progression are challenging. Short clamp times are used to study the mechanisms of tubular repair after IRI-AKI[62], but in the experience of the de Caestecker laboratory, these injuries are insufficient to cause progression to CKD (M.P.D.C., unpublished work). However, a persistent reduction in GFR has been reported in mice a year after bilateral IRI[63]. These differing findings underscore the fact that minor differences in mouse strain, age, fluid resuscitation and even altitude may account for heterogeneity in bilateral IRI-AKI outcomes.

Unilateral IRI enables longer ischaemic times than bilateral IRI, and can involve cold or warm ischaemia[64]. This approach models the effects of IRI in patients undergoing nephron-sparing surgery in which ischaemia times >25 min are associated with progression to CKD[65,66]. Functional recovery can be evaluated by performing a contralateral nephrectomy after the initial injury[67]. Rodents undergoing unilateral IRI develop more severe injury and delayed recovery, which is partially reversed after nephrectomy, than those undergoing bilateral IRI with similar clamp times[68–70]. A study that used a mouse model of unilateral cold IRI-AKI to induce intra-operative renal histology similar to that of patients undergoing nephron-sparing surgery showed that this level of injury led to CKD progression in the mice[71].

Most IRI-AKI studies are performed in 8- to 12-week-old male mice, whereas human AKI commonly occurs in old age[9]. Following kidney IRI, aged mice develop similar levels of injury to younger mice but have increased mortality and delayed recovery[72]. Female sex is protective in IRI-AKI, partly owing to a protective effect of low testosterone levels[73]. This finding is important because cadaveric male donor kidneys have a higher incidence of delayed graft function than female donor kidneys[74]. This sex difference is recapitulated in mouse transplantation studies[74], emphasizing the importance of studying both sexes[75]. Mouse strain and environment also affect susceptibility to IRI-AKI[57,67], underscoring the importance of establishing IRI-AKI conditions for each individual mouse line.

Effects of comorbidities. Diabetic Akita mice with *Ins2* mutations and mice treated with streptozotocin have increased susceptibility to IRI-AKI[67,76]. This susceptibility is associated with increased mitochondrial damage and is reversed by short-term correction of hyperglycaemia with insulin[76], suggesting that it is the result of hyperglycaemia-induced changes to metabolism and/or mitochondrial function, rather than structural changes to the kidney. Db/db mice (a model of type 2 diabetes mellitus) also have increased susceptibility to IRI-AKI[77], which is associated with delayed restoration of renal blood flow after release of clamps[78], suggesting a contribution of vascular dysfunction.

Few studies exist of IRI-AKI in rodents with pre-existing CKD. One such study evaluated the effects of IRI-AKI in rats that had undergone subtotal nephrectomy (a 75% surgical reduction in renal mass) 2 weeks before IRI; although the severity of early injury was similar in rats that had undergone subtotal nephrectomy and sham surgery controls, functional recovery was impaired in the subtotal nephrectomy group[79]. This impairment was associated with a marked reduction in peritubular capillary density, suggesting that peritubular capillary rarefaction has an role in promoting CKD progression after AKI in the setting of pre-existing CKD.

Cardiorenal syndrome type 1

Cardiorenal syndrome type 1 (CRS-1), is an AKI syndrome precipitated by acute cardiac dysfunction. Clinically, the most common cause of CRS-1 is acute heart failure, followed by cardiac surgery and acute myocardial infarction (AMI)[80]. AKI might be driven by reduced cardiac output or venous congestion and exacerbated by diuretic-induced hypotension and hypovolaemia[4]. CRS-1 is a strong predictor of both short-term and long-term mortality[80,81]. Women are at a lower risk of developing CRS-1 following AMI than men; however, studies of sex difference in CRS-1 due to cardiac surgery have produced conflicting results[82,83]. Pre-existing CKD, diabetes, old age, hypertension and prior AKI are common comorbidities that increase the risk of CRS-1 (REF.[81]). In clinical practice, distinguishing between cardiorenal syndrome type 2 (that is, chronic heart failure resulting in CKD) and CRS-1 with

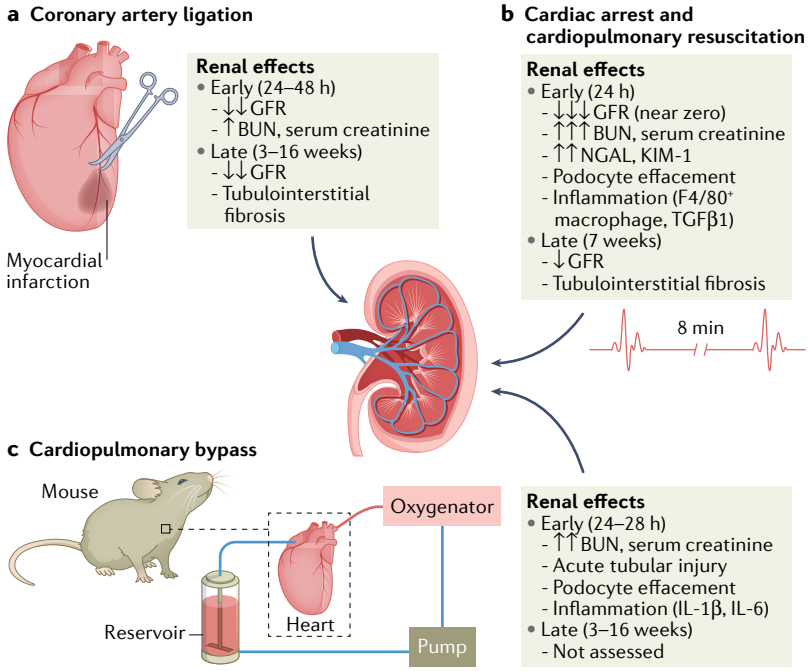


Fig. 1 | **Rodent models of cardiorenal syndrome type 1. a** | The most common cause of cardiorenal syndrome type 1 (CRS-1) is acute myocardial infarction, which can be modelled using coronary artery ligation. The left anterior descending artery is commonly suture ligated, resulting in severe acute heart failure that does not resolve. Acute renal effects include a reduction in glomerular filtration rate (GFR) with accompanying increases in blood urea nitrogen (BUN) and serum creatinine levels. Late outcomes include tubulointerstitial fibrosis. **b** | Cardiac arrest and cardiopulmonary resuscitation is a model of whole-body ischaemia–reperfusion that models cardiac arrest-induced CRS-1. The arrest time can be varied to titrate injury. The model results in severe AKI with near-zero GFR, increased serum and kidney tissue biomarkers of injury such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), renal inflammation with infiltrating macrophages and an increase in tissue transforming factor-β1 (TGFβ1) in the first 24 h. Late outcomes include reduced GFR and tubulointerstitial fibrosis. **c** | Cardiopulmonary bypass is a component of cardiac surgery that itself induces CRS-1. In rodent models of cardiopulmonary bypass, tubing is placed in the aorta and vena cava and connected to a pump and membrane oxygenator for circulation. This model requires considerable surgical expertise and a thoracotomy. Time on the pump can be varied. Acute renal effects include acute tubular necrosis with increased BUN and serum creatinine as well as podocyte injury and inflammation (demonstrated by increased interleukin (IL)-1β and IL-6). Studies with prolonged survival reporting late outcomes are rare.

pre-existing CKD is often difficult. The development of defined models could lead to improvements in the diagnostic evaluation of CRS-1 and -2.

Acute myocardial infarction. Several models of CRS-1 have been developed in rodents (FIG. 1). The most widely cited is a model of AMI induced by ligation of the left anterior descending coronary artery. The ligation induces renal cortical and medullary hypoxia[84] with robust inflammatory responses[85]. Kidney dysfunction is mild, but long-term follow-up indicates that rodents have reduced GFR and/or renal fibrosis after AMI[85–87]. Whether these findings represent defective renal repair and true AKI to CKD transition or a transition from CRS-1 to CRS-2 due to irreversible heart failure[85], resulting in reduced renal blood flow and secondary renal ischaemia, is unclear. Important limitations to these models include <70% survival for >24 h such that short-term outcomes are most frequently reported and the surgical complexity of left anterior descending coronary artery ligation, which results in additional stress and inflammatory responses to those induced by the AMI. We are unaware of any studies that have reported sex differences in AMI-induced CRS-1 models.

Cardiac arrest and resuscitation. A mouse model of cardiac arrest and cardiopulmonary resuscitation (CA–CPR) has also been developed. Cardiac arrest is induced with potassium chloride or electrical fibrillation, and after a period of no-flow, cardiac function is restored with adrenaline and chest compressions. Mice have no renal blood flow during arrest, but this flow normalizes after return of spontaneous circulation[88]. Despite the complexity of the model, 24-h survival in experienced laboratories is approximately 80% and survival up to 7 weeks has been reported[89]. Unlike the AMI model, AKI is severe: 8 min of CA–CPR results in near-zero GFR after 24 h, with markedly elevated blood urea nitrogen (BUN) and serum creatinine levels[90]. Urinary NGAL and renal Kim-1 levels are elevated, and histology shows PTEC injury and inflammatory infiltrates consistent with clinical specimens[89,91,92]. Toll-like receptor 4 (TLR4) deletion and systemic T cell deletion ameliorate AKI after CA–CPR, the latter without changing intrarenal monocyte activity, suggesting a role for exacerbating non-renal factors such as immune activation and systemic inflammation[92,93]. Kidney injury is sex and age dependent and oestrogen-mediated female protection is lost with age in mice, similar to clinical observations[91,94]. Strain effects in the CA–CPR model have not been investigated. The limitations of this model include the surgical challenge and the necessity for diligent thermal regulation as body temperature has profound effects on the severity of AKI following CA–CPR[95].

Cardiopulmonary bypass. Cardiopulmonary bypass (CPB) contributes to cardiac surgery-induced AKI[96]. Rodent models of CBP have been developed to explore the mechanisms of CBP-induced CRS-1. The development of small-animal CBP has been technically challenging, but rat[97], rabbit[98] and mouse models have now been established[99]. The necessary pharmacological manipulation includes high-dose heparin and protamine sulphate, with off-target effects, including pulmonary hypertension and coagulopathy.

Rat models show that CBP causes acute tubular necrosis and moderate elevation in BUN and serum creatinine, resulting from reduced renal perfusion, and endothelial, platelet, complement and immune activation, intravascular haemolysis and dilutional anaemia[100,101]. Other rodent models reproduce these findings with varying fidelity, in part owing to non-scaling physiological parameters such as blood rheology, cell size to tubing ratio and viscosity. Physiological changes during CBP mirror their clinical correlates, including development of pulmonary interstitial oedema, coagulopathy, systemic inflammation and renal hypoxia associated with increased haemodilution[102]. To our knowledge, sex or strain differences and long-term kidney outcomes have not been investigated in CBP

models despite considerable clinical interest in these high-impact areas.

Effects of comorbidities. The translational success of CRS-1 models requires consideration of comorbidities, such as CKD, diabetes, old age and hypertension, that increase the risk of CRS-1 and might influence renal responses to therapy. Although studies in rodents are limited, type 2 diabetes mellitus was shown to increase susceptibility to CRS-1 after AMI in a rat model[103] and treatment with SGLT2 inhibitors ameliorated AMI-induced CRS-1 in diabetic rats[104].

Toxin-induced injury

The effects of various nephrotoxic agents, including radiocontrast agents, non-steroidal anti-inflammatory agents and gentamycin, have been modelled in rodents[105,106]. These models have been optimized in rats but are unreliable in mice and require non-physiological dosing and/or combinations of insults that do not occur in patient populations, such as dehydration and inhibition of nitric oxide synthase to induce radiocontrast-induced AKI[105]. High-dose folic acid and aristolochic acid have also been used to model the AKI to CKD transition in mice[106], but these agents are not common causes of AKI in humans. In our experience, folic acid induces highly variable injury[107,108] and aristolochic acid induces variable mortality with marked systemic toxicity[109], necessitating large cohort numbers to evaluate the effects of interventions. Here we focus on two commonly used mouse models that induce more predictable injury with less systemic toxicity, and model common mechanisms of human AKI in patients: cisplatin-induced AKI[110–112] and rhabdomyolysis-induced AKI (rhabdo-AKI)[113].

