



## CKJ REVIEW

# Novel strategies in nephrology: what to expect from the future?

Sidar Copur<sup>1</sup>, Cem Tanriover<sup>1</sup>, Furkan Yavuz<sup>1</sup>, Maria J. Soler<sup>2,3</sup>, Alberto Ortiz <sup>4</sup>, Adrian Covic<sup>5</sup> and Mehmet Kanbay <sup>6</sup>

<sup>1</sup>Department of Medicine, Koc University School of Medicine, Istanbul, Turkey, <sup>2</sup>Department of Nephrology, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Spain, <sup>3</sup>Nephrology and Kidney Transplant Research Group, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain, <sup>4</sup>Department of Medicine, Universidad Autonoma de Madrid and IIS-Fundacion Jimenez Diaz, Madrid, Spain, <sup>5</sup>Nephrology Clinic, Dialysis and Renal Transplant Center, 'C.I. PARHON' University Hospital, and 'Grigore T. Popa' University of Medicine, Iasi, Romania and <sup>6</sup>Department of Medicine, Division of Nephrology, Koc University School of Medicine, Istanbul, Turkey

Correspondence to: Mehmet Kanbay; E-mail: [mkanbay@ku.edu.tr](mailto:mkanbay@ku.edu.tr)

## ABSTRACT

Chronic kidney disease (CKD) will become the fifth global cause of death by 2040. Its largest impact is on premature mortality but the number of persons with kidney failure requiring renal replacement therapy (RRT) is also increasing dramatically. Current RRT is suboptimal due to the shortage of kidney donors and dismal outcomes associated with both hemodialysis and peritoneal dialysis. Kidney care needs a revolution. In this review, we provide an update on emerging knowledge and technologies that will allow an earlier diagnosis of CKD, addressing the current so-called blind spot (e.g. imaging and biomarkers), and improve renal replacement therapies (wearable artificial kidneys, xenotransplantation, stem cell-derived therapies, bioengineered and bio-artificial kidneys).

**Keywords:** artificial kidney, bioengineering, chronic kidney disease, induced pluripotent stem cells, xenotransplantation

## INTRODUCTION

There are currently 850 million persons in the world with chronic kidney disease (CKD), and CKD is predicted to become the fifth global cause of death by 2040 and the second cause of death in countries with long life expectancy by 2100 [1]. Moreover, CKD is the leading chronic condition with increased incidence, prevalence and overall health impact. The terminal stage of CKD, referred as end-stage renal disease (ESRD) or kidney failure, is defined by an estimated glomerular filtration rate (eGFR) below 15 mL/min/1.73 m<sup>2</sup> by the Kidney Disease: Improving Global Outcomes (KDIGO), affecting approximately 800 000 patients in

the USA (71% on dialysis and 29% with a kidney transplant) [2], whereas, in Europe the estimated ESRD population is over 1 million people, with considerable variations across individual countries [3, 4].

Kidney transplantation was first performed in 1954 by Dr Joseph Murray and is the current gold standard for treatment. However, there are still fewer donors than the relentlessly increasing waiting list [5]. Chronic hemodialysis was introduced in 1960 by Dr Belding Scribner, and despite being the major form of renal replacement therapy (RRT) it is associated with numerous short- and long-term complications. Importantly, the life expectancy of patients in dialysis in their twenties is 40 years

Received: 30.7.2022; Editorial decision: 14.9.2022

© The Author(s) 2022. Published by Oxford University Press on behalf of the ERA. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

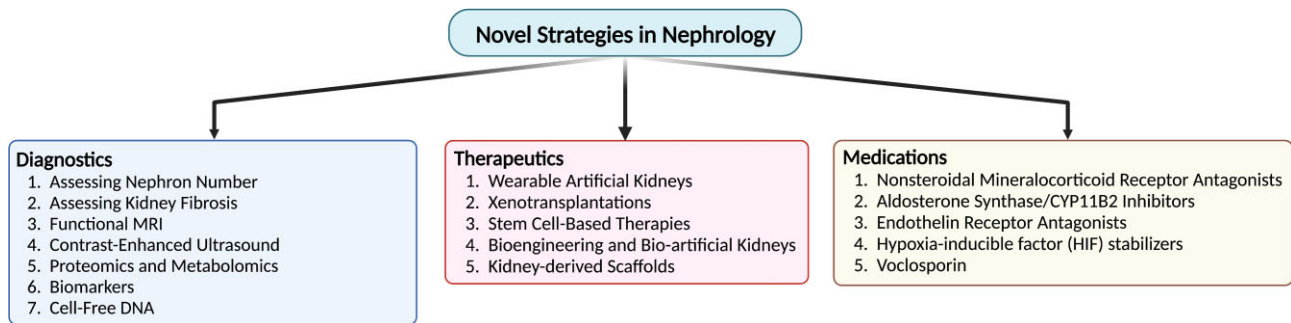


Figure 1: General overview of the novel strategies in nephrology. (Diagnostics will be further elaborated in detail in Fig. 2.)

shorter than for the general population [6]. Peritoneal dialysis is less frequently utilized, and beside specific complications (peritonitis, cellulitis, metabolic disturbances) it is limited by almost inexorable membrane failure [7].

Thus, overall, the treatment of ESRD patients remains sub-optimal, due to a shortage of kidney donors and multiple significant complications associated with both dialysis modalities, although multiple alternatives have been considered over the years. Some of the recent developments have the potential to revolutionize the field of nephrology in the upcoming decades. In addition, a large proportion of patients with CKD, especially those with stage 2 and stage 3 CKD, are older individuals who will never require any form of renal replacement therapy mainly since these patients die earlier of cardiovascular diseases. Thus, the real increase in the need of RRT is much less. The main benefits of new drugs such as sodium–glucose cotransporter 2 (SGLT2) inhibitors and mineralocorticoid receptor antagonists (MRAs) are in this group of kidney disease patients.

In this review, we aim to describe the novel diagnostic methods in nephrology such as the advancements in kidney imaging and modalities utilized to estimate renal function as well as the novel therapeutic approaches in kidney disease including wearable artificial kidneys, xenotransplantation, stem cell-derived therapies, bioengineering models and medications on the rise (Fig. 1).

## NOVEL DIAGNOSTIC METHODS

Novel diagnostic methods that would allow an earlier diagnosis of CKD are an unmet clinical need. Reliance on the current eGFR threshold to diagnose CKD means that by the time CKD is diagnosed, over 50% of the functional kidney mass has been lost and the combined risk of CKD progression and premature death is already increased by around 2- to 7-fold, while current interventions decrease the risk of adverse outcomes by 20%–40% [8, 9]; in other words, diagnosis is too late. While CKD may be diagnosed earlier based on high albuminuria values, most patients progress to CKD category G3 while having physiological albuminuria, as evidenced by epidemiological data that show that G3 is the most common category of CKD [10], i.e. albuminuria did not allow an earlier diagnosis (i.e. G1 or G2) for most patients that progressed to G3. This subclinical stage of CKD progression, potentially lasting decades, as evidenced by those forms of CKD in which we have a tool that allows an earlier diagnosis (e.g. sonography for autosomal dominant polycystic kidney disease) is in fact the blind spot for CKD diagnosis [11–13]. Several approaches are under study to address the blind spot in CKD, mainly using imaging and assessment of biomarkers in biological fluids (Fig. 2).