Cisplatin. AKI is the main dose-limiting toxicity in patients treated with cisplatin[114], which is still the most effective chemotherapeutic used to treat many cancers. Cisplatin dose and frequency vary according to the cancer type, but it is often given intravenously over four to six, 3–4-week cycles, often in combination with other chemotherapies. Cisplatin is concentrated in PTECs after uptake by the basolateral organic cation transporters, resulting in cell death. IV fluids are often used to hydrate patients and mitigate the renal toxicity. Despite this intervention, 30% of cisplatin-treated patients develop AKI requiring dose alterations or switching to less effective therapeutic regimens. Although very few patients develop severe, dialysis-dependent AKI during treatment (the incidence is unclear as dialysis-dependent AKI might be under-reported), they are at an increased long-term risk of CKD[115]. Apart from cisplatin dose and hydration status, factors that determine the severity of cisplatin AKI are poorly understood. Moreover, kidney function can remain decreased >5 years after cisplatin treatment, regardless of whether the patient had a clinical AKI event[115].

Cisplatin AKI is often studied in rodents after a single intraperitoneal, high-dose bolus. The peak of injury occurs after 48–72 h at which point animals are moribund; thus, long-term studies cannot be performed. The kidneys show extensive tubular damage with mitochondrial dysfunction, oxidative stress, cell death and inflammation[110]. Kidney biopsies are rarely performed in cisplatin-treated patients, so whether these events occur in humans is unknown.

To better model human cisplatin-induced AKI, several laboratories have used repeated-dosing models in which mice are given weekly injections of low-dose cisplatin for 2–4 weeks[111]. Kidney function, as measured by BUN, is not substantially altered in these models, but urinary AKI biomarkers such as NGAL and albuminuria are increased after 1–3 days of cisplatin treatment[116,117]. These findings are consistent with clinical data indicating that urinary AKI biomarkers are elevated after cisplatin treatment even in patients without AKI[118]. The mice show a marked reduction in GFR with variable degrees of kidney fibrosis and peritubular capillary rarefaction[68,117], which persists for months after treatment[117].

Sensitivity to cisplatin varies between inbred mouse strains. For example, C57BL/6 mice require higher doses to induce kidney fibrosis than FVB/N mice[119]. Genes involved in circadian rhythm also impact the severity of cisplatin-AKI[120], so maintaining consistent dosing schedules is important. Like other toxin models of AKI, hydration status affects cisplatin-AKI severity, and many laboratories give subcutaneous saline before and 24 h after cisplatin dosing. This protocol also models the clinical practice of giving IV fluids to prevent dehydration and mitigate renal toxicity resulting from cisplatin treatment.

Effects of comorbidities. Most rodent models of cisplatin AKI employ 6–12-week-old male mice without cancer. Models with comorbidities are needed to recapitulate the clinical scenario more accurately, particularly as therapeutics that improve kidney function might have undesirable effects on tumour responses to cisplatin[121]. Moreover, using a genetically engineered model of lung cancer, the Siskind laboratory has observed that aged mice with lung tumours are sensitized to cisplatin AKI compared with non-tumour-bearing controls (L.J.S., unpublished work). The mechanisms by which distant tumours affect cisplatin AKI represent an important knowledge gap in the field.

Rhabdomyolysis. Rhabdomyolysis occurs when the breakdown of skeletal muscle releases myoglobin into the circulation. This breakdown may result from direct trauma, strenuous exercise, drug use, ischaemia caused by arterial obstruction, viral myositis and acquired or inherited myopathies[113]. Circulating myoglobin is filtered and reabsorbed by PTECs via apical membrane megalin receptors[122,123] and oxidation of ferrous iron in the haem moiety promotes redox cycling, inducing cell damage and AKI[124]. Rhabdomyolysis also models mechanisms of AKI resulting from intravascular haemolysis[122] and increased circulating cell-free haemoglobin after CPB and sepsis[125,126]. Kidney injury is compounded by vasoconstriction caused by myoglobin and exacerbated by associated volume depletion; tubular obstruction due to precipitation of myoglobin with Tamm–Horsfall

urinary glycoprotein (also known as uromodulin); and inflammation caused by necrosis and the release of inflammatory mediators[123].

The most widely used model of rhabdo-AKI in rodents uses intramuscular injection of glycerol to damage muscle fibre membranes and release myoglobin into the circulation[127]. The severity of injury is affected by hydration status, as myoglobin is concentrated in the tubular lumen with dehydration. For this reason, some investigators water-restrict animals overnight prior to glycerol injection. Female sex, old age and the cause of rhabdomyolysis are predictive of adverse outcomes in patients[128], but to our knowledge, no published data are available on the effects of animal strain or sex on sensitivity to rhabdo-AKI. Oxidant environments may increase sensitivity to haem-induced oxidant injury[129]. However, to our knowledge no data are available on the effects of comorbidities such as old age, diabetes or CKD in models of rhabdo-AKI.

Most work in rodents has evaluated short-term outcomes after rhabdo-AKI, avoiding the confounding effects of mortality, which peaks 2–4 days after injury in mice[68,130]. Long-term studies show that despite extensive tubulointerstitial fibrosis GFR returns to normal 6 to 8 weeks after injury[68,130,131]. Unlike severe kidney IRI and repeated-dosing cisplatin-AKI, peritubular capillary density is preserved after severe rhabdo-AKI in mice[68], suggesting that preservation of the microvasculature is a better determinant of recovery than fibrosis. This finding is consistent with data in patients with combat injuries who show restoration of kidney function following dialysis-dependent rhabdo-AKI[132,133].

Sepsis

Sepsis results from a maladaptive response to systemic progression of a local infection and can lead to multiple organ failure, including AKI[134], and death. The maladaptive response involves systemic inflammatory response syndrome; coagulopathy; reduced micro- and macro-circulatory renal blood flow; and changes in kidney metabolism[134,135]. The causative pathogen, site of infection and inter-individual differences in responses to the same pathogen can affect outcomes. Factors such as older age, male sex and comorbidities, including CKD, diabetes, cancer, and liver disease, are associated with increased mortality[136]. In addition, sepsis responses change over time; a hyperinflammatory response is a risk factor for early death, whereas sepsis-associated immunosuppression is associated with increased mortality after long hospitalizations[137].

Lipopolysaccharide. The simplest rodent model of SA-AKI involves injection of lipopolysaccharide (LPS), which induces sterile inflammation, mimicking the hyperinflammatory state of sepsis. Once batch-to-batch variations of LPS are addressed, this model is simple and reproducible. However, there is a narrow range between the doses that are required to induce AKI and those that are lethal. Unlike SA-AKI, LPS-induced AKI is easily reversed by fluids[138]. Moreover, therapeutics that target LPS responses might not be effective or might impair bacterial clearance in patients with sepsis.

Faecal slurry. Another simple model of SA-AKI involves intraperitoneal injection of faecal slurry[139]. In contrast to LPS, which acts through binding to TLR4, which is expressed principally in mononuclear phagocytic cells (i.e. macrophages and neutrophils)[140] and is induced in the renal tubular epithelium and vasculature in sepsis[141], faecal slurry components activate innate immune responses through multiple receptors. Although the LPS and faecal slurry models are both straightforward and scalable, their rapid kinetics make it difficult for these models to replicate the clinical trajectory of SA-AKI.

Caecal ligation and puncture. The caecal ligation and puncture model (CLP) of sepsis more accurately resembles human sepsis than the LPS and faecal slurry models. The CLP model differs from the faecal slurry model in that caecal pressure drives sustained release of caecal content, causing a progressive increase in bacterial load. Organ failure is variable, with liver failure typically occurring early and AKI a later response. Serum creatinine is a poor marker of AKI because sepsis decreases creatinine production[142]; however, measured GFR declines within a few hours of CLP surgery[143]. Renal histological damage is mild, with vacuole-like structures observed in PTECs. As end-organ damage is variable and therapy that benefits one organ might increase damage in another, survival studies are often used to give a composite view of therapeutic responses in sepsis, balancing competing causes of death.

Several factors affect the severity of the CLP model, including the length of the caecum, the gauge of the needle used for puncture, the number of puncture holes, the microbiome, fluid and antibiotic administration, age and sex[144,145]. Mouse strain is also important; old but not young C57BL/6 mice[146] and young outbred CD-1 mice are susceptible to AKI[147].

Effects of comorbidities. Two studies have shown that pre-existing CKD exacerbates CLP-induced SA-AKI[107,148]. One of these studies reported differential responses to treatments between CLP mice with and those without CKD; those without CKD responded to soluble FMS-like tyrosine kinase 1 (sFLT1), but not anti-high mobility group protein B1 (anti-HMGB1), whereas those with CKD responded to anti-HMGB1 but not sFLT1[148]. This finding underscores the importance of developing models of SA-AKI that enable the evaluation of therapeutic responses in the setting of pre-existing CKD.

Remote organ effects of AKI

AKI is a risk factor for mortality that is independent of the overall severity of illness of the patient[149] and the modality and timing of dialysis initiation has only a limited impact on mortality[150]. As hyperkalaemia, acidosis, and fluid overload are easily corrected by dialysis, other factors that are not improved by dialysis must be contributing to mortality. Identification of these non-traditional complications of AKI has been aided by studies in rodent models that revealed deleterious effects of AKI in a variety of organs, including the brain, liver, intestine, lung and heart[151].

Remote effects of AKI might be due to direct effects of renal injury (such as cytokine production and the release of damage-associated molecular patterns and mitochondrial DNA) and/or the accumulation of metabolites and proteins as a result of increased production and/or decreased renal clearance[152]. These factors are important when considering the interpretation of models used to study the remote effects of AKI. The most commonly used model for these studies is bilateral IRI-AKI as the majority of the other AKI models (including CA-CPR, SA-AKI, cisplatin AKI; rhabdo-AKI and CPB-AKI) have independent effects on organs other than the kidney that would confound the analysis. As surgery promotes a systemic inflammatory response, sham-operated controls should be used to study the remote organ effects of IRI-AKI[153].

Bilateral nephrectomy studies provide useful complementary data that enable the effects of kidney failure versus tissue injury to be distinguished and highlight the importance of extra-renal production of plasma mediators that promote remote organ damage in AKI. For example, plasma IL-6 levels increase in mice after bilateral nephrectomy, indicating that the main source of this cytokine is extra-renal and suggesting that the kidneys have a role in IL-6 clearance[153–156]. The value of using complementary models is further highlighted by studies showing that bilateral IRI and bilateral nephrectomy result in distinct functional and transcriptional changes in remote organs, suggesting that the remote effects of injured kidneys are distinct from the effects of loss of kidney function[157,158].

Lung inflammation. Respiratory failure and the need for prolonged mechanical ventilation are common in patients with AKI and are not simply explained by complications such as fluid overload[159,160]. In animal models, AKI-mediated lung inflammation is similar to acute lung inflammation of other causes, such as sepsis[161]. This inflammation is thought to contribute to higher rates of respiratory complications (such as respiratory failure requiring mechanical ventilation, prolonged mechanical ventilation and prolonged weaning) in patients with AKI than in similarly ill patients without AKI[162]. However, most animal studies of AKI-mediated lung injury have only examined the short-term effects of AKI (usually with 24 h of the initiating injury), whereas human AKI is likely to contribute to lung inflammation over days. Models of AKI with sustained lung injury are therefore needed to develop and test therapeutics in the setting of established disease. The Faubel laboratory has shown that reducing daily fluid administration after bilateral IRI-AKI in mice increases AKI severity and lung inflammation 7 days after injury[163]. These studies demonstrate the feasibility of this approach, but the experiments are challenging because severe AKI and lung inflammation increase mortality.

Cardiac dysfunction. AKI has well-documented adverse effects on cardiac function[164–167]. The clinical syndrome by which AKI leads to acute cardiac dysfunction is termed cardiorenal syndrome type 3 (CRS-3)[165]. Several rodent studies have reported cardiac dysfunction demonstrated by echocardiography 24–72 h after AKI[168–170]. Mitochondrial dysfunction is a consistent theme that explains CRS-3 in rodent models and therapies to improve mitochondrial function are associated with improved cardiac function[168,170]. Only two studies have examined the long-term effects of AKI on the heart. In the first, galectin-3 was implicated in the development of cardiac fibrosis and dysfunction 28 days post-AKI[171]. This finding is notable because plasma galectin-3 and biomarkers of cardiac injury are elevated in patients with AKI[171]. In the second study, diastolic dysfunction was observed 1 year post-AKI and associated with reduced levels of cardiac ATP[63]. This finding is consistent with accumulating clinical data showing that patients with AKI are at an increased risk of heart failure in the long term[172]. The possibility that short-term interventions for AKI and/or AKI-mediated cardiac dysfunction could have long-term beneficial effects on patient outcomes is of considerable interest and could be tested using currently available models of IRI-AKI.

Large animal models

AKI has been studied in several large animal models (LAMs), including dogs, cats, sheep, pigs and non-human primates. These models have both pros and cons compared with rodent models. For example, pig kidneys are multi-lobular with a similar vasculature[173] and similar innate immune responses to human kidneys[174] (e.g. the levels of renal TLR4 are comparable in pigs and humans[140]), whereas inflammatory responses differ in mice and humans[175]. However, genetic tools are not as advanced in LAMs as in mice. Larger blood volumes in LAMs enable more blood samples to be obtained for longitudinal biomarker analyses, but increased model size increases costs and limits sample size. Historically, the high costs of LAMs have prohibited analysis of the effects of comorbidities, such as diabetes, CKD and older age, on AKI susceptibility. Early LAMs used dogs and sheep to explore mechanisms of AKI, but pigs are now the most commonly used model[176]. A well-developed sheep model continues to provide advances in the understanding of SA-AKI.

Ischaemia–reperfusion injury

IRI-AKI has been studied in LAMs for decades and early studies provided fundamental insights, for example, into renal autoregulation of blood flow[177]. LAMs have been used to evaluate the effects of warm and cold ischaemia times and cold preservation solutions on kidney transplant function after auto-transplantation, xenotransplantation[178,179] and allotransplantation following donation after cardiac arrest[180].

Unilateral IRI with contralateral nephrectomy has been performed in dogs[181] and sheep[182] to test potential therapeutics. However, renal pedicle clamp times vary, making comparisons between studies difficult. In dogs, 60 min of renal pedicle clamping has been shown to result in a 5-fold increase in serum creatinine[183] and similar effects have been reported in sheep[184]. Longer occlusion times (i.e. 90 min) cause severe AKI in sheep[182]. Bilateral renal artery clamping has also been performed in sheep, enabling the effects of local injection

of therapies via the renal artery to be evaluated in one kidney with the contralateral kidney providing a control for comparison[185].

Pig models often use 60 min of warm ischaemia combined with contralateral nephrectomy, which results in elevation of serum creatinine for 3 days[186]. Ischaemic preconditioning protects against kidney IRI in this model[187]. In pigs, clamp times of 120 min that result in ~50% reductions in GFR have been used to study the long-term effects of AKI[188]. A clamp time of 30 min resulted in histopathological evidence of kidney damage but kidney function was not evaluated[189]. However, 45 min of renal artery clamping has been shown to reduce the GFR of the affected kidney in pigs[190].

Cardiopulmonary bypass

As CPB is surgically complex and scalability is difficult in small animals, LAMs have been used to characterize CPB-AKI. CPB was first established in a cat model[191]. Most studies report immediate (during CPB and immediately after) or short-term (up to 48 h) results. Critical variables that influence AKI severity include duration of CPB, pump flow and circuit priming volume. Models vary in the use of drugs and anaesthetics and in blood transfusion and intravenous fluid rates. Although most studies specify duration of CPB, targeting a clinically relevant 2.5 h, reporting of other parameters is variable.

Anatomical similarities between sheep and humans make them an attractive model for studying CPB[192]. Sheep studies have shown reduced renal blood flow and oxygen delivery during CPB, validating clinical findings and suggesting the fidelity of the models[193–196]. In pigs, CPB induces renal inflammation similar to that observed in patients. For example, a pig CPB model recapitulated the renal endotheliopathy that has been observed in human CPB[197], and urinary markers of inflammation that are increased following CPB in humans are also increased in pig models[198].

Effects of comorbidities. Clinical studies of the effect of obesity on the risk of AKI following cardiac surgery have reported either no effect, increased risk, decreased risk or a U-shaped association with body mass index[199–202]. Following CPB and sham surgery, obese pigs fed a high-fat diet had higher GFR, renal blood flow and PTEC proliferation than lean controls, suggesting that obesity is protective in this model[203]. However, not all results suggested a protective effect, for example, the obese pigs had increased renal inflammation and urinary NGAL levels that were around double those of lean controls. These conflicting findings may reflect the clinical complexity of obesity as a risk factor for AKI after cardiac surgery.

Toxin-induced injury

Toxin-induced AKI has not been extensively studied in LAMs. In piglets, a cisplatin dosage of 3 mg/kg was shown to cause a rise in serum creatinine levels[204]. An adult pig model of cisplatin-induced AKI has also been developed; in this model IV injection of cisplatin (4 mg/kg) was superior to IP injections[205]. Repeated subcutaneous injection of gentamicin induces AKI in dogs[206] and sheep[207], and daily gentamicin injection (80 mg/kg) for 10 days induced an approximately 5-fold increase in serum creatinine in pigs[208]. Other toxin-induced AKI models include radiocontrast AKI in dogs using 7 ml/kg vascoray[209] and in minipigs using 25 ml/kg Iohexol IV, which resulted in elevated serum creatinine levels for several days[210]. Rhabdo-AKI has been investigated in dogs using intramuscular injection of glycerol[211]. More studies of toxin-induced AKI in LAMs are needed to inform dose selections and the frequency of nephrotoxin injections to enable standardized approaches.

Haemorrhage

Mice are not suitable for studying the haemodynamic effects of haemorrhagic shock because of their small blood volume. Although rats have been used in such studies, LAMs have larger blood volumes, which makes it easier to titrate the severity of haemorrhagic shock, are easier to manipulate and have more similarities to humans in cardiovascular and haemodynamic responses to haemorrhagic shock than do rodents. In 1983, a study in dogs was the first to show the sensitive nature of renal cortical oxygen changes in response to haemorrhagic shock[212], which has since been reported in non-human primates[213]. The effects of controlled haemorrhage on renal function have been studied in pigs for approximately 30 years[214]. Studies in a pig model of haemorrhagic shock showed that a novel haemostatic approach, endovascular balloon occlusion of the aorta, may worsen AKI when the balloon is inflated proximal to the renal arteries[215]. Another pig model of pulmonary contusion and haemorrhagic shock showed that higher IV fluid rates and lower thresholds for hyperkalaemia reduced BUN and creatinine levels[216].

Major trauma

Importantly, trauma is often multifactorial, and the lethal triad of hypothermia, coagulopathy and acidosis is not always achieved experimentally. Complex trauma models are of specific relevance to severe injuries seen in military medicine but require intense resources and specific expertise. A polytrauma model of haemorrhage and femur fracture in pigs with artificially induced hypothermia resulted in serum creatinine levels of >3 mg/dl (265 µmol/l) over a 16-h time course[217]. Although the aim of this model was to recapitulate the lethal triad with the assumption that each component contributes to organ dysfunction secondary to polytrauma, the injury pattern was not evaluated under normothermic conditions. Another pig polytrauma model of blunt chest injury, liver laceration and haemorrhage showed a marked protective effect of hypothermia (34 °C) on liver dysfunction but only a slight protective effect on the kidney, as determined by a slight reduction in BUN compared with controls[218]. A clinical study in patients who underwent successful CPR showed a moderate protective effect of mild therapeutic hypothermia on AKI[219].

Burns

Pigs are often used in burns studies in LAM, as pig and human skin have similar structure and wound healing processes[220]. These models have also been used to examine burn-induced AKI. An early study that focused on

AKI after scalding covering 30% of the total body surface area (TBSA) reported only renal histological damage[221]. A subsequent study that used a pig model of 40% TBSA burns and traumatic brain injury reported that a high volume IV fluid resuscitation strategy improved urine output and BUN compared with a more restrictive fluid resuscitation strategy[222]. A similar burns only model showed transient AKI that normalized without IV fluids, presumably by autoresuscitation via the animals drinking[223]. A follow-up study reported that oral fluid resuscitation preserved kidney function in pigs with 40% TBSA burns[224]. These LAM studies have sparked on-going debate about fluid resuscitation volumes and the use of IV versus enteral fluids in patients with extensive burns[225].

Sepsis

Sheep models are often used to study SA-AKI induced by LPS infusion[226], intraperitoneal faeces injection[227] or CLP[228]. However, the most widely used LAM of SA-AKI involves delivering a single IV bolus of *E. coli* to sheep[229]. This model mimics the effects of IV access sepsis, which increases serum creatinine and reduces urine output[230,231]. Studies are usually conducted over a 30-h time course. This model has been used to investigate the effects of sepsis and fluid resuscitation on renal oxygenation and AKI[232].

SA-AKI models have not been as extensively characterized in pigs as in sheep because pigs show inconsistent responses to sepsis[233]. In pigs, CLP has been used in combination with IRI to increase the severity of AKI[234]. Comparing pigs that do and do not develop AKI following the induction of sepsis has been used to provide insight into the factors that predispose septic patients to AKI[233]. A study in pigs with sepsis induced by IV infusion of *Pseudomonas aeruginosa* or peritoneal faecal injection showed a role for inflammation (that is, higher levels of circulating cytokines and local expression of inflammatory genes) in AKI susceptibility[235]. Results are discordant with respect to the amount of *P. aeruginosa* needed to induce SA-AKI in pigs, some studies report AKI after a single injection[236], whereas others required continuous infusion of bacteria to reduce GFR[237]. Autologous faecal infusion into the peritoneum induces a rapid decline in kidney function in pigs[238] and sheep[239]. Faecal infusion can also be combined with mesenteric artery ligation to exacerbate AKI[240]. In sheep, direct infusion of *P. aeruginosa* into the lungs results in AKI within 24 h[241,242].

Several critical variables are often overlooked in SA-AKI LAMs, including the dose and strain of bacteria used and the bacterial origin and lot number of LPS. For example, LPS derived from *E. coli* strains O111:B4 and O55:B5 have different dose-dependent effects on AKI[243–245]. Importantly, the animal strain used for pig studies is not always reported, and disclosure of age and weight is paramount. As many strains of swine can reach over 200 kg, minipigs are attractive models, as they reach sexually maturity within 4–6 months and at much lower body weights than other breeds. Age and sex differences have not been investigated extensively in swine. However, most pig AKI studies are performed in females. Although it has been suggested that female pigs demonstrate stronger innate immune responses than males[246], evidence of such a difference is unclear. Anecdotal reasons for female preference include ease of inserting bladder catheters. At minimum, LAM studies should disclose the strain, sex, weight and age of the animals used.

Recommendations for future studies

Despite advances in the molecular understanding of human AKI, we continue to rely on experimental models to develop targeted therapeutics. However, successful translation of therapies from experimental models of AKI to humans has not been achieved. This failure suggests a need to re-think past approaches. To address this need, we have developed a series of opinion-based recommendations based on our own experiences for investigators involved in preclinical development of AKI therapeutics. Our aim is to provide a framework for discussion rather than a consensus statement for the field. However, we believe that a multidisciplinary approach is key to optimizing the development of effective therapies for AKI.

Mechanistic and target validation studies

The principles driving the selection of experimental models for mechanistic and target validation studies should include consideration of model variability and end points. In addition, more than one model of AKI should be used for these studies.