## Imaging: assessing nephron number as a determinant of kidney disease and kidney fibrosis

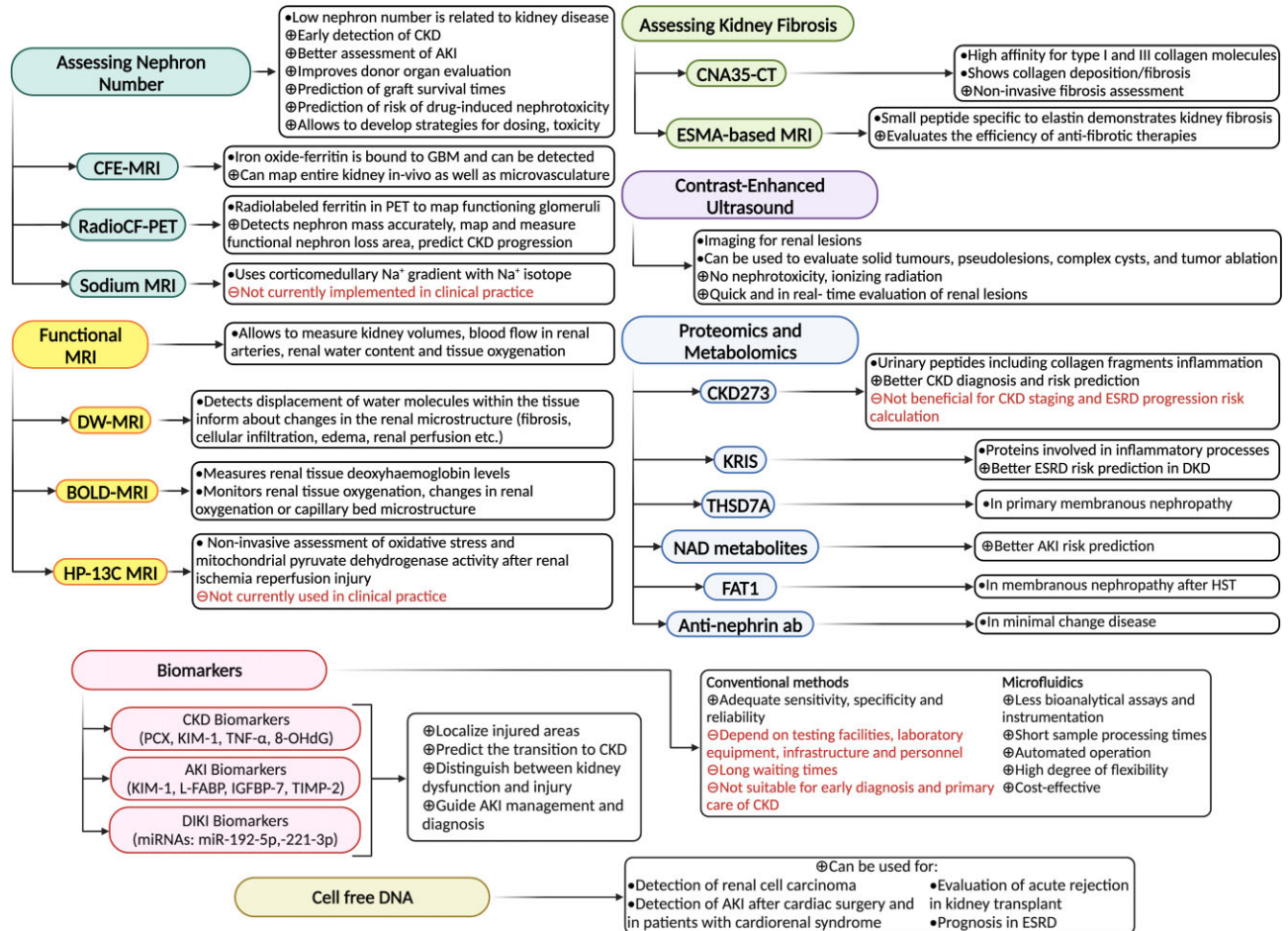
Imaging techniques have the advantage of being non-invasive and, thus, may be safely repeated to evaluate changes, providing information for both kidneys and potentially combining functional with morphological information. The most interesting advances relate to estimation of nephron number, overall kidney functions, fibrosis and new functional magnetic resonance imaging (MRI), as well as ultrasound techniques such as diffusion-weighted MRI (DWI or DW-MRI), blood oxygenation level-dependent MRI (BOLD-MRI), perfusion MRI, hyperpolarized (HP) carbon 13 MRI (13C MRI) and contrast-enhanced ultrasound (CEUS).

Functional nephron number is considered an important determinant of kidney health and disease susceptibility throughout life [14, 15]. In humans, nephron number varies widely and a low nephron endowment at birth and/or a loss of nephrons throughout life is strongly associated with kidney disease [15]. Novel technologies to measure nephron number are under development, and functional nephron number has the potential to be used as a clinical biomarker [14]. Nephron number, when used as a biomarker, could provide important information regarding the progression of kidney disease and provide early detection of CKD onset or assessment of recovery after acute kidney injury, improve the evaluation and assessment of donor organs, predict graft survival times, predict the risk of drug-induced nephrotoxicity, and help develop strategies for dosing and toxicity testing for a wide range of therapeutic drugs [14].

New methods have been suggested to measure nephron number *ex vivo* in the intact kidney: cationized ferritin-enhanced MRI (CFE-MRI) [16], light sheet microscopy after optical clearing [17] and computed tomography (CT) [18]. However, the utilization of these tools *in vivo* and in the clinics necessitates them being non-destructive and relatively non-invasive, and thus far only CFE-MRI has been used *in vivo* [14].

CFE-MRI uses ferritin filled with an iron oxide [14]. Following intravenous injection, the ferritin is cationized and bound to the glomerular basement membrane. The accumulation of ferritin in the glomeruli allows its detection, mapping of the entire kidney *in vivo* and co-localization of glomeruli with other structures such as the microvasculature. To support the use of nephron number as a clinical parameter, the cationized ferritin molecule of CFE-MRI has been modified to form radiolabeled cationic ferritin (RadioCF), a radiotracer used in positron emission tomography (PET) to map functioning glomeruli *in vivo* in the kidney [19]. RadioCF-PET accurately quantifies nephron mass in animals and had the potential for clinical translation [19]. Radio-CF is formed by integration of a radioisotope,

## Novel Diagnostic Methods



**Figure 2:** Novel diagnostic methods in nephrology. CFE-MRI: cationized ferritin-enhanced MRI; RadioCF-PET: radiolabeled cationic ferritin-positron emission tomography; CKD: chronic kidney disease; AKI: acute kidney injury; GBM: glomerular basement membrane; KRIS: Kidney Risk Inflammatory Signature; THSD7A: thrombospondin type-1 domain-containing 7A antibodies; NAD: nicotinamide adenine dinucleotide; FAT1: glomerular antigen-protocadherin FAT1; ESRD: end-stage renal disease; DKD: diabetic kidney disease; HST: hematopoietic stem cell transplantation; PCX: podocalyxin; KIM-1: kidney injury molecule 1; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; 8-OHdG: 8-oxo-7,8-dihydro-2'-deoxyguanosine; L-FABP: liver-type fatty acid binding protein; IGFBP-7: insulin-like growth factor-binding protein-7; TIMP-2: tissue inhibitor of metalloprotease-2; DKI: drug-induced kidney injury; CNA35-CT: collagen-binding adhesion protein-35 CT; ESMA-based MRI: elastin-specific contrast agent MRI; DW-MRI: diffusion-weighted MRI; BOLD-MRI: blood oxygen level-dependent MRI; HP-13C MRI: hyperpolarized carbon 13 MRI; plus sign: advantages; minus sign: disadvantages/limitations (indicated in red color).

Cu-64, to the cationic ferritin (CF) and has been shown to bind functional glomeruli when given intravenously [19]. RadioCF-PET can map and measure the areas of functional nephron loss making it a diagnostic tool that can also predict CKD progression [14].

MRI has been used to assess the functional status of both kidneys. Multiparametric MRI may evaluate diverse aspects of kidney function and vascularization [20]. A novel imaging modality is sodium MRI. Compared with “normal MRI,” which is a map of hydrogen atoms in the body, sodium MRI is a map of sodium atoms in the body. The kidney has a baseline gradient of sodium concentrations from the cortex to the medulla (corticomedullary sodium gradient) [21, 22]. The first study of sodium MRI on human kidneys demonstrated that the sodium gradient increases linearly from cortex to medulla which is followed by linear decrease until renal pelvis [23, 24]. Although alterations in the sodium gradient have been demonstrated on multiple clinical and pre-clinical trials, the sodium MRI technique is far from be-

ing integrated into clinical practice [25–27] mainly due to the cost and technical factors involved in MRI recalibration and operation. An additional major setback of this technique for now is the lack of adequate characterization of findings in different CKD stages and in various kidney diseases.

Specific techniques have been designed to assess kidney fibrosis and its dynamics in both kidneys simultaneously *in vivo* [28]. These include fluorescent CNA35 CT, which takes advantage of a collagen binding peptide, and elastin-specific contrast agent MRI (ESMA)-based molecular MRI of elastin [28]. For instance, collagen-binding adhesion protein (CNA35) which has high affinity for type I and III collagen molecules has been utilized as a tool to visualize collagen deposition in mouse subjects and has a potential to be utilized in human subjects as a non-invasive method of fibrosis assessment [29, 30]. On the other hand, ESMA-based MRI, which is a small peptide specific to elastin, component of extracellular matrix, has been shown to demonstrate kidney fibrosis in mouse models as well as the



efficiency of anti-fibrotic therapies [31]. These techniques will eventually also test a potential impact of anti-fibrotic therapies, a current unmet need.

In addition, novel MRI techniques allow to generate imaging biomarkers that can improve the management of kidney disease [20]. MRI enables to measure kidney volumes, blood flow in renal arteries, renal water content and tissue oxygenation [20]. DWI, or DW-MRI, can detect the displacement of water molecules within the tissue architecture [20]. This technique can inform about any changes in the renal microstructure such as renal fibrosis, cellular infiltration (inflammatory or tumorous) or edema as well as changes in renal perfusion and in the water handling in the tubular compartment [20]. BOLD-MRI, which measures renal tissue deoxyhaemoglobin levels voxel by voxel, is a promising technique to monitor renal tissue oxygenation in humans [32]. It allows the monitoring of changes in renal oxygenation or changes in the capillary bed microstructure. In addition, T2 is altered by several other factors such as hydration status, dietary sodium and susceptibility effects [20]. In addition, HP 13C MRI is a potential tool, not currently used in the clinic, for the non-invasive assessment of oxidative stress and mitochondrial pyruvate dehydrogenase activity following renal ischemia–reperfusion injury [33].

Furthermore, CEUS has been a promising imaging modality for renal lesions. CEUS lacks nephrotoxicity, ionizing radiation, and has the ability to evaluate the enhancement pattern of renal lesions quickly and in real time [34]. Some of the well-defined applications of CEUS are the differentiation of solid tumors, pseudolesions and complex cysts; characterization of complex cysts with different malignant potential; and evaluation of tumor ablation [34]. Microbubble contrast agents are safe with rare adverse reactions [34].

These approaches are promising tools to assess nephron number, diverse kidney functions and kidney fibrosis and may be transitioned to clinical use if their safety, efficacy and regulatory requirements are established.

### Biological fluid biomarkers

Proteomics and metabolomics have recently been tested in the field of nephrology with intriguing and promising results. In some cases, proteomic or metabolomic signatures themselves are used as biomarkers. In others, they are tools that are used to identify individual biomarkers that are then assessed using more conventional techniques. Additionally, both RNA and DNA in biological fluids may serve as biomarkers. These biomarkers should correlate well with kidney disease, histopathology, progression, outcomes or early disease, and allow for rapid, non-invasive and specific measurements with high sensitivity and specificity.

### Proteomic and metabolomic analysis for the detection of biomarkers

Pontillo and Mischak identified 273 urinary peptides that differ between patients with CKD and healthy subjects, namely the marker CKD273 which includes fragments of collagen and of proteins involved in inflammation and tissue repair, by using capillary electrophoresis-mass spectroscopy (CE-MS) on 230 CKD patients and 379 control subjects [35, 36]. Further studies have implicated the potential superiority of CKD273 in the prediction and diagnosis of CKD over traditional markers leading to a letter of support of the US Food and Drug Administration (FDA). According to a cross-sectional study conducted on 1990

participants, CKD273 performed better than traditional markers and correlated better than albuminuria with eGFR and better predicted rapid CKD progression [37]. Similar findings were observed in another study conducted on 2087 participants, in which CKD273 added to the prediction of CKD G3 after accounting for baseline eGFR, albuminuria and covariables [38]. In a randomized controlled trial in diabetic patients without albuminuria, CKD273 predicted the development of albuminuria [39]. Furthermore, the same CE-MS analysis of a single urine sample may be used to derive other peptidomics markers that predict the rapid loss of eGFR better than albuminuria in patients who do not fulfill current eGFR criteria for CKD (i.e. may allow an earlier diagnosis of CKD than albuminuria) [40], or correlate with kidney fibrosis as detected in kidney biopsy [41] or provide information on the underlying cause of CKD and its prognosis [42, 43].

The Kidney Risk Inflammatory Signature (KRIS) includes 17 proteins directly involved in inflammation and correlated with the 10-year risk of ESRD in diabetic kidney disease [44]. In this study 194 circulating inflammatory proteins have been evaluated in three different cohorts comprised of type 1 and 2 diabetic patients, revealing that 17 novel proteins enriched for TNF Receptor Superfamily members are linked to early and late renal function decline leading to ESRD in diabetic subjects. Even though the major source, hypothesized to be white blood cells, of those KRIS proteins is yet to be determined, their appearance years prior to the onset of ESRD appears to be related to overproduction rather than disrupted renal clearance. In addition to their predictive role in ESRD, KRIS proteins provide a potential therapeutic intervention point as evidenced by a clinical trial conducted in one of those three cohorts demonstrating decline in albuminuria consistent with the decline in KRIS proteins in response to 24-week trial with 4 mg of baricitinib, a JAK-1/2 inhibitor [45]. Additionally, certain metabolites of nicotinamide adenine dinucleotide (NAD) have recently been correlated with acute kidney injury (AKI) risk [46].

There are multiple ongoing clinical trials (NCT01550393, NCT02743273, NCT00690586, NCT04851145) investigating the role of proteomics and metabolomics in the field of nephrology. However, these trials should demonstrate that proteomics or metabolomics biomarkers offer additional information over conventional biomarkers that are clinically relevant and may change therapeutic decision-making in a cost-effective manner. It is important to emphasize that studies investigating novel biomarkers need not only a detection cohort but also an independent (second) validation cohort.

In addition, multiple novel biomarkers have been identified recently, sometimes using proteomics, although they are in diverse stages of translation to the clinic [47]. More than 40 potential biomarkers have emerged in recent years and most can be sorted based on their association with features such as glomerular injury [podocalyxin (PCX)], tubular injury [kidney injury molecule 1 (KIM-1)], inflammation [tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), TNF receptors-1 and -2] [48] and oxidative stress [8-oxo-7,8-dihydro-2'-deoxyguanosine (8-OHdG)] [47]. CKD biomarkers can be identified by a variety of conventional methods such as solid-phase fluorescent immunoassay, liquid chromatography-mass spectrometry, liquid chromatography-mass spectrometry, high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) [47]. Additionally, microfluidics allow for short sample processing times along with a small footprint, automated operation and a high degree of flexibility, and may potentially offer a robust, cost-effective and simple-to-operate instrument for early diagnosis of CKD and other pathological events [47].

Membranous nephropathy is the most common cause of nephrotic syndrome in adults and in hematopoietic stem cell transplant (HST) patients. A flurry of autoantigens has been identified recently, following the description of anti-phospholipase A2 receptor (PLA2R) antibodies, a biomarker that according to guidelines can now replace kidney biopsy in patients with membranous nephropathy [49]. Anti-protocadherin FAT1 antibodies are found in over 80% of cases of membranous nephropathy following HST [50]. Patients with anti-PLA2R antibody-negative primary membranous nephropathy may have anti-thrombospondin type-1 domain-containing 7A antibodies [51, 52]. Additionally, anti-nephrin antibodies have been detected via serological and immunohistochemical studies in a subset of biopsy-proven minimal change disease patients [53].

The field of biomarkers of AKI is also very active; urinary insulin-like growth factor-binding protein-7 (IGFBP-7) and tissue inhibitor of metalloprotease-2 (TIMP-2) testing are now in clinical use under the brand name Nephrocheck [47, 54]. Kidney tubular injury results in the release of blood and urine biomarkers [55]. Urinary KIM-1, liver-type fatty acid binding protein (L-FABP), IGFBP-7 and TIMP-2 are released from the proximal tubule, whereas uromodulin (UMOD) is secreted from the loop of Henle and neutrophil gelatinase-associated lipocalin (NGAL) originates from distal tubules [55]. These biomarkers could potentially localize specific segments of injured tubules. Biomarkers involved in inflammation, repair and fibrosis [55] could also predict the transition from AKI to CKD, help distinguish between kidney dysfunction and injury, guide AKI management, and improve the diagnosis of diseases such as acute interstitial nephritis [55]. Furthermore, some biomarkers are ready for use in clinical trials of AKI and could guide management in certain clinical settings. The Kidney Precision Medicine Project is an ongoing effort to build a kidney tissue atlas and increase the use of biomarkers to evaluate nephron health [55].

### RNA biomarkers

microRNAs (miRNA) are stable molecules and there are several examples of their potential use in the context of kidney injury, including drug-induced kidney injury (DIKI) [56]. A cross-laboratory program to identify urinary miRNA patterns associated with cell- or cause-specific DIKI characterized biomarkers of glomerular, proximal tubule, thick ascending limb (TAL) of the loop of Henle or collecting duct (CD) injury in rats by exposing them to cell-specific toxins and confirming the location of increased expression by laser capture microdissection of nephron segments [56]. Urinary miR-192-5p was identified as potentially proximal tubule-specific. Urinary miR-221-3p, miR-222-3p and miR-210-3p increased following exposure to TAL toxins, and miR-23a-3p following the podocyte toxin doxorubicin. Thus, urinary miRNA panels from different nephron regions may contribute to the exploring the DIKI potential of novel drugs [56].