Robust models and end points. We recommend choosing models that have low variability and robust end points, even if these do not reflect the goal of the intervention in humans (for example, long-term improvement in renal function, which can be challenging to achieve in preclinical model systems). In rodents, we suggest short-term models using robust measures of kidney function, injury and/or repair. The availability of genetic and/or validated pharmacological tools to evaluate target function might limit the choice of models to mice, organoids and/or zebrafish. In mouse genetic models, background strains should be defined, and investigators must consider how genetic and environmental effects might influence individual responses to injury. Sex might be a less important consideration at this early stage of therapeutic development unless an effect of sex on the target mechanism is suspected. Inter-assay variability in human kidney organoid systems[10] also necessitates investigators choosing robust end points that provide clear signals over background noise.

Multiple models. We recommend using more than one model to determine whether a target is involved in regulating a common pathophysiological response in AKI. Although the models used at this stage of therapeutic development do not need to reflect the heterogeneity of human AKI, molecular targets that have effects in more than one model are more likely to have a role in different AKI scenarios in humans. For this reason, we suggest that researchers evaluate at least two different injury models for rodent studies based on their laboratory's expertise (for example, IRI and toxin-induced AKI).

Ideally, studies will have been performed to determine whether the molecular target is involved in human AKI. Often evidence that a molecular pathway is perturbed in patients with AKI is provided by gene expression studies. However, despite their current limitations (such as lack of blood flow and primitive tubules), CRISPR-Cas9-mediated gene targeting of PSC-derived kidney organoids provides a powerful tool for the functional assessment of molecular targets in human AKI. Many laboratories might not have the expertise to perform both rodent and organoid AKI studies, underscoring the importance of developing collaborative approaches at all stages of therapeutic development.

Drug screening

Validated 'hits' from drug screening campaigns need to be evaluated in experimental models before undergoing further preclinical development. As such studies require scaled chemical synthesis and consideration of drug stability and metabolism, the selection of models used at this stage of therapeutic development must include consideration of scale and throughput in addition to the principles discussed above.

Scale, reproducibility and throughput. Depending on the anticipated throughput, scale and reproducibility might have the greatest impact on the choice of models used for drug screening. If thousands of compounds need to be screened, whether a model is amenable to high-throughput, automated analysis will be an important consideration. However, the pathophysiological relevance of the model must also be considered. Simple, two-dimensional cell culture systems are amenable to high-throughput analyses but the limited pathophysiological relevance of the end points that can be assayed must be weighed against the importance of having a reliable, automated readout. Larval zebrafish and human kidney organoid models provide attractive alternatives to these simple cell culture systems. Both are amenable to automated screening analyses (for example, using fluorescent markers and automated image analyses[10,46,247]) and provide more complex readouts that are more likely to reflect the complexity of the cellular responses and interactions that occur in patients with AKI. As compounds can be added to small volumes of water in larval zebrafish assays and kidney organoids are generally immersed in cell culture media, it can be assumed that the steady-state levels of these compounds in the media reflect tissue levels, avoiding the need for more detailed compound stability and pharmacokinetics studies at this stage. In addition, zebrafish have the advantage of providing a readout for unanticipated in vivo drug toxicities, either by direct observation or with the use of fluorescent readouts[248].

Secondary models, timing of interventions and end points. Following screening, selected compounds need to be tested in mammalian models. As with target validation studies, we recommend the selection of at least two, short-term AKI models with robust end points that can be used to identify therapeutic effect sizes that are predicted from prior mechanistic studies. In addition, we recommend testing the interventions at clinically relevant time points. Most therapeutics tested in animal models are administered prophylactically prior to the induction of injury. Although important in terms of elucidating pathophysiological mechanisms, the potential for therapeutic translation of this approach is limited. Therapeutic translatability is improved if interventions are tested after the onset of AKI at a time when the injury might be recognized clinically (at least 24 h after onset). We recommend inclusion of both sexes at this stage of preclinical research to evaluate for sexual dimorphism in major outcomes. Results for both sexes should be reported separately using data that have not been pooled[75].

In practical terms, cost and size constraints generally limit studies at this stage of drug development to rodents. Mice are often used rather than rats because of their smaller size and scale for synthetic chemistry. However, drugs are metabolized more rapidly in mice than in rats because of their greater liver-to-body-weight ratios[249], which is important because drug exposure might be shorter in mice than in rats and pharmacokinetics must be considered. This issue can be circumvented if studies are accompanied by analysis of biomarkers reflective of target engagement in the kidney[250], which might be discovered in mechanistic target validation studies. However, given the uncertainty about drug exposure, particularly if biomarkers of dose-dependent target engagement have not been identified, multiple drug doses should be evaluated to identify therapeutic effects.

Late-stage preclinical studies

The patients with AKI who will ultimately be included in Phase 3 hypothesis-testing clinical trials must be considered when designing studies in the final stages of preclinical development. This reverse translational approach requires bench-to-bedside expertise and is bolstered by having research teams with complementary basic research and clinical skills. To date, patient recruitment for most AKI clinical trials has been based on the selection of hospital in-patients at an increased risk of developing AKI. Studies have been performed in patients undergoing coronary angiography or coronary artery bypass surgery and in patients with SA-AKI in the ICU. Most of these studies selected patients at an increased risk of adverse AKI outcomes owing to older age and pre-existing CKD, diabetes and/or heart failure[251,252]. Late-stage preclinical studies should model the renal insults that these patients are exposed to, the clinically significant end points that are used in clinical trials and the effects of age, sex, genetic heterogeneity and comorbidities on therapeutic responses.

In addition, we recommend that late-stage preclinical studies evaluate therapeutic responses in human models, including iPSC-derived kidney organoids from male and female donors and diverse demographic groups, and in LAMs of AKI, which are essential to determine whether therapeutic effects are preserved in physiological systems that are more closely aligned to those of humans. As in clinical trials, defined primary end points and scientific rigour, including power analyses, randomization and investigator blinding, should be clearly established before these studies are initiated to ensure reproducibility[250].

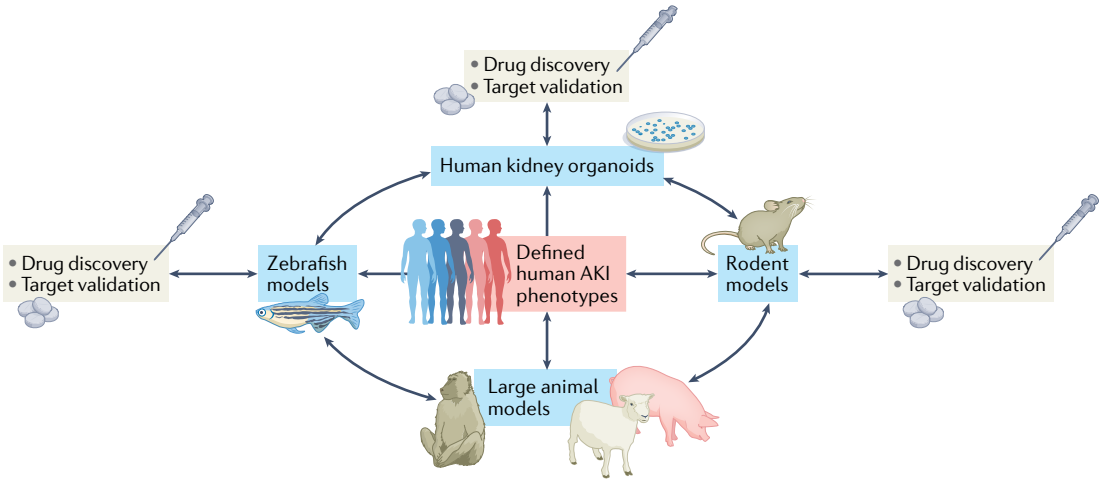


Fig. 2 | **Individualized therapeutic development plans for AKI.** A multidisciplinary, team science approach in which multiple, complementary models of acute kidney injury (AKI) are selected based on their ability to recapitulate molecular and cellular pathophysiology in patients with distinct AKI phenotypes, as well as the therapeutic development stage, is needed to capture the heterogeneity of human disease and optimize the development of effective therapeutics for these patients. Results from these animal model studies will provide iterative feedback for therapeutic development.

Multidisciplinary collaboration

Although late-stage preclinical development studies for AKI therapies are costly and resource intensive, these challenges are minor when compared with the cost and participant risk associated with a failed phase III clinical trial. This point is pertinent because the scientific foundation for these hypothesis-testing trials is largely based on preclinical data[9,250]. Resource limitations and the expertise required for the development of experimental models has resulted in disjointed efforts at therapeutic development for AKI, with silos of expertise reporting individual findings that are rarely part of coordinated efforts. For this reason, we recommend that investigators and their funding agencies take a more holistic approach to therapeutic development. Teams of investigators with expertise in different organisms and disease models should work more closely with clinical investigators to delineate clear therapeutic goals targeting defined patterns of disease seen in patients with AKI. Although challenging, we believe that following this blueprint for a multidisciplinary approach to AKI will set the field up for the best chance of success at developing effective therapies (FIG. 2).

In practical terms, this multidisciplinary approach will require the establishment of stronger collaborative relationships between clinical and basic science investigators in the AKI community. Such relationships can be facilitated by establishing regular, in-person scientific conferences in which clinical and basic science investigators have opportunities to interact and share new AKI research findings (for example, the FASEB AKI Bench to Bedside Conference Series uses this approach[253]). In addition, funding opportunities for translational research should encourage collaborative interactions between basic and clinical scientists in study design and execution as well as in the grant review process. For example, we suggest that scientific input from clinical investigators involved in phenotyping patients with AKI should be sought when selecting the most appropriate preclinical AKI models and study end points for the evaluation of novel molecular targets.

Conclusions

The development of novel and more precisely defined experimental models of AKI holds the promise of recapitulating the diversity of human AKI pathophysiology. However, there is a need to better integrate use of this panel of AKI models with emerging data on the molecular phenotypes in human AKI. This integration will require the development of flexible, team-based approaches in which preclinical investigators with expertise in different AKI models work closely together with AKI clinical investigators to design individualized therapeutic development plans for subsets of patients with distinct AKI phenotypes.

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1. Al-Jaghbeer, M., Dealmeida, D., Bilderback, A., Ambrosino, R. & Kellum, J. A. Clinical Decision Support for In-Hospital AKI. *J. Am. Soc. Nephrol.* **29**, 654–660 (2018).
2. Kellum, J. A., Lameire, N. & Group, K. A. G. W. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit. Care* **17**, 204 (2013).
3. Kellum, J. A. et al. Acute kidney injury. *Nat. Rev. Dis. Prim.* **7**, 52 (2021).
4. Ronco, C., Bellomo, R. & Kellum, J. A. Acute kidney injury. *Lancet* **394**, 1949–1964 (2019).
5. Hoste, E. A. J. et al. Global epidemiology and outcomes of acute kidney injury. *Nat. Rev. Nephrol.* **14**, 607–625 (2018).
6. Tenzi, J. et al. Renal histopathology in critically ill patients with septic acute kidney injury (S-AKI). *J. Crit. Care* **68**, 38–41 (2021).
7. Neugarten, J. & Golestaneh, L. Female sex reduces the risk of hospital-associated acute kidney injury: a meta-analysis. *BMC Nephrol.* **19**, 314 (2018).
8. Neugarten, J. & Golestaneh, L. Influence of sex on the progression of chronic kidney disease. *Mayo Clin. Proc.* **94**, 1339–1356 (2019).
9. Skrypnyk, N. I., Siskind, L. J., Faubel, S. & de Caestecker, M. P. Bridging translation for acute kidney injury with better preclinical modeling of human disease. *Am. J. Physiol. Renal Physiol.* **310**, F972–F984 (2016).
10. Przepiorski, A., Crunk, A. E., Espiritu, E. B., Hukriede, N. A. & Davidson, A. J. The utility of human kidney organoids in modeling kidney disease. *Semin. Nephrol.* **40**, 188–198 (2020).
11. Combes, A. N., Zappia, L., Er, P. X., Oshlack, A. & Little, M. H. Single-cell analysis reveals congruence between kidney organoids and human fetal kidney. *Genome Med.* **11**, 3 (2019).