### Cell-free DNA

Cell-free DNA methods have increasingly been used, along with other methods, especially in the field of hematology and oncology to detect malignancies and evaluate recurrences. Similarly, they have potential for use in the detection of renal cell carcinoma [57]. Though it is too early to draw conclusions, there is potential for cell-free DNA to be used in the detection of acute rejection in kidney transplant recipients [58, 59]. Additionally, high levels of cell-free DNA were associated with adverse outcomes

in one study of 131 CKD patients not on dialysis and in another study conducted on 289 patients on hemodialysis [60, 61]. Additionally, cell-free DNA was associated with AKI after cardiac surgery or in patients with type 1 cardiorenal syndrome [62, 63]. However, these data should be validated in large scale multi-center clinical studies that address the added benefit on patient management of assessing cell-free DNA.

## NOVEL THERAPEUTIC ALTERNATIVES

Novel therapeutic alternatives for ESRD include wearable artificial kidneys, xenotransplantation, stem cell-based therapy, and bioengineered and bio-artificial kidneys. Of note, one of the main objectives of these novel therapeutic approaches should be to maintain patients at home and to avoid dialysis centers. Additionally, novel medications to prevent CKD progression or treat CKD complications are at advanced stages of clinical development or have already been approved for clinical use in some countries (Fig. 3).

The main aim for the development of novel therapeutic markers is to create a time frame for earlier treatment with possible reversal of disease or prevention of disease progression. Even though current therapeutic alternatives are mostly unbeneficial in this regard, novel therapeutic modalities have been developed and testes along with the diagnostic modalities in hope of reach that goal.

### Wearable artificial kidneys

Existing hemodialysis is an imperfect treatment. It is socially obtrusive to the patient, necessitating them to take several days out of the week to undergo a long treatment using bulky equipment requiring significant water and electricity supply. Also, because it only occurs 3 days a week, it is at best a very imperfect approximation of the continuously functioning normal kidneys [64]. Thus, current hemodialysis modalities cannot be considered green techniques and are associated with dismal outcomes [6].

The wearable artificial kidney (WAK) is an innovative approach to renal replacement therapy aiming to improve patients' quality of life by allowing mobility and continuous clearance of toxins without accumulation during inter-dialysis periods, with better hemodynamic stability, similar to healthy kidneys. The minimum requirements for the dialysis system of a WAK are pumping systems, a dialysate regeneration system, dialysis membrane, batteries and a patient monitoring system [65]. The initial effort for ambulation during hemodialysis sessions consisted of a WAK system enabling ambulation for up to one-third of the dialysis session [66]. Nevertheless, it lacked enough sorbent for full time treatment and patients had to be connected to a classical hemodialysis device for at least two-thirds of the session duration. Next, another WAK system was developed that had sorbent-containing minicartridges instead of requiring large amounts of dialysate; however, this system was limited by the requirement of minicartridges change four times daily, and by high infection risk due to transcutaneous arteriovenous shunts/fistula [67]. A few other WAK designs over the years did not undergo clinical trials [66, 68, 69]. Current WAK models weigh under 5 kg and have long-lasting small batteries that avoid the need for continuous electrical connection, improved permeability membranes and advanced filtration materials that enable the reuse of dialysate solutions without needing large quantities of water as in conventional hemodialysis [70]. Among the five different WAK models currently under

## Novel Therapeutic Alternatives

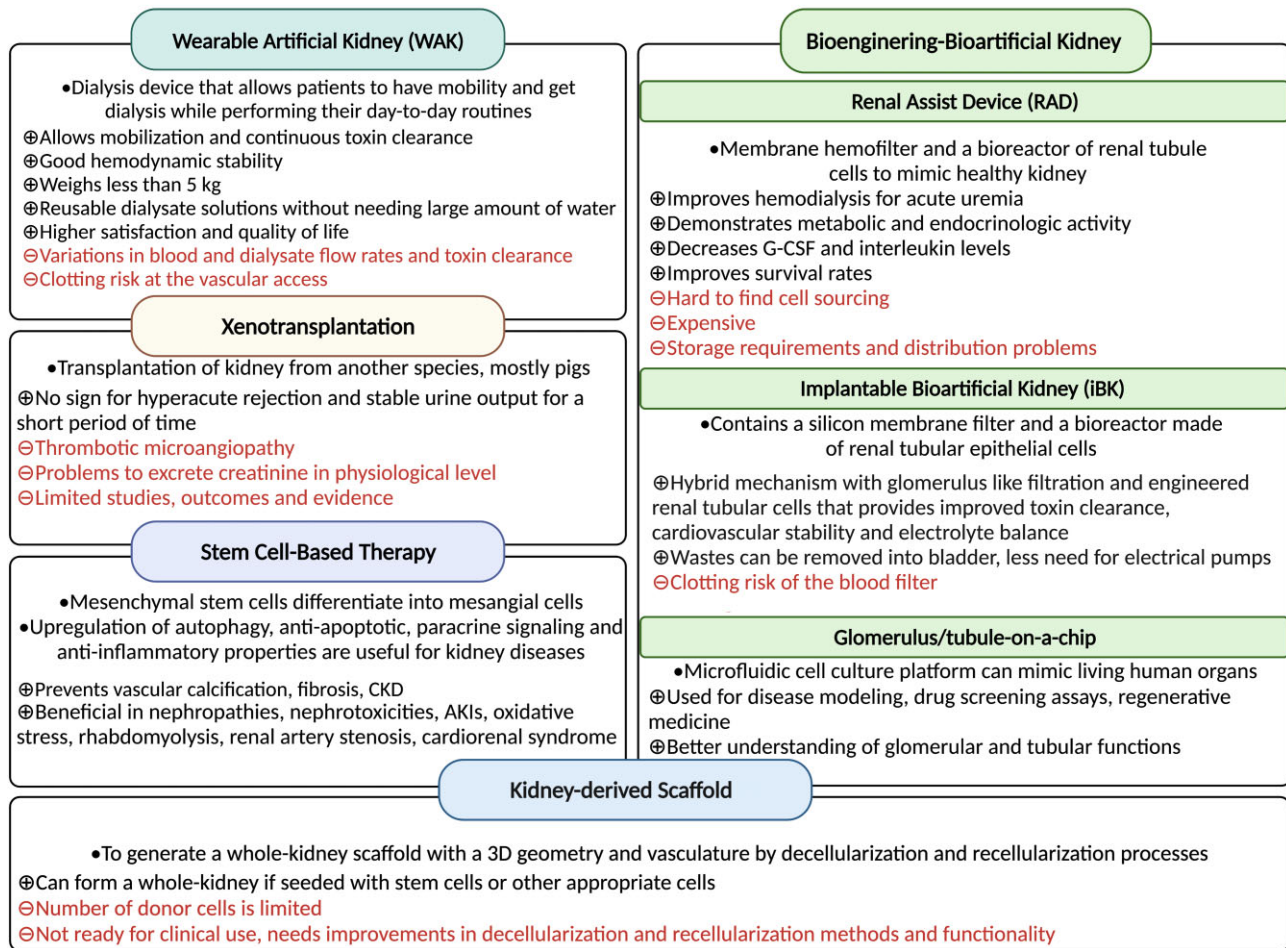


Figure 3: Novel therapeutic alternatives in nephrology. G-CSF: granulocyte colony stimulating factor. plus sign: advantages; minus sign: disadvantages/limitations (indicated in red color).

development, three are based on peritoneal dialysis, one on hemodialysis and one is a combined model [71, 72].

Only a single human clinical trial (prospective, non-randomized) involving 10 subjects with a duration of 24 h has been conducted regarding the efficiency and adverse effects of WAK [73]. No serious adverse events such as hemodynamic instability were observed and overall toxin clearance was achieved, but the trial was terminated because of the development of excessive amounts of carbon dioxide air bubbles. The targeted ultrafiltration rates were achieved with a mean blood flow rate of  $42 \pm 24$  mL/min and a mean dialysate flow rate of  $43 \pm 20$  mL/min. Additionally, self-reported treatment satisfaction and treatment-related quality of life were higher than for traditional hemodialysis sessions. Major limitations in addition to carbon dioxide bubbles were the considerable variations in blood and dialysate flow rates and in toxin clearance. Additionally, a pilot study with eight patients demonstrated similar outcomes [74]. Targeted ultrafiltration rates were achieved with a mean blood flow of  $58.6 \pm 11.7$  mL/min and mean dialysate flow of  $47.1 \pm 7.8$  mL/min without significant hemodynamic adverse effects. Two patients experienced clotting at the vascular access despite the use of heparin similar to hemodialysis sessions. In summary, WAK systems are promising but their qual-

ity should be improved and confirmed in longer and larger scale studies.

### Xenotransplantation: an old concept revisited

Xenotransplantation was first postulated in 1667. However, clinical trials in the early 20th century were marred by limited success due to high rates of rejection, thrombotic complications and infection [75–77]. Given the ever-expanding waiting lists for kidney transplantation, which is now over 100 000 patients compared with a much lower number (~35 000) of annual kidney transplantations performed in the USA, the concept of xenotransplantation has re-gained clinical and research attention [78]. CRISPR/Cas9 and advances in the field of bioengineering have allowed xenotransplantation across non-human species with higher success and lower complication rates [79]. Pigs are the common choice as a source for kidneys due to their short maturation period, low risk of zoonosis, advancement in bioengineering studies, and relative physiology and size similarity to humans. Two 2022 clinical reports have been promising [80, 81]. In a brain-dead patient, both kidneys were transplanted from pigs with 10 genetic modifications aimed at preventing



rejection. Despite the lack of hyperacute rejection and stable urine output for 72 h until the termination of the trial, the transplanted kidneys were unable to excrete creatinine at physiological levels and histological examination revealed evidence for thrombotic microangiopathy with no evidence of cellular rejection or deposition of antibody or complement proteins [80]. In two further cases of genetically modified pig-to-human kidney transplantations, GFR significantly improved within minutes after transplantation [81]. Kidney biopsies performed at the 6th, 24th, 48th and 54th hours post-transplantation revealed no signs of a hyperacute or antibody-mediated rejection reaction. However, the follow-up period was limited to 54 h [81]. Overall, these cases indicate that prevention of antibody-mediated hyperacute rejection is feasible. However, the recent death of the recipient of the first ever heart transplant from genetically modified pigs, likely from a porcine virus and after initial apparent success of the procedure, adds a note of caution to the current limitations of our understanding of the procedure and its complications.

An alternative strategy would entail human organ generation in animals whose organ size, anatomy and physiology are closer to humans (e.g. in pigs) [82]. With the demonstration of potential interspecies organ generation with the use of xenogenic pluripotent stem cells, as shown by rat pluripotent stem cell-derived on organogenesis-disabled mice via blastocyst complementation method, the potential role for xenotransplantation has been questioned for human subjects without any clinical or pre-clinical study in that field. In any case, the ethical issues posed by xenotransplantation remain controversial.

### Stem cell-based therapy: an alternative approach on the rise

Stem-cell based therapies became popular after the discovery of induced pluripotent stem cells by Yamanaka and colleagues in 2006 [83]. Pluripotent stem cells have the potential to differentiate into almost all cell types in the body and are derived from somatic cells by the introduction of Yamanaka factors (four specific genes encoding transcription factors, namely Oct3/4, Klf4, Sox2 and Myc) [84]. Stem-cell-based therapies have been on the rise in the last few decades. Multipotent stromal cells are the choice so far and are derived from bone marrow (BM-MSCs), amniotic fluid (AFSCs), urine (USCs) or umbilical cord (UC-MSCs) [85]. Mesenchymal stem cells have been shown to differentiate into mesangial cells when they are cultured with injured mesangial cells *in vitro* [86]. The main mechanisms of stem cell therapy in kidney diseases include upregulation of autophagy, anti-apoptotic properties, paracrine signaling and anti-inflammatory properties. Extracellular vesicles secreted from stem cells may prevent high phosphorus-induced vascular calcification in CKD patients and may prevent fibrosis and the resultant CKD in unilateral ureteral obstruction through paracrine mechanisms [87, 88]. Stem cell therapy protects from cisplatin-induced nephrotoxicity, AKI, diabetic nephropathy and oxidative stress through the activation of autophagy [89, 90]. Anti-apoptotic and anti-inflammatory properties of stem cells are beneficial for the treatment of rhabdomyolysis or chromium- or adriamycin-induced AKI, diabetic nephropathy, renal artery stenosis, cardiorenal syndrome, lupus nephritis and obesity-induced AKI [91–100]. Few clinical trials are yet to investigate the safety, efficiency and applicability of stem cell therapy in human subjects with CKD, though results appear promising as shown by a placebo-controlled clinical trial conducted on 200 Chinese patients with CKD and eGFR below 60 mL/min/1.73 m<sup>2</sup> demonstrating efficiency of bone marrow-derived mesenchymal stem cell therapy

as intravenous infusion in terms of serum creatinine, cystatine C and reactive oxygen species after 8 weeks of therapy [101].

Kidney organoids may be produced from pluripotent stem cells. Efficient bioengineering protocols differentiate human pluripotent stem cells into multipotent nephron progenitors and, hopefully in the end, functional kidneys. Kidney organoids formed in this procedure are organized into nephron-like structures and express markers for podocytes, proximal tubules, loops of Henle and distal tubules [102–104]. Organoids may also be considered to develop kidney disease models. For now, they are not able to mature beyond the second trimester stage. Eventually, progress in this field is aimed at contributing to renal replacement therapies in the future.

Finally, kidney rejuvenation strategies have been envisioned using Yamanaka factors *in vivo* (e.g. through kidney artery catheterization) or *ex vivo* (e.g. for kidneys from older transplant donors) [105].

### Bioengineered and bio-artificial kidneys

The concept of bioengineering an artificial kidney has been studied for at least three decades [106]. The bio-artificial Renal Assist Device (RAD) was a hybrid system where a multi-fiber bioreactor consisting of the synthetic hollow fibers of a high-flux hemofiltration cartridge combined with porcine primary tubular epithelial cells [107]. The RAD conducted active transport (differential reabsorption and secretion) as well as metabolic and endocrine functions [108], and improved hemodialysis performance in acutely uremic dogs [109]. In two clinical trials in the intensive care unit setting, the RAD maintained viability, durability and functionality in the clinical setting, and performed metabolic and endocrinologic activity [108]. All but one treated patient improved 1–7 days following therapy, and 6 of the 10 patients survived past 30 days. One patient died within 12 h of RAD because of voluntary withdrawal from ventilatory life support and three other patients expired due to complications of comorbidities unrelated to AKI or RAD therapy. Treatment with RAD decreased granulocyte colony stimulating factor (G-CSF), interleukin (IL)-6, IL-10 and IL-6/IL-10 ratios [108]. A second study compared continuous venovenous hemofiltration together with RAD in 40 patients versus conventional continuous renal replacement therapy (CRRT) alone in 18 patients, observing a mortality rate of 33% in the RAD group and 61% in the CRRT group at Day 28 [110]. Furthermore, survival through Day 180 was significantly higher in the RAD group and the risk for death was approximately 50% of that in the CRRT group [110].

The RAD is the only bio-artificial kidney device tested in humans. There were limitations to its widespread use for kidney disease such as cell sourcing, times and costs of device manufacturing, storage requirements and distribution related problems [107]. The Bioartificial Renal Epithelial Cell System (BRECS) is an alternative technology, based on niobium-coated carbon and cryopreservable polycarbonate seeded with human renal tubular epithelial cells derived from adult progenitor cells [111–113]. BRECS showed promising results in a porcine septic shock model [113] and in an anephric sheep model [114]. However, BRECS is yet to be tested in human trials but there are no publications since 2017.

Implantable bioartificial kidneys (iBKs) combine the previous experiences with RAD bioengineering and the new developments in miniaturization technology [115]. Similar to wearable artificial kidneys, iBK is potentially capable of providing continuous dialysis throughout the day [116]. The iBK is composed of a mechanical blood filter made with a silicon

membrane and a bioreactor containing engineered renal tubular epithelial cells making it a hybrid device capable of increased toxin clearance with improved cardiovascular stability and a higher quality of life. The bioreactor is responsible for electrolyte balance as well as metabolic functions and the silicon membrane provides glomerulus-like filtration. The iBK is intended to connect into the patient's systemic circulation allowing the filtered waste to be removed directly into the bladder, thus eliminating the need for electrical pumps [116]. The iBK is currently in preclinical testing. The silicon cartridge was patent without an electrical pump or anticoagulants for up to 1 month in canine models [116, 117]. One of the major drawbacks of iBK could be the durability and clotting of the blood filter, requiring patients to undergo frequent surgeries to manipulate or change the device. In addition, the tubular cells would need to maintain stability and viability against the high blood shear force that would be encountered in clinical use [115]. The bioengineered artificial kidney, despite its limitations and drawbacks, could become a potential alternative to renal replacement therapy and transplantation.