12. Freedman, B. S. et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat. Commun.* **6**, 8715 (2015).
13. Homan, K. A. et al. Flow-enhanced vascularization and maturation of kidney organoids in vitro. *Nat. Methods* **16**, 255–262 (2019).
14. Morizane, R. et al. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat. Biotechnol.* **33**, 1193–1200 (2015).
15. Przepiorski, A. et al. A simple bioreactor-based method to generate kidney organoids from pluripotent stem cells. *Stem Cell Rep.* **11**, 470–484 (2018).
16. Subramanian, A. et al. Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation. *Nat. Commun.* **10**, 5462 (2019).
17. Takasato, M. et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* **526**, 564–568 (2015).
18. Wu, H. et al. Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. *Cell Stem Cell* **23**, 869–881.e8 (2018).
19. Taguchi, A. & Nishinakamura, R. Higher-order kidney organogenesis from pluripotent stem cells. *Cell Stem Cell* **21**, 730–746.e6 (2017).
20. Tsujimoto, H. et al. A modular differentiation system maps multiple human kidney lineages from pluripotent stem cells. *Cell Rep.* **31**, 107476 (2020).
21. Uchimura, K., Wu, H., Yoshimura, Y. & Humphreys, B. D. Human pluripotent stem cell-derived kidney organoids with improved collecting duct maturation and injury modeling. *Cell Rep.* **33**, 108514 (2020).
22. Bajaj, P. et al. Human pluripotent stem cell-derived kidney model for nephrotoxicity studies. *Drug Metab. Dispos.* **46**, 1703–1711 (2018).
23. Czerniecki, S. M. et al. High-throughput screening enhances kidney organoid differentiation from human pluripotent stem cells and enables automated multidimensional phenotyping. *Cell Stem Cell* **22**, 929–940.e4 (2018).
24. Kang, H. M. et al. Effective reconstruction of functional organotypic kidney spheroid for in vitro nephrotoxicity studies. *Sci. Rep.* **9**, 17610 (2019).
25. Digby, J. L. M., Vanichapol, T., Przepiorski, A., Davidson, A. J. & Sander, V. Evaluation of cisplatin-induced injury in human kidney organoids. *Am. J. Physiol. Renal Physiol.* **318**, F971–F978 (2020).
26. Lemos, D. R. et al. Interleukin-1beta Activates a MYC-dependent metabolic switch in kidney stromal cells necessary for progressive tubulointerstitial fibrosis. *J. Am. Soc. Nephrol.* **29**, 1690–1705 (2018).
27. Sweeney, D. E. et al. Functional maturation of drug transporters in the developing, neonatal, and postnatal kidney. *Mol. Pharmacol.* **80**, 147–154 (2011).
28. Soo, J. Y., Jansen, J., Masereeuw, R. & Little, M. H. Advances in predictive in vitro models of drug-induced nephrotoxicity. *Nat. Rev. Nephrol.* **14**, 378–393 (2018).
29. Bantounas, I. et al. Generation of functioning nephrons by implanting human pluripotent stem cell-derived kidney progenitors. *Stem Cell Rep.* **10**, 766–779 (2018).
30. Garreta, E. et al. Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells. *Nat. Mater.* **18**, 397–405 (2019).
31. Sharmin, S. et al. Human induced pluripotent stem cell-derived podocytes mature into vascularized glomeruli upon experimental transplantation. *J. Am. Soc. Nephrol.* **27**, 1778–1791 (2016).
32. van den Berg, C. W. et al. Renal subcapsular transplantation of PSC-derived kidney organoids induces neo-vasculogenesis and significant glomerular and tubular maturation in vivo. *Stem Cell Rep.* **10**, 751–765 (2018).
33. Lin, N. Y. C. et al. Renal reabsorption in 3D vascularized proximal tubule models. *Proc. Natl Acad. Sci. USA* **116**, 5399–5404 (2019).
34. Reimschuessel, R., Bennett, R. O., May, E. B. & Lipsky, M. M. Development of newly formed nephrons in the goldfish kidney following hexachlorobutadiene-induced nephrotoxicity. *Toxicol. Pathol.* **18**, 32–38 (1990).
35. Reimschuessel, R. & Williams, D. Development of new nephrons in adult kidneys following gentamicin-induced nephrotoxicity. *Ren. Fail.* **17**, 101–106 (1995).
36. Reimschuessel, R. A fish model of renal regeneration and development. *ILAR J.* **42**, 285–291 (2001).
37. Wingert, R. A. & Davidson, A. J. The zebrafish pronephros: a model to study nephron segmentation. *Kidney Int.* **73**, 1120–1127 (2008).
38. Davidson, A. J. Uncharted waters: nephrogenesis and renal regeneration in fish and mammals. *Pediatr. Nephrol.* **26**, 1435–1443 (2011).
39. Diep, C. Q. et al. Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. *Nature* **470**, 95–100 (2011).
40. Zhou, W., Boucher, R. C., Bollig, F., Englert, C. & Hildebrandt, F. Characterization of mesonephric development and regeneration using transgenic zebrafish. *Am. J. Physiol. Renal Physiol.* **299**, F1040–F1047 (2010).
41. Brilli Skvarca, L. et al. Enhancing regeneration after acute kidney injury by promoting cellular dedifferentiation in zebrafish. *Dis. Model Mech.* **12**, dmm037390 (2019).
42. Cianciolo Cosentino, C. et al. Histone deacetylase inhibitor enhances recovery after AKI. *J. Am. Soc. Nephrol.* **24**, 943–953 (2013).
43. Hentschel, D. M. et al. Acute renal failure in zebrafish: a novel system to study a complex disease. *Am. J. Physiol. Renal Physiol.* **288**, F923–F929 (2005).
44. Chiba, T. et al. Retinoic acid signaling coordinates macrophage-dependent injury and repair after AKI. *J. Am. Soc. Nephrol.* **27**, 495–508 (2016).
45. Yin, W. et al. Mammalian target of rapamycin mediates kidney injury molecule 1-dependent tubule injury in a surrogate model. *J. Am. Soc. Nephrol.* **27**, 1943–1957 (2016).
46. Sanker, S. et al. Development of high-content assays for kidney progenitor cell expansion in transgenic zebrafish. *J. Biomol. Screen.* **18**, 1193–1202 (2013).
47. Skrypnyk, N. I. et al. Delayed treatment with PTBA analogs reduces postinjury renal fibrosis after kidney injury. *Am. J. Physiol. Renal Physiol.* **310**, F705–F716 (2016).
48. Wen, X. et al. Time-dependent effects of histone deacetylase inhibition in sepsis-associated acute kidney injury. *Intensive Care Med. Exp.* **8**, 9 (2020).
49. Clatworthy, A. E. et al. Pseudomonas aeruginosa infection of zebrafish involves both host and pathogen determinants. *Infect. Immun.* **77**, 1293–1303 (2009).
50. Wen, X. et al. A zebrafish model of infection-associated acute kidney injury. *Am. J. Physiol. Renal Physiol.* **315**, F291–F299 (2018).
51. Emmerich, C. H. et al. Improving target assessment in biomedical research: the GOT-IT recommendations. *Nat. Rev. Drug Discov.* **20**, 64–81 (2021).
52. Opsahl, J. A., Abraham, P. A. & Keane, W. F. Angiotensin-converting enzyme inhibitors in chronic renal failure. *Drugs* **39** (Suppl. 2), 23–32 (1990).
53. Sharfuddin, A. A. & Molitoris, B. A. Pathophysiology of ischemic acute kidney injury. *Nat. Rev. Nephrol.* **7**, 189–200 (2011).
54. Heyman, S. N., Rosen, S. & Rosenberger, C. Animal models of renal dysfunction: acute kidney injury. *Expert Opin. Drug Discov.* **4**, 629–641 (2009).
55. Kirita, Y., Wu, H., Uchimura, K., Wilson, P. C. & Humphreys, B. D. Cell profiling of mouse acute kidney injury reveals conserved cellular responses to injury. *Proc. Natl Acad. Sci. USA* **117**, 15874–15883 (2020).
56. Cippa, P. E. et al. Transcriptional trajectories of human kidney injury progression. *JCI Insight* **3**, e123151 (2018).
57. Wei, Q. & Dong, Z. Mouse model of ischemic acute kidney injury: technical notes and tricks. *Am. J. Physiol. Renal Physiol.* **303**, F1487–F1494 (2012).
58. Skrypnyk, N. I., Harris, R. C. & de Caestecker, M. P. Ischemia-reperfusion model of acute kidney injury and post injury fibrosis in mice. *J. Vis. Exp.* https://doi.org/10.3791/50495 (2013).
59. Zager, R. A. & Altschuld, R. Body temperature: an important determinant of severity of ischemic renal injury. *Am. J. Physiol.* **251**, F87–F93 (1986).
60. Shanley, P. F. et al. Topography of focal proximal tubular necrosis after ischemia with reflow in the rat kidney. *Am. J. Pathol.* **122**, 462–468 (1986).
61. Lee, H. T., Ota-Setlik, A., Fu, Y., Nasr, S. H. & Emala, C. W. Differential protective effects of volatile anesthetics against renal ischemia-reperfusion injury in vivo. *Anesthesiology* **101**, 1313–1324 (2004).
62. Ferenbach, D. A. & Bonventre, J. V. Mechanisms of maladaptive repair after AKI leading to accelerated kidney ageing and CKD. *Nat. Rev. Nephrol.* **11**, 264–276 (2015).
63. Soranno, D. E. et al. Acute kidney injury results in long-term diastolic dysfunction that is prevented by histone deacetylase inhibition. *JACC Basic. Transl. Sci.* **6**, 119–133 (2021).
64. Le Clef, N., Verhulst, A., D'Haese, P. C. & Vervaet, B. A. Unilateral renal ischemia-reperfusion as a robust model for acute to chronic kidney injury in mice. *PLoS One* **11**, e0152153 (2016).
65. Thompson, R. H. et al. Every minute counts when the renal hilum is clamped during partial nephrectomy. *Eur. Urol.* **58**, 340–345 (2010).
66. Thompson, R. H. et al. Renal function after partial nephrectomy: effect of warm ischemia relative to quantity and quality of preserved kidney. *Urology* **79**, 356–360 (2012).
67. Scarfe, L. et al. Long-term outcomes in mouse models of ischemia-reperfusion-induced acute kidney injury. *Am. J. Physiol. Renal Physiol.* **317**, F1068–F1080 (2019).
68. Menshikh, A. et al. Capillary rarefaction is more closely associated with CKD progression after cisplatin, rhabdomyolysis, and ischemia-reperfusion-induced AKI than renal fibrosis. *Am. J. Physiol. Renal Physiol.* **317**, F1383–F1397 (2019).
69. Yang, L., Besschetnova, T. Y., Brooks, C. R., Shah, J. V. & Bonventre, J. V. Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat. Med.* **16**, 535–543 (2010). 1p following 143.
70. Finn, W. F. Enhanced recovery from postischemic acute renal failure. Micropuncture studies in the rat. *Circ. Res.* **46**, 440–448 (1980).
71. Soranno, D. E. et al. Matching human unilateral AKI, a reverse translational approach to investigate kidney recovery after ischemia. *J. Am. Soc. Nephrol.* **30**, 990–1005 (2019).
72. Clements, M. E., Chaber, C. J., Ledbetter, S. R. & Zuk, A. Increased cellular senescence and vascular rarefaction exacerbate the progression of kidney fibrosis in aged mice following transient ischemic injury. *PLoS One* **8**, e70464 (2013).
73. Kim, J. et al. Orchiectomy attenuates post-ischemic oxidative stress and ischemia/reperfusion injury in mice. A role for manganese superoxide dismutase. *J. Biol. Chem.* **281**, 20349–20356 (2006).
74. Aufhauser, D. D. Jr et al. Improved renal ischemia tolerance in females influences kidney transplantation outcomes. *J. Clin. Invest.* **126**, 1968–1977 (2016).
75. Tannenbaum, C., Ellis, R. P., Eyssel, F., Zou, J. & Schiebinger, L. Sex and gender analysis improves science and engineering. *Nature* **575**, 137–146 (2019).
76. Peng, J. et al. Hyperglycemia, p53, and mitochondrial pathway of apoptosis are involved in the susceptibility of diabetic models to ischemic acute kidney injury. *Kidney Int.* **87**, 137–150 (2015).
77. Gao, G. et al. TNF-alpha mediates increased susceptibility to ischemic AKI in diabetes. *Am. J. Physiol. Renal Physiol.* **304**, F515–F521 (2013).
78. Shi, H., Patschan, D., Epstein, T., Goligorsky, M. S. & Winaver, J. Delayed recovery of renal regional blood flow in diabetic mice subjected to acute ischemic kidney injury. *Am. J. Physiol. Renal Physiol.* **293**, F1512–F1517 (2007).
79. Polichnowski, A. J. et al. Severe renal mass reduction impairs recovery and promotes fibrosis after AKI. *J. Am. Soc. Nephrol.* **25**, 1496–1507 (2014).
80. Vandenberghe, W. et al. Acute kidney injury in cardiorenal syndrome type 1 patients: a systematic review and meta-analysis. *Cardiorenal Med.* **6**, 116–128 (2016).
81. Uduman, J. Epidemiology of cardiorenal syndrome. *Adv. Chronic Kidney Dis.* **25**, 391–399 (2018).
82. Vallabhajosyula, S. et al. Sex disparities in acute kidney injury complicating acute myocardial infarction with cardiogenic shock. *ESC Heart Fail.* **6**, 874–877 (2019).
83. Neugarten, J., Sandilya, S., Singh, B. & Golestaneh, L. Sex and the risk of AKI following cardio-thoracic surgery: a meta-analysis. *Clin. J. Am. Soc. Nephrol.* **11**, 2113–2122 (2016).
84. Chang, D. et al. Noninvasive identification of renal hypoxia in experimental myocardial infarctions of different sizes by using BOLD MR imaging in a mouse model. *Radiology* **286**, 129–139 (2018).
85. Lekawanvijit, S. et al. Myocardial infarction impairs renal function, induces renal interstitial fibrosis, and increases renal KIM-1 expression: implications for cardiorenal syndrome. *Am. J. Physiol. Heart Circ. Physiol.* **302**, H1884–H1893 (2012).
86. Lu, J. et al. Abrogation of lectin-like oxidized LDL receptor-1 attenuates acute myocardial ischemia-induced renal dysfunction by modulating systemic and local inflammation. *Kidney Int.* **82**, 436–444 (2012).
87. Ranganathan, P. et al. MicroRNA-150 deletion in mice protects kidney from myocardial infarction-induced