An organ-on-a-chip is a microfluidic cell culture platform that mimics the activities, mechanics and physiology of living human organs. The microfluidics system offers a physiological cell microenvironment allowing long-term culturing while maintaining cell phenotypes [116]. The organ-on-a-chip technology has provided the development of more advanced three-dimensional (3D) organ-level structures *in vitro* and the incorporation of dynamic mechanical and chemical signals [107]. It has been used to replicate tubular or glomerular structures. Various glomerulus-on-a-chip and tubule-on-a-chip models have been utilized for disease modeling, drug screening assays and *in vivo* regenerative medicine applications [116]. The glomerulus-on-a-chip models the glomerular filtration barrier as both physical forces and chemical stimuli influencing glomerular cell functions can be modulated [107]. In the tubule-on-a-chip, tubular cells that are cultured in a 3D channel that represent the microenvironment of human kidney tubules and their functions of reabsorption and secretion [107].

The chip-based technology has helped conduct experiments to better understand glomerular and tubular functions. Future studies to produce next-generation chips combining both glomerular and tubular elements to generate a functional nephron that incorporates both filtration and reabsorption are necessary [116].

A further bioengineering technology aims to generate a whole-kidney scaffold with a 3D geometry and vasculature [118]. After the decellularization of a kidney gathered from a patient with CKD, the recellularization of the scaffold with patient-specific progenitor cells would potentially result in the generation of a new transplantable organ [118]. Kidney-derived scaffolds are obtained by a process called 'decellularization' in which detergents and enzymes remove the cellular components without affecting the extracellular matrix (ECM) [107, 119, 120]. The re-cellularization of the acellular matrix of the kidney is then performed via different cell seeding processes [107]. Biological scaffolds allow signal exchange between the matrix and the cells resulting in the induction of migration, proliferation and differentiation [107]. However, the number of donor cells is limited, and proper tissue/organ function requires advancements in the re-cellularization technology [107]. Therefore, the correct repopulation and alignment of billions of cells for proper kidney function is still in its early stages of development.

The use of either epithelial cells in combination with endothelial cells or pluripotent stem cells that have the potential

to differentiate into every other cell type has yielded the most promising results for kidney re-cellularization [107]. Song *et al.* reported that the infusion of human umbilical venous endothelial cells and rat neonatal kidney cells in a rat kidney scaffold showed promising results [96]. After the decellularization of rat, porcine and human kidneys by detergents the authors seeded rat kidney scaffolds with epithelial and endothelial cells and perfused these constructs in a whole-organ bioreactor to regenerate functional tissue. This novel structure produced rudimentary urine both *in vitro* and *in vivo* following transplantation into rats [121].

Even though Song *et al.* and several other authors [122, 123] have reported promising findings, the translation of kidney-derived scaffolds into the clinics still requires further technological advancements such as improvements in decellularization methods to avoid the disruption of the remaining matrix and strategies to prevent the host immunological response to the decellularized scaffold [107].

### Medications on the rise

In addition to the kidney and cardioprotective effects of SGLT2 inhibitors and GLP1 receptor agonists, novel drugs have been recently approved or have provided promising results in clinical trials for kidney protection (nonsteroidal MRAs, endothelin receptor antagonists), treatment of consequences of CKD [hypoxia-inducible factor (HIF) stabilizers] or specific causes of CKD, such as voclosporin for lupus nephritis (Table 1).

### Nonsteroidal MRA

Finerenone is a non-steroidal selective MRA that has emerged as a kidney and cardiovascular protective medication in patients with diabetic kidney disease on angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB) that may also add protection to that offered by SGLT2 inhibitors [124]. A double-blind clinical trial with a median follow-up period of 3.4 years and conducted on 7437 subjects with type 2 diabetes mellitus and CKD with eGFR of 25–90 mL/min/1.73 m<sup>2</sup> and microalbuminuria demonstrates that finerenone improved cardiovascular outcomes including hospitalization for heart failure, myocardial infarction, stroke and death from cardiovascular cause [125]. Additionally, the risk for CKD progression has been reduced with finerenone [126, 127]. Further analyses confirmed a beneficial impact of finerenone on proteinuria, CKD progression and cardiovascular outcomes [128, 129]. Indeed, finerenone has been approved by the US FDA and the European Medicines Agency (EMA) for the treatment of CKD G3 and G4 with albuminuria in type 2 diabetes in adults [130]. Given the mechanism of action of finerenone, its beneficial impact is expected to extend beyond diabetic kidney disease to other forms of CKD. In addition, other non-steroidal selective MRAs, such as apararenone (MT-3995), KBP-5074 and AZD9977, are undergoing clinical trials while esaxerenone (CS3150) is approved in Japan for the treatment of hypertension [131]. Of note, as with other agents which antagonize the renin-angiotensin-aldosterone system hyperkalemia is a risk and should be kept in mind while utilizing these medications.

Aldosterone synthase inhibitors, mainly the inhibitor of aldosterone synthase enzyme or CYP11B2, have been investigated for their potential role in the treatment of heart failure, hypertension and kidney diseases [132, 133]. LCI699, the first orally administered aldosterone synthase inhibitor (ASI), has shown to lead a reduction in plasma aldosterone concentration along



Table 1: Medications that can be potentially used in nephrology.

Medication	Mechanism of action	Advantages
Finerenone	Non-steroidal selective MRA	<ul style="list-style-type: none"> <li>• Better cardiovascular outcomes:</li> <li>• Decreases hospitalization for HF, MI, stroke and death risk</li> <li>• Reduces CKD progression</li> <li>• Declines proteinuria</li> </ul>
LCI699, fadrozole, RO6836191	Aldosterone synthase/CYP11B2 inhibitors	<ul style="list-style-type: none"> <li>• Lead to reduction in plasma aldosterone concentration along with an increase in plasma renin and deoxycortisol concentration</li> <li>• Beneficial in the treatment of primary hypertension</li> </ul>
Sparsentan, sitaxentan, zibotentan	Endothelin receptor antagonists	<ul style="list-style-type: none"> <li>• Can be used to decrease proteinuria and kidney progression in CKD, FSGS, patients with scleroderma</li> </ul>
Daprodustat	HIF prolyl hydroxylase inhibitor (HIF stabilizer)	<ul style="list-style-type: none"> <li>• Similar efficiency and cardiovascular event risk with darbepoetin alfa or epoetin alfa in anemia treatment</li> <li>• Can be administered orally</li> </ul>
Voclosporin	Calcineurin inhibitor	<ul style="list-style-type: none"> <li>• Voclosporin with MMF and low-dose steroids shows better kidney outcomes versus MMF and low-dose steroids</li> </ul>

HF: heart failure; MI: myocardial infarction; MMF: mycophenolate mofetil; FSGS: focal segmental glomerulosclerosis.

with an increase in plasma renin and deoxycortisol concentration [134]. Additionally, it has shown to be beneficial in the treatment of primary hypertension. Similar effects have been observed with others ASIs such as fadrozole and RO6836191 in phase 1 and 2 clinical trials [135, 136]. Future large-scale human randomized clinical trials are required for better understanding of the clinical effects of ASIs and potential integration into clinical practice.

### Endothelin receptor antagonists

Endothelin receptor antagonists (ERAs) have a kidney protective effect on CKD and proteinuria but are marred by sodium retention and volume overload as a key safety issue. Bosentan and atrasentan are older ERA drugs that displayed some kidney protective actions in patients with CKD [137–139]. However, the pivotal phase 3 trial of atrasentan for diabetic kidney disease was terminated and despite kidney protection, it did not offer the cardiovascular protection associated with SGLT2 inhibition even though patients were carefully selected to minimize the adverse impact of sodium retention [139, 140]. Sparsentan is both an endothelin receptor-A inhibitor and an angiotensin receptor blocker and was superior to irbesartan in reducing proteinuria in patients with focal segmental glomerular sclerosis, without any significant adverse effect [141]. A clinical trial in immunoglobulin A nephropathy is ongoing (NCT03762850). Sitaxentan reduced proteinuria and pulse wave velocity compared with placebo in non-diabetic patients with proteinuria [142]. Another clinical trial investigating zibotentan in patients with scleroderma demonstrated beneficial effects on kidney disease progression [143]. Since the main concern for ERA is sodium retention, it may be hypothesized that co-administration with SGLT2 inhibitors may improve the safety profile and potentially enhance kidney protection. Indeed, a phase 2 clinical trial of zibotentan and dapagliflozin for the treatment of CKD (ZENITH-CKD Trial) is ongoing (NCT04724837). Sodium retention is a

major problem in ERA, however patients on these medications are reported to respond well to diuretic therapy. It is important to understand that the observed sodium—and water—retention using ERA is attributed to the effect of ERA-B blockade and that selective ERA-A blockers are non-selective at the peak levels. [144, 145] More selective ERA-A blockade might be the future and is currently under investigation.