acute kidney injury. *Am. J. Physiol. Renal Physiol.* **309**, F551–F558 (2015).
88. Rutledge, C. A. et al. A novel ultrasound-guided mouse model of sudden cardiac arrest. *PLoS One* **15**, e0237292 (2020).
89. Matsushita, K. et al. The acute kidney injury to chronic kidney disease transition in a mouse model of acute cardiorenal syndrome emphasizes the role of inflammation. *Kidney Int.* **97**, 95–105 (2020).
90. Li, X. et al. Acute renal venous obstruction is more detrimental to the kidney than arterial occlusion: implication for murine models of acute kidney injury. *Am. J. Physiol. Renal Physiol.* **302**, F519–F525 (2012).
91. Hutchens, M. P. et al. Estrogen is renoprotective via a non-receptor dependent mechanism after cardiac arrest in vivo. *Anesthesiology* **112**, 395–405 (2010).
92. Burne-Taney, M. J. et al. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. *Am. J. Physiol. Renal Physiol.* **285**, F87–F94 (2003).
93. Zhang, Q. et al. Tolllike receptor 4 contributes to acute kidney injury after cardiopulmonary resuscitation in mice. *Mol. Med. Rep.* **14**, 2983–2990 (2016).
94. Ikeda, M. et al. Estrogen administered after cardiac arrest and cardiopulmonary resuscitation ameliorates acute kidney injury in a sex- and age-specific manner. *Crit. Care* **19**, 332 (2015).
95. Zeiner, A. et al. The effect of mild therapeutic hypothermia on renal function after cardiopulmonary resuscitation in men. *Resuscitation* **60**, 253–261 (2004).
96. Neyra, J. A. et al. Kidney tubular damage and functional biomarkers in acute kidney injury following cardiac surgery. *Kidney Int. Rep.* **4**, 1131–1142 (2019).
97. Ballaux, P. K., Gourlay, T., Ratnatunga, C. P. & Taylor, K. M. A literature review of cardiopulmonary bypass models for rats. *Perfusion* **14**, 411–417 (1999).
98. Yu, L. et al. The deep hypothermic circulatory arrest causes more kidney malfunctions based on a novel rabbit model. *Ann. Saudi Med.* **34**, 532–540 (2014).
99. Madrahimov, N. et al. Cardiopulmonary bypass in a mouse model: a novel approach. *J. Vis. Exp.* https://doi.org/10.3791/56017 (2017).
100. Wang, G. et al. Erythropoietin attenuates cardiopulmonary bypass-induced renal inflammatory injury by inhibiting nuclear factor-kappaB p65 expression. *Eur. J. Pharmacol.* **689**, 154–159 (2012).
101. Koning, N. J. et al. Impaired microcirculatory perfusion in a rat model of cardiopulmonary bypass: the role of hemodilution. *Am. J. Physiol. Heart Circ. Physiol.* **310**, H550–H558 (2016).
102. Darby, P. J. et al. Anemia increases the risk of renal cortical and medullary hypoxia during cardiopulmonary bypass. *Perfusion* **28**, 504–511 (2013).
103. Ohno, K. et al. Diabetes increases the susceptibility to acute kidney injury after myocardial infarction through augmented activation of renal Toll-like receptors in rats. *Am. J. Physiol. Heart Circ. Physiol.* **313**, H1130–H1142 (2017).
104. Kimura, Y. et al. Canagliflozin, a sodium-glucose cotransporter 2 inhibitor, normalizes renal susceptibility to type 1 cardiorenal syndrome through reduction of renal oxidative stress in diabetic rats. *J. Diabetes Investig.* **10**, 933–946 (2019).
105. Singh, A. P. et al. Animal models of acute renal failure. *Pharmacol. Rep.* **64**, 31–44 (2012).
106. Ortiz, A. et al. Translational value of animal models of kidney failure. *Eur. J. Pharmacol.* **759**, 205–220 (2015).
107. Doi, K. et al. Pre-existing renal disease promotes sepsis-induced acute kidney injury and worsens outcome. *Kidney Int.* **74**, 1017–1025 (2008).
108. Souza, A. C. et al. TLR4 mutant mice are protected from renal fibrosis and chronic kidney disease progression. *Physiol. Rep.* **3**, e12558. (2015).
109. Novitskaya, T. et al. A PTBA small molecule enhances recovery and reduces postinjury fibrosis after aristolochic acid-induced kidney injury. *Am. J. Physiol. Renal Physiol.* **306**, F496–F504 (2014).
110. Pabla, N. & Dong, Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney Int.* **73**, 994–1007 (2008).
111. Sharp, C. N. & Siskind, L. J. Developing better mouse models to study cisplatin-induced kidney injury. *Am. J. Physiol. Renal Physiol.* **313**, F835–F841 (2017).
112. Ghosh, S. Cisplatin: the first metal based anticancer drug. *Bioorg. Chem.* **88**, 102925 (2019).
113. Bosch, X., Poch, E. & Grau, J. M. Rhabdomyolysis and acute kidney injury. *N. Engl. J. Med.* **361**, 62–72 (2009).
114. Stewart, J. H. et al. The pattern of excess cancer in dialysis and transplantation. *Nephrol. Dial. Transpl.* **24**, 3225–3231 (2009).
115. Latcha, S. et al. Long-term renal outcomes after cisplatin treatment. *Clin. J. Am. Soc. Nephrol.* **11**, 1173–1179 (2016).
116. Sharp, C. N. et al. Repeated administration of low-dose cisplatin in mice induces fibrosis. *Am. J. Physiol. Renal Physiol.* **310**, F560–F568 (2016).
117. Sharp, C. N. et al. Subclinical kidney injury induced by repeated cisplatin administration results in progressive chronic kidney disease. *Am. J. Physiol. Renal Physiol.* **315**, F161–F172 (2018).
118. George, B., Joy, M. S. & Aleksunes, L. M. Urinary protein biomarkers of kidney injury in patients receiving cisplatin chemotherapy. *Exp. Biol. Med.* **243**, 272–282 (2018).
119. Sears, S. M. et al. C57BL/6 mice require a higher dose of cisplatin to induce renal fibrosis and CCL2 correlates with cisplatin-induced kidney injury. *Am. J. Physiol. Renal Physiol.* **319**, F674–F685 (2020).
120. Zha, M. et al. The circadian clock gene Bmal1 facilitates cisplatin-induced renal injury and hepatization. *Cell Death Dis.* **11**, 446 (2020).
121. Ravichandran, K. et al. CD4 T cell knockout does not protect against kidney injury and worsens cancer. *J. Mol. Med.* **94**, 443–455 (2016).
122. Van Avondt, K., Nur, E. & Zeerleder, S. Mechanisms of haemolysis-induced kidney injury. *Nat. Rev. Nephrol.* **15**, 671–692 (2019).
123. Panizo, N., Rubio-Navarro, A., Amaro-Villalobos, J. M., Egido, J. & Moreno, J. A. Molecular mechanisms and novel therapeutic approaches to rhabdomyolysis-induced acute kidney injury. *Kidney Blood Press. Res.* **40**, 520–532 (2015).
124. Boutaud, O. & Roberts, L. J. 2nd Mechanism-based therapeutic approaches to rhabdomyolysis-induced renal failure. *Free Radic. Biol. Med.* **51**, 1062–1067 (2011).
125. Kerchberger, V. E. & Ware, L. B. The role of circulating cell-free hemoglobin in sepsis-associated acute kidney injury. *Semin. Nephrol.* **40**, 148–159 (2020).
126. O'Neal, J. B., Shaw, A. D. & Billings, F. T. 4th Acute kidney injury following cardiac surgery: current understanding and future directions. *Crit. Care* **20**, 187 (2016).
127. Kawai, H. et al. Experimental glycerol myopathy: a histological study. *Acta Neuropathol.* **80**, 192–197 (1990).
128. McMahon, G. M., Zeng, X. & Waikar, S. S. A risk prediction score for kidney failure or mortality in rhabdomyolysis. *JAMA Intern. Med.* **173**, 1821–1828 (2013).
129. Okamura, D. M. & Pennathur, S. The balance of powers: redox regulation of fibrogenic pathways in kidney injury. *Redox Biol.* **6**, 495–504 (2015).
130. Belliere, J. et al. Specific macrophage subtypes influence the progression of rhabdomyolysis-induced kidney injury. *J. Am. Soc. Nephrol.* **26**, 1363–1377 (2015).
131. Soares, T. J., Costa, R. S., Volpini, R. A., Da Silva, C. G. & Coimbra, T. M. Long-term evolution of the acute tubular necrosis (ATN) induced by glycerol: role of myofibroblasts and macrophages. *Int. J. Exp. Pathol.* **83**, 165–172 (2002).
132. Faulk, T. et al. Rhabdomyolysis among critically Ill combat casualties: long-term outcomes. *Am. J. Nephrol.* **48**, 399–405 (2018).
133. Bolanos, J. A. et al. Outcomes after post-traumatic AKI requiring RRT in United States military service members. *Clin. J. Am. Soc. Nephrol.* **10**, 1732–1739 (2015).
134. Peerapornratana, S., Manrique-Caballero, C. L., Gomez, H. & Kellum, J. A. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. *Kidney Int.* **96**, 1083–1099 (2019).
135. Manrique-Caballero, C. L., Del Rio-Pertuz, G. & Gomez, H. Sepsis-associated acute kidney injury. *Crit. Care Clin.* **37**, 279–301 (2021).
136. Iskander, K. N. et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiol. Rev.* **93**, 1247–1288 (2013).
137. Hotchkiss, R. S., Coopersmith, C. M., McDunn, J. E. & Ferguson, T. A. The sepsis seesaw: tilting toward immunosuppression. *Nat. Med.* **15**, 496–497 (2009).
138. Legrand, M. et al. The role of renal hypoperfusion in development of renal microcirculatory dysfunction in endotoxemic rats. *Intensive Care Med.* **37**, 1534–1542 (2011).
139. Brealey, D. et al. Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**, R491–R497 (2004).
140. Vaure, C. & Liu, Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Front. Immunol.* **5**, 316 (2014).
141. El-Achkar, T. M. et al. Sepsis induces changes in the expression and distribution of Toll-like receptor 4 in the rat kidney. *Am. J. Physiol. Renal Physiol.* **290**, F1034–F1043 (2006).
142. Doi, K. et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. *J. Am. Soc. Nephrol.* **20**, 1217–1221 (2009).
143. Street, J. M. et al. The role of adenosine 1a receptor signaling on GFR early after the induction of sepsis. *Am. J. Physiol. Renal Physiol.* **314**, F788–F797 (2018).
144. Alverdy, J. C., Keskey, R. & Thewissen, R. Can the cecal ligation and puncture model be repurposed to better inform therapy in human sepsis? *Infect. Immun.* https://doi.org/10.1128/IAI.00942-19 (2020).
145. Rittirsch, D., Huber-Lang, M. S., Flierl, M. A. & Ward, P. A. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nat. Protoc.* **4**, 31–36 (2009).
146. Miyaji, T. et al. Ethyl pyruvate decreases sepsis-induced acute renal failure and multiple organ damage in aged mice. *Kidney Int.* **64**, 1620–1631 (2003).
147. Doi, K. et al. AP214, an analogue of alpha-melanocyte-stimulating hormone, ameliorates sepsis-induced acute kidney injury and mortality. *Kidney Int.* **73**, 1266–1274 (2008).
148. Leelahavanichkul, A. et al. Chronic kidney disease worsens sepsis and sepsis-induced acute kidney injury by releasing High Mobility Group Box Protein-1. *Kidney Int.* **80**, 1198–1211 (2011).
149. Levy, E. M., Viscoli, C. M. & Horwitz, R. I. The effect of acute renal failure on mortality. A cohort analysis. *JAMA* **275**, 1489–1494 (1996).
150. Faubel, S. Have we reached the limit of mortality benefit with our approach to renal replacement therapy in acute kidney injury? *Am. J. Kidney Dis.* **62**, 1030–1033 (2013).
151. Faubel, S. & Shah, P. B. Immediate consequences of acute kidney injury: the impact of traditional and nontraditional complications on mortality in acute kidney injury. *Adv. Chronic Kidney Dis.* **23**, 179–185 (2016).
152. Faubel, S. & Edelstein, C. L. Mechanisms and mediators of lung injury after acute kidney injury. *Nat. Rev. Nephrol.* **12**, 48–60 (2015).
153. Hoke, T. S. et al. Acute renal failure after bilateral nephrectomy is associated with cytokine-mediated pulmonary injury. *J. Am. Soc. Nephrol.* **18**, 155–164 (2007).
154. Andres-Hernando, A. et al. Cytokine production increases and cytokine clearance decreases in mice with bilateral nephrectomy. *Nephrol. Dial. Transpl.* **27**, 4339–4347 (2012).
155. Dennen, P. et al. Urine interleukin-6 is an early biomarker of acute kidney injury in children undergoing cardiac surgery. *Crit. Care* **14**, R181 (2010).
156. Klein, C. L. et al. Interleukin-6 mediates lung injury following ischemic acute kidney injury or bilateral nephrectomy. *Kidney Int.* **74**, 901–909 (2008).
157. Hassoun, H. T. et al. Ischemic acute kidney injury induces a distant organ functional and genomic response distinguishable from bilateral nephrectomy. *Am. J. Physiol. Renal Physiol.* **293**, F30–F40 (2007).
158. Karimi, Z. et al. Renal ischemia/reperfusion against nephrectomy for induction of acute lung injury in rats. *Ren. Fail.* **38**, 1503–1515 (2016).
159. Walcher, A., Faubel, S., Keniston, A. & Dennen, P. In critically Ill patients requiring CRRT, AKI Is associated with increased respiratory failure and death versus ESRD. *Ren. Fail.* **33**, 935–942 (2011).
160. Waikar, S. S., Liu, K. D. & Chertow, G. M. The incidence and prognostic significance of acute kidney injury. *Curr. Opin. Nephrol. Hypertens.* **16**, 227–236 (2007).
161. Bhargava, R. et al. Acute lung injury and acute kidney injury are established by four hours in experimental sepsis and are improved with pre, but not post, sepsis administration of TNF-alpha antibodies. *PLoS One* **8**, e79037 (2013).
162. Teixeira, J. P., Ambruso, S., Griffin, B. R. & Faubel, S. Pulmonary consequences of acute kidney injury. *Semin. Nephrol.* **39**, 3–16 (2019).
163. Andres-Hernando, A. et al. Prolonged acute kidney injury exacerbates lung inflammation at 7 days post-acute kidney injury. *Physiol. Rep.* **2**, e12084 (2014).
164. Cruz, D. N., Gheorghiade, M., Palazzuoli, A., Ronco, C. & Bagshaw, S. M. Epidemiology and outcome of the cardio-renal syndrome. *Heart Fail. Rev.* **16**, 531–542 (2011).

165. Ronco, C., Haapio, M., House, A. A., Anavekar, N. & Bellomo, R. Cardiorenal syndrome. *J. Am. Coll. Cardiol.* **52**, 1527–1539 (2008).
166. Ronco, C., House, A. A. & Haapio, M. Cardiorenal and renocardiac syndromes: the need for a comprehensive classification and consensus. *Nat. Clin. Pract. Nephrol.* **4**, 310–311 (2008).
167. Gammelager, H. et al. Three-year risk of cardiovascular disease among intensive care patients with acute kidney injury: a population-based cohort study. *Crit. Care* **18**, 492 (2014).
168. Sumida, M. et al. Regulation of mitochondrial dynamics by dynamin-related protein-1 in acute cardiorenal syndrome. *J. Am. Soc. Nephrol.* **26**, 2378–2387 (2015).
169. Fox, B. M. et al. Metabolomics assessment reveals oxidative stress and altered energy production in the heart after ischemic acute kidney injury in mice. *Kidney Int.* **95**, 590–610 (2019).
170. Kelly, K. J. Distant effects of experimental renal ischemia/reperfusion injury. *J. Am. Soc. Nephrol.* **14**, 1549–1558 (2003).
171. Martinez-Martinez, E. et al. Galectin-3 blockade inhibits cardiac inflammation and fibrosis in experimental hyperaldosteronism and hypertension. *Hypertension* **66**, 767–775 (2015).
172. Go, A. S. et al. Acute kidney injury and risk of heart failure and atherosclerotic events. *Clin. J. Am. Soc. Nephrol.* **13**, 833–841 (2018).
173. Giraud, S. et al. Contribution of large pig for renal ischemia-reperfusion and transplantation studies: the preclinical model. *J. Biomed. Biotechnol.* **2011**, 532127 (2011).
174. Fairbairn, L., Kapetanovic, R., Sester, D. P. & Hume, D. A. The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. *J. Leukoc. Biol.* **89**, 855–871 (2011).
175. Seok, J. et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl Acad. Sci. USA* **110**, 3507–3512 (2013).
176. Packialakshmi, B., Stewart, I. J., Burmeister, D. M., Chung, K. K. & Zhou, X. Large animal models for translational research in acute kidney injury. *Ren. Fail.* **42**, 1042–1058 (2020).
177. Adams, P. L., Adams, F. F., Bell, P. D. & Navar, L. G. Impaired renal blood flow autoregulation in ischemic acute renal failure. *Kidney Int.* **18**, 68–76 (1980).
178. Snoeijs, M. G. et al. Addition of a water-soluble propofol formulation to preservation solution in experimental kidney transplantation. *Transplantation* **92**, 296–302 (2011).
179. Ekser, B., Rigotti, P., Gridelli, B. & Cooper, D. K. Xenotransplantation of solid organs in the pig-to-primate model. *Transpl. Immunol.* **21**, 87–92 (2009).
180. Xu, M. et al. Anti-CD47 monoclonal antibody therapy reduces ischemia-reperfusion injury of renal allografts in a porcine model of donation after cardiac death. *Am. J. Transpl.* **18**, 855–867 (2018).
181. Neumayer, H. H., Blossei, N., Seherr-Thohs, U. & Wagner, K. Amelioration of postischaemic acute renal failure in conscious dogs by human atrial natriuretic peptide. *Nephrol. Dial. Transpl.* **5**, 32–38 (1990).
182. Nilsson, K. F., Sandin, J., Gustafsson, L. E. & Frithiof, R. The novel nitric oxide donor PDNO attenuates ovine ischemia-reperfusion induced renal failure. *Intensive Care Med. Exp.* **5**, 29 (2017).
183. Zahran, M. H. et al. Renoprotective effect of local sildenafil administration in renal ischaemia-reperfusion injury: a randomised controlled canine study. *Arab. J. Urol.* **17**, 150–159 (2019).
184. O'Kane, D. et al. Zinc preconditioning protects against renal ischaemia reperfusion injury in a preclinical sheep large animal model. *Biometals* **31**, 821–834 (2018).
185. Woolley, J. L. et al. Effect of the calcium entry blocker verapamil on renal ischemia. *Crit. Care Med.* **16**, 48–51 (1988).
186. Favreau, F. et al. Expression and modulation of translocator protein and its partners by hypoxia reoxygenation or ischemia and reperfusion in porcine renal models. *Am. J. Physiol. Renal Physiol.* **297**, F177–F190 (2009).
187. Hunter, J. P., Hosgood, S. A., Barlow, A. D. & Nicholson, M. L. Ischaemic conditioning reduces kidney injury in an experimental large-animal model of warm renal ischaemia. *Br. J. Surg.* **102**, 1517–1525 (2015).
188. Amdisen, C. et al. Testing danegaptide effects on kidney function after ischemia/reperfusion injury in a new porcine two week model. *PLoS One* **11**, e0164109 (2016).
189. Castellano, G. et al. Therapeutic targeting of classical and lectin pathways of complement protects from ischemia-reperfusion-induced renal damage. *Am. J. Pathol.* **176**, 1648–1659 (2010).
190. Sølling, C. et al. Erythropoietin administration is associated with short-term improvement in glomerular filtration rate after ischemia-reperfusion injury. *Acta Anaesthesiol. Scand.* **55**, 185–195 (2011).
191. GIBBON, J. H. Jr Artificial maintenance of circulation during experimental occlusion of pulmonary artery. *Arch. Surg.* **34**, 1105–1131 (1937).
192. DiVincenti, L. Jr, Westcott, R. & Lee, C. Sheep (Ovis aries) as a model for cardiovascular surgery and management before, during, and after cardiopulmonary bypass. *J. Am. Assoc. Lab. Anim. Sci.* **53**, 439–448 (2014).
193. Lankadeva, Y. R. et al. Reversal of renal tissue hypoxia during experimental cardiopulmonary bypass in sheep by increased pump flow and arterial pressure. *Acta Physiol.* **231**, e13596 (2021).
194. Lannemyr, L. et al. Effects of cardiopulmonary bypass on renal perfusion, filtration, and oxygenation in patients undergoing cardiac surgery. *Anesthesiology* **126**, 205–213 (2017).
195. Lankadeva, Y. R. et al. Strategies that improve renal medullary oxygenation during experimental cardiopulmonary bypass may mitigate postoperative acute kidney injury. *Kidney Int.* **95**, 1338–1346 (2019).
196. Lankadeva, Y. R. et al. Influence of blood hemoglobin concentration on renal hemodynamics and oxygenation during experimental cardiopulmonary bypass in sheep. *Acta Physiol.* **231**, e13583 (2020).
197. Qureshi, S. H., Patel, N. N. & Murphy, G. J. Vascular endothelial cell changes in postcardiac surgery acute kidney injury. *Am. J. Physiol. Renal Physiol.* **314**, F726–F735 (2018).
198. Murphy, G. J. et al. An initial evaluation of post-cardiopulmonary bypass acute kidney injury in swine. *Eur. J. Cardiothorac. Surg.* **36**, 849–855 (2009).
199. Shi, N. et al. The association between obesity and risk of acute kidney injury after cardiac surgery. *Front. Endocrinol.* **11**, 534294 (2020).
200. O'Sullivan, K. E. et al. The effect of obesity on acute kidney injury after cardiac surgery. *J. Thorac. Cardiovasc. Surg.* **150**, 1622–1628 (2015).
201. Billings, F. T. et al. Obesity and oxidative stress predict AKI after cardiac surgery. *J. Am. Soc. Nephrol.* **23**, 1221–1228 (2012).
202. Stamou, S. C. et al. Effect of body mass index on outcomes after cardiac surgery: is there an obesity paradox? *Ann. Thorac. Surg.* **91**, 42–47 (2011).
203. Sleeman, P. et al. High fat feeding promotes obesity and renal inflammation and protects against post cardiopulmonary bypass acute kidney injury in swine. *Crit. Care* **17**, R262 (2013).
204. Santiago, M. J. et al. Cisplatin-induced non-oliguric acute kidney injury in a pediatric experimental animal model in piglets. *PLoS One* **11**, e0149013 (2016).
205. Wang, S. Y., Zhang, C. Y., Cai, G. Y. & Chen, X. M. Method used to establish a large animal model of drug-induced acute kidney injury. *Exp. Biol. Med.* **246**, 986–995 (2021).
206. Zheng, J. S. et al. Screening of early diagnostic markers of gentamicin-induced acute kidney injury in canines. *J. Vet. Res.* **63**, 405–411 (2019).
207. Garry, F., Chew, D. J. & Hoffsis, G. F. Urinary indices of renal function in sheep with induced aminoglycoside nephrotoxicosis. *Am. J. Vet. Res.* **51**, 420–427 (1990).
208. Cui, J. et al. Rapamycin protects against gentamicin-induced acute kidney injury via autophagy in mini-pig models. *Sci. Rep.* **5**, 11256 (2015).
209. Margulies, K. B., McKinley, L. J., Cavero, P. G. & Burnett, J. C. Jr. Induction and prevention of radiocontrast-induced nephropathy in dogs with heart failure. *Kidney Int.* **38**, 1101–1108 (1990).
210. Wu, J. et al. Retinoic acid attenuates contrast-induced acute kidney injury in a miniature pig model. *Biochem. Biophys. Res. Commun.* **512**, 163–169 (2019).
211. Tang, W. et al. Renal protective effects of early continuous venovenous hemofiltration in rhabdomyolysis: improved renal mitochondrial dysfunction and inhibited apoptosis. *Artif. Organs* **37**, 390–400 (2013).
212. Rajagopalan, P. R., Reines, H. D., Pulliam, C., Fitts, C. T. & LeVeen, H. H. Reversal of acute renal failure using hemodilution with hydroxyethyl starch. *J. Trauma.* **23**, 795–800 (1983).
213. van Griensven, M. et al. Protective effects of the complement inhibitor compstatin cp40 in hemorrhagic shock. *Shock* **51**, 78–87 (2019).
214. Sondeen, J. L., Gonzaludo, G. A., Loveday, J. A., Rodkey, W. G. & Wade, C. E. Hypertonic saline/dextran improves renal function after hemorrhage in conscious swine. *Resuscitation* **20**, 231–241 (1990).
215. Hoareau, G. L. et al. Renal effects of three endoaortic occlusion strategies in a swine model of hemorrhagic shock. *Injury* **50**, 1908–1914 (2019).
216. Smith, S. et al. Aggressive treatment of acute kidney injury and hyperkalemia improves survival in a combat relevant trauma model in swine. *Am. J. Surg.* **219**, 860–864 (2020).
217. Wong, Y. C. et al. Potential biomarker panel for predicting organ dysfunction and acute coagulopathy in a polytrauma porcine model. *Shock* **43**, 157–165 (2015).
218. Fröhlich, M. et al. Induced hypothermia reduces the hepatic inflammatory response in a swine multiple trauma model. *J. Trauma. Acute Care Surg.* **76**, 1425–1432 (2014).
219. Hasslacher, J. et al. Acute kidney injury and mild therapeutic hypothermia in patients after cardiopulmonary resuscitation — a post hoc analysis of a prospective observational trial. *Crit. Care* **22**, 154 (2018).
220. Sullivan, T. P., Eaglstein, W. H., Davis, S. C. & Mertz, P. The pig as a model for human wound healing. *Wound Repair. Regen.* **9**, 66–76 (2001).
221. Khorram-Sefat, R. et al. The therapeutic effect of C1-inhibitor on gut-derived bacterial translocation after thermal injury. *Shock* **9**, 101–108 (1998).
222. Guenther, T. M. et al. High versus low volume fluid resuscitation strategies in a porcine model (sus scrofa) of combined thermal and traumatic brain injury. *Shock* **55**, 536–544 (2020).
223. Burmeister, D. M. et al. Impact of isolated burns on major organs: a large animal model characterized. *Shock* **46**, 137–147 (2016).
224. Gómez, B. I. et al. Enteral resuscitation with oral rehydration solution to reduce acute kidney injury in burn victims: evidence from a porcine model. *PLoS One* **13**, e0195615 (2018).
225. Mason, S. A., Nathens, A. B. & Jeschke, M. G. "Hold the pendulum: rates of acute kidney injury are increased in patients who receive resuscitation volumes less than predicted by the Parkland Equation". *Ann. Surg.* **266**, e108 (2017).
226. Christenson, J. T. & Owunwanne, A. Leucocyte sequestration in endotoxemia and the effect of low-molecular-weight dextran. *Eur. J. Nucl. Med.* **17**, 28–33 (1990).
227. Kampmeier, T. G. et al. Effects of resuscitation with human albumin 5%, hydroxyethyl starch 130/0.4 6%, or crystalloid on kidney damage in an ovine model of septic shock. *Br. J. Anaesth.* **121**, 581–587 (2018).
228. Linton, A. L., Walker, J. F., Lindsay, R. M. & Sibbald, W. J. Acute renal failure and tubular damage due to sepsis in an animal model. *Proc. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* **21**, 837–842 (1985).
229. Di Giantomasso, D., Morimatsu, H., May, C. N. & Bellomo, R. Intrarenal blood flow distribution in hyperdynamic septic shock: Effect of norepinephrine. *Crit. Care Med.* **31**, 2509–2513 (2003).
230. Langenberg, C., Gobe, G., Hood, S., May, C. N. & Bellomo, R. Renal histopathology during experimental septic acute kidney injury and recovery. *Crit. Care Med.* **42**, e58–e67 (2014).
231. Lankadeva, Y. R., Kosaka, J., Evans, R. G., Bellomo, R. & May, C. N. Urinary oxygenation as a surrogate measure of medullary oxygenation during angiotensin II therapy in septic acute kidney injury. *Crit. Care Med.* **46**, e41–e48 (2018).
232. Lankadeva, Y. R. et al. Effects of fluid bolus therapy on renal perfusion, oxygenation, and function in early experimental septic kidney injury. *Crit. Care Med.* **47**, e36–e43 (2019).
233. Benes, J. et al. Searching for mechanisms that matter in early septic acute kidney injury: an experimental study. *Crit. Care* **15**, R256 (2011).
234. Wang, H. et al. Improvement of cytokine response and survival time by bioartificial kidney therapy in acute uremic pigs with multi-organ dysfunction. *Int. J. Artif. Organs* **33**, 526–534 (2010).
235. Matejovic, M. et al. Molecular differences in susceptibility of the kidney to sepsis-induced kidney injury. *BMC Nephrol.* **18**, 183 (2017).
236. Vassal, O. et al. Renal haemodynamic response to amino acids infusion in an experimental porcine model of septic shock. *Acta Anaesthesiol. Scand.* **59**, 598–608 (2015).
237. Matejovic, M. et al. Renal proteomic responses to severe sepsis and surgical trauma: dynamic analysis of porcine tissue biopsies. *Shock* **46**, 453–464 (2016).
238. Merz, T. et al. Cystathionine-γ-lyase expression is associated with mitochondrial respiration during