### HIF stabilizers

HIF prolyl hydroxylase inhibitors, also termed HIF stabilizers, are oral agents to treat CKD-associated anemia. Indeed, their major advantage over erythropoietin (EPO) analogs is the oral route of administration instead of subcutaneous injections. Some of them are in clinical use in China and Japan, and roxadustat was recently approved by the EMA. However, the FDA recently rejected roxadustat and vadadustat based on safety concerns. HIF stabilizers do not appear to preserve kidney function and their cardiovascular safety record has been inconclusive regarding blood pressure effects and cardiac hypertrophy. Although it was initially hypothesized that by providing more stable and lower EPO levels, they may be safer than current EPO derivatives, the cardiovascular safety appears to depend on the population being studied and the type of HIF stabilizer being utilized for as yet unclear reasons. Thus, roxadustat may even be safer than EPO derivatives in non-dialysis and incident dialysis patients [hazard ratio (95% confidence interval) 0.79 (0.61, 1.02), 0.78 (0.62, 0.98) and 0.78 (0.57, 1.05) for major adverse cardiovascular events (MACE), MACE+ and all-cause mortality, respectively] [146] while the most recent phase 3 clinical trials in dialysis and non-dialysis patients randomized to either daprodustat or injectable erythropoiesis-stimulating agents (ESA) treatment evidenced an influence of the geographic region regarding cardiovascular safety: daprodustat was safer than ESA in Western Europe and Asia [147, 148]. The dialysis trial randomized 2964 dialysis patients to either daprodustat or injectable ESA treatment and

showed that daprodustat was non-inferior to either darbepoetin alfa or epoetin alfa from an efficacy point of view [147]. Similar findings have also been established over the course of few other large scale clinical trials [149–152]. In 3872 CKD patients not undergoing dialysis, a randomized clinical trial with a median follow-up of 1.9 years demonstrated that daprodustat was also non-inferior to darbepoetin alfa for the treatment of anemia without any overall change in major cardiovascular event risk [148]. Such finding has been validated in another clinical trial [153]. HIF stabilizers are novel agents that require further investigation with large-scale studies covering different populations.

### Voclosporin

A randomized double-blind and placebo-controlled phase 3 clinical trial randomized 357 subjects with biopsy-proven class III-IV-V active lupus nephritis to receive either placebo or voclosporin, a novel calcineurin inhibitor, with background mycophenolate mofetil (MMF) and rapidly tapered low-dose corticosteroids. Voclosporin provided better kidney outcomes including urine protein to creatinine ratio and eGFR without additional adverse effects [154]. Another clinical trial validated the superior kidney outcomes (complete renal remission) both in low and high dose (23.7 mg or 39.5 mg, each twice daily, respectively) voclosporin plus MMF (2 g/day) groups as compared with placebo plus MMF, while demonstrating a higher risk for adverse events including deaths [155]. Surprisingly, more deaths were observed in the low-dose than in the placebo or high-dose groups (11.2%, 1.1% and 2.3%, respectively). Voclosporin recently became the first drug approved by the FDA with an indication for active lupus nephritis.

### CONCLUSION

In conclusion, multiple advances in several fronts of the fight against kidney disease are likely to result in earlier diagnosis and intervention, increasing the chances of long-term success, more sustainable and patient-friendly modes of kidney replacement therapy, and novel interventions to delay CKD progression and improve the management of CKD complications. Up until the development of new models for diagnostics and therapeutics it is crucial to define and identify the target groups and the patients at those groups. We believe that with the development and clinical use of diagnostics markers patients may get diagnosed earlier in the disease course, potentially at a reversible state. Furthermore, novel therapeutic alternatives have a potential to slow the progression of certain disease, reduce the complications of CKD and need for RRT or kidney transplantation. However, further large-scale clinical studies with credible outcomes are required before clinical use of such therapeutic and diagnostic modalities and it is difficult to pinpoint a timeline for such developments.

### FUNDING

Research by A.O. is supported by IS/Fondos FEDER (PI18/01 366, PI19/00 588, PI19/00 815, DTS18/00 032, ERA-PerMed-JTC2018 (KIDNEY ATTACK AC18/00 064) and PERSTIGAN AC18/00 071, ISCIII-RETIC REDinREN RD016/0009), Sociedad Española de Nefrología, FRIAT, Comunidad de Madrid en Biomedicina B2017/BMD-3686 CIFRA2-CM, Instituto de Salud Carlos III (ISCIII) RICORS program to RICORS2040 (RD21/0005/0001) and SPACKDC PMP21/00 109, FEDER funds. RD16/0009.

### AUTHORS' CONTRIBUTIONS

S.C., C.T., F.Y. and M.K. contributed substantially to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work. M.J.S, A.C., A.O. and M.K. drafted the work or revised it critically for important intellectual content.

### ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

### DATA AVAILABILITY STATEMENT

No new data were generated or analyzed in support of this research.

### CONFLICT OF INTEREST STATEMENT

A.O. has received grants from Sanofi, and consultancy or speaker fees or travel support from Advicciene, Astellas, AstraZeneca, Amicus, Amgen, Fresenius Medical Care, GSK, Bayer, Sanofi-Genzyme, Menarini, Mundipharma, Kyowa Kirin, Alexion, Freeline, Idorsia, Chiesi, Otsuka, Novo-Nordisk, Sysmex and Vifor Fresenius Medical Care Renal Pharma, and is Director of the Catedra Mundipharma-UAM of diabetic kidney disease and the Catedra Astrazeneca-UAM of chronic kidney disease and electrolytes. A.O. is the previous Editor-in-Chief of CKJ. M.K. is member of the CKJ Editorial Board. M.J.S. is the current Editor-in-Chief of CKJ. The other authors declare that they have no conflict of interest.

### REFERENCES

- Jager KJ, Kovesdy C, Langham R et al. A single number for advocacy and communication-worldwide more than 850 million individuals have kidney diseases. *Nephrol Dial Transplant* 2019;34:1803–5. <http://dx.doi.org/10.1093/ndt/gfz174>
- Johansen KL, Chertow GM, Foley RN et al. US Renal Data System 2020 annual data report: epidemiology of kidney disease in the United States. *Am J Kidney Dis* 2021;77:A7–8. <http://dx.doi.org/10.1053/j.ajkd.2021.01.002>
- Kramer A, Pippias M, Noordzij M et al. The European Renal Association – European Dialysis and Transplant Association (ERA-EDTA) registry annual report 2015: a summary. *Clin Kidney J* 2018;11:108–22. <http://dx.doi.org/10.1093/ckj/sfx149>
- Heaf J. Current trends in European renal epidemiology. *Clin Kidney J* 2017;10:149–53. <http://dx.doi.org/10.1093/ckj/sfw150>
- Hart A, Lentine KL, Smith JM et al. OPTN/SRTR 2019 annual data report: kidney. *Am J Transplant* 2021;21 Suppl 2:21–137. <http://dx.doi.org/10.1111/ajt.16502>
- Ortiz A, et al. Asociación Información Enfermedades Renales Genéticas (AIRG-E), European Kidney Patients' Federation (EKPF) RICORS2040: the need for collaborative research in chronic kidney disease. *Clin Kidney J* 2022;15: 372–87. <http://dx.doi.org/10.1093/ckj/sfab170>
- Mehrotra R, Devuyt O, Davies SJ et al. The current state of peritoneal dialysis. *J Am Soc Nephrol* 2016;27:3238–52. <http://dx.doi.org/10.1681/ASN.2016010112>