sepsis-induced acute kidney injury in swine with atherosclerosis. *Intensive Care Med. Exp.* **6**, 43 (2018).
239. Post, E. H. et al. The effects of acute renal denervation on kidney perfusion and metabolism in experimental septic shock. *BMC Nephrol.* **18**, 182 (2017).
240. Kubiak, B. D. et al. A clinically applicable porcine model of septic and ischemia/reperfusion-induced shock and multiple organ injury. *J. Surg. Res.* **166**, e59–e69 (2011).
241. Maybauer, M. O. et al. Recombinant human activated protein C attenuates cardiovascular and microcirculatory dysfunction in acute lung injury and septic shock. *Crit. Care* **14**, R217 (2010).
242. Lange, M. et al. Effects of early neuronal and delayed inducible nitric oxide synthase blockade on cardiovascular, renal, and hepatic function in ovine sepsis. *Anesthesiology* **113**, 1376–1384 (2010).
243. Fenhammar, J. et al. Endothelin receptor A antagonism attenuates renal medullary blood flow impairment in endotoxemic pigs. *PLoS One* **6**, e21534 (2011).
244. Fenhammar, J. et al. Toll-like receptor 4 inhibitor TAK-242 attenuates acute kidney injury in endotoxemic sheep. *Anesthesiology* **114**, 1130–1137 (2011).
245. Mazzola, S. et al. Carbon monoxide pretreatment prevents respiratory derangement and ameliorates hyperacute endotoxic shock in pigs. *FASEB J.* **19**, 2045–2047 (2005).
246. Netti, G. S. et al. LPS removal reduces CD80-mediated albuminuria in critically ill patients with Gram-negative sepsis. *Am. J. Physiol. Renal Physiol.* **316**, F723–F731 (2019).
247. Sander, V. et al. Protocol for large-scale production of kidney organoids from human pluripotent stem cells. *Star. Protoc.* **1**, 100150 (2020).
248. Hukriede, N., Vogt, A. & de Caestecker, M. Drug discovery to halt the progression of acute kidney injury to chronic kidney disease: a case for phenotypic drug discovery in acute kidney injury. *Nephron* **137**, 268–272 (2017).
249. Martignoni, M., Groothuis, G. M. & de Kanter, R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert. Opin. Drug Metab. Toxicol.* **2**, 875–894 (2006).
250. de Caestecker, M. et al. Bridging translation by improving preclinical study design in AKI. *J. Am. Soc. Nephrol.* **26**, 2905–2916 (2015).
251. Weisbord, S. D. & Palevsky, P. M. Design of clinical trials in acute kidney injury: lessons from the past and future directions. *Semin. Nephrol.* **36**, 42–52 (2016).
252. Faubel, S. et al. Ongoing clinical trials in AKI. *Clin. J. Am. Soc. Nephrol.* **7**, 861–873 (2012).
253. de Caestecker, M. P., Siew, E. D., Harris, R. C. & Hukriede, N. A. Introduction: The 2019 Federation of American societies for experimental biology acute kidney injury from bench to bedside conference. *Semin. Nephrol.* **40**, 99–100 (2020).
254. Hale, L. J. et al. 3D organoid-derived human glomeruli for personalised podocyte disease modelling and drug screening. *Nat. Commun.* **9**, 5167 (2018).
255. Kumar, S. V. et al. Kidney micro-organoids in suspension culture as a scalable source of human pluripotent stem cell-derived kidney cells. *Development* **146**, dev172361 (2019).
256. Lawlor, K. T. et al. Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation. *Nat. Mater.* **20**, 260–271 (2021).

Author contributions
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