8. Perez-Gomez MV, Bartsch LA, Castillo-Rodriguez E et al. Clarifying the concept of chronic kidney disease for non-nephrologists. *Clin Kidney J* 2019;12:258–61. <http://dx.doi.org/10.1093/ckj/sfz007>
9. Fernandez-Fernandez B, Sarafidis P, Kanbay M et al. SGLT2 inhibitors for non-diabetic kidney disease: drugs to treat CKD that also improve glycaemia. *Clin Kidney J* 2020;13:728–33. <http://dx.doi.org/10.1093/ckj/sfaa198>
10. Gorostidi M, Sanchez-Martinez M, Ruilope LM et al. Chronic kidney disease in Spain: prevalence and impact of accumulation of cardiovascular risk factors. *Nefrologia (Engl Ed)* 2018;38:606–15. <http://dx.doi.org/10.1016/j.nefro.2018.04.010>
11. Ruilope LM, Ruiz-Hurtado G, Miranda B et al. Use of chronic kidney disease blind spot to prevent cardiorenal outcomes. *Eur Heart J* 2022;43:257–60. <http://dx.doi.org/10.1093/eurheartj/ehab456>
12. Sanchez-Nino MD, Sanz AB, Ramos AM et al. Clinical proteomics in kidney disease as an exponential technology: heading towards the disruptive phase. *Clin Kidney J* 2017;10:188–91. <http://dx.doi.org/10.1093/ckj/sfx023>
13. Sanchez-Nino MD, Fernandez-Fernandez B, Ortiz A. Klotho, the elusive kidney-derived anti-ageing factor. *Clin Kidney J* 2020;13:125–7. <http://dx.doi.org/10.1093/ckj/sfz125>
14. Bennett KM, Baldelomar EJ, Charlton JR. Delivering on the potential of measuring nephron number in the clinic. *Nat Rev Nephrol* 2022;18:271–2. <http://dx.doi.org/10.1038/s41581-022-00560-5>
15. Luyckx VA, Rule AD, Tuttle KR et al. Nephron overload as a therapeutic target to maximize kidney lifespan. *Nat Rev Nephrol* 2022;18:171–83. <http://dx.doi.org/10.1038/s41581-021-00510-7>
16. Baldelomar EJ, Charlton JR, Beeman SC et al. Measuring rat kidney glomerular number and size in vivo with MRI. *Am J Physiol Renal Physiol* 2018;314:F399–f406. <http://dx.doi.org/10.1152/ajprenal.00399.2017>
17. Puelles VG, Combes AN, Bertram JF. Clearly imaging and quantifying the kidney in 3D. *Kidney Int* 2021;100:780–6. <http://dx.doi.org/10.1016/j.kint.2021.04.042>
18. Xie L, Koukos G, Barck K et al. Micro-CT imaging and structural analysis of glomeruli in a model of adriamycin-induced nephropathy. *Am J Physiol Renal Physiol* 2019;316:F76–89. <http://dx.doi.org/10.1152/ajprenal.00331.2018>
19. Baldelomar EJ, Reichert DE, Shoghi KI et al. Mapping nephron mass in vivo using positron emission tomography. *Am J Physiol Renal Physiol* 2021;320:F183–92. <http://dx.doi.org/10.1152/ajprenal.00418.2020>
20. Selby NM, Blankestijn PJ, Boor P et al. Magnetic resonance imaging biomarkers for chronic kidney disease: a position paper from the European Cooperation in Science and Technology Action PARENCHIMA. *Nephrol Dial Transplant* 2018;33:ii4–14. <http://dx.doi.org/10.1093/ndt/gfy152>
21. Grist JT, Hansen ES, Zöllner FG et al. Sodium (<sup>23</sup>Na) MRI of the kidney: basic concept. *Methods Mol Biol* 2021;2216:257–66. [http://dx.doi.org/10.1007/978-1-0716-0978-1\\_15](http://dx.doi.org/10.1007/978-1-0716-0978-1_15)
22. Zöllner FG, Konstandin S, Lommen J et al. Quantitative sodium MRI of kidney. *NMR Biomed* 2016;29:197–205. <http://dx.doi.org/10.1002/nbm.3274>
23. Maril N, Rosen Y, Reynolds GH et al. Sodium MRI of the human kidney at 3 Tesla. *Magn Reson Med* 2006;56:1229–34. <http://dx.doi.org/10.1002/mrm.21031>
24. Grist JT, Riemer F, Hansen ESS et al. Visualization of sodium dynamics in the kidney by magnetic resonance imaging in a multi-site study. *Kidney Int* 2020;98:1174–8. <http://dx.doi.org/10.1016/j.kint.2020.04.056>
25. Qi H, Nørtinger TS, Nielsen PM et al. Early diabetic kidney maintains the corticomedullary urea and sodium gradient. *Physiol Rep* 2016;4:e12714.
26. Liu J, Shelton EL, Crescenzi R et al. Kidney injury causes accumulation of renal sodium that modulates renal lymphatic dynamics. *Int J Mol Sci* 2022;23.
27. Lemoine S, Salerno FR, Akbari A et al. Tissue sodium storage in patients with heart failure: a new therapeutic target? *Circ Cardiovasc Imaging* 2021;14:e012910. <http://dx.doi.org/10.1161/CIRCIMAGING.121.012910>
28. Klinkhammer BM, Lammers T, Mottaghy FM et al. Non-invasive molecular imaging of kidney diseases. *Nat Rev Nephrol* 2021;17:688–703. <http://dx.doi.org/10.1038/s41581-021-00440-4>
29. Sanders HM, Iafisco M, Pouget EM et al. The binding of CNA35 contrast agents to collagen fibrils. *Chem Commun (Camb)* 2011;47:1503–5. <http://dx.doi.org/10.1039/C0CC02901G>
30. Megens RT, Oude Egbrink MG, Cleutjens JP et al. Imaging collagen in intact viable healthy and atherosclerotic arteries using fluorescently labeled CNA35 and two-photon laser scanning microscopy. *Mol Imaging* 2007;6:247–60. <http://dx.doi.org/10.2310/7290.2007.00021>
31. Sun Q, Baues M, Klinkhammer BM et al. Elastin imaging enables noninvasive staging and treatment monitoring of kidney fibrosis. *Sci Transl Med* 2019;11. <http://dx.doi.org/10.1126/scitranslmed.aat4865>
32. Pruijm M, Mendichovszky IA, Liss P et al. Renal blood oxygenation level-dependent magnetic resonance imaging to measure renal tissue oxygenation: a statement paper and systematic review. *Nephrol Dial Transplant* 2018;33:ii22–8. <http://dx.doi.org/10.1093/ndt/gfy243>
33. Baligand C, Qin H, True-Yasaki A et al. Hyperpolarized (<sup>13</sup>C) magnetic resonance evaluation of renal ischemia reperfusion injury in a murine model. *NMR Biomed* 2017;30:e3765. <http://dx.doi.org/10.1002/nbm.3765>
34. Bertolotto M, Bucci S, Valentino M et al. Contrast-enhanced ultrasound for characterizing renal masses. *Eur J Radiol* 2018;105:41–48. <http://dx.doi.org/10.1016/j.ejrad.2018.05.015>
35. Pontillo C, Mischak H. Urinary peptide-based classifier CKD273: towards clinical application in chronic kidney disease. *Clin Kidney J* 2017;10:192–201. <http://dx.doi.org/10.1093/ckj/sfx002>
36. Good DM, Zürgbig P, Argilés A et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. *Mol Cell Proteomics* 2010;9:2424–37. <http://dx.doi.org/10.1074/mcp.M110.001917>
37. Schanstra JP, Zürgbig P, Alkhalaf A et al. Diagnosis and prediction of CKD progression by assessment of urinary peptides. *J Am Soc Nephrol* 2015;26:1999–2010. <http://dx.doi.org/10.1681/ASN.2014050423>
38. Pontillo C, Zhang ZY, Schanstra JP et al. Prediction of chronic kidney disease stage 3 by CKD273, a urinary proteomic biomarker. *Kidney Int Rep* 2017;2:1066–75. <http://dx.doi.org/10.1016/j.ekir.2017.06.004>
39. Tofte N, Lindhardt M, Adamova K et al. Early detection of diabetic kidney disease by urinary proteomics and subsequent intervention with spironolactone to delay progression (PRIORITY): a prospective observational study and embedded randomised placebo-controlled trial. *Lancet Diabetes Endocrinol* 2020;8:301–12. [http://dx.doi.org/10.1016/S2213-8587\(20\)30026-7](http://dx.doi.org/10.1016/S2213-8587(20)30026-7)



40. Rodriguez-Ortiz ME, Pontillo C, Rodriguez M et al. Novel urinary biomarkers for improved prediction of progressive eGFR loss in early chronic kidney disease stages and in high risk individuals without chronic kidney disease. *Sci Rep* 2018;8:15940. <http://dx.doi.org/10.1038/s41598-018-34386-8>
41. Magalhaes P, Pejchinovski M, Markoska K et al. Association of kidney fibrosis with urinary peptides: a path towards non-invasive liquid biopsies? *Sci Rep* 2017;7:16915. <http://dx.doi.org/10.1038/s41598-017-17083-w>
42. Siwy J, Zurbig P, Argiles A et al. Noninvasive diagnosis of chronic kidney diseases using urinary proteome analysis. *Nephrol Dial Transplant* 2017;32:2079–89. <https://www.ncbi.nlm.nih.gov/pubmed/27984204>
43. Rudnicki M, Siwy J, Wendt R et al. Urine proteomics for prediction of disease progression in patients with IgA nephropathy. *Nephrol Dial Transplant* 2021;37:42–52. <http://dx.doi.org/10.1093/ndt/gfaa307>
44. Niewczasz MA, Pavkov ME, Skupien J et al. A signature of circulating inflammatory proteins and development of end-stage renal disease in diabetes. *Nat Med* 2019;25:805–13. <http://dx.doi.org/10.1038/s41591-019-0415-5>
45. Tuttle KR, Brosius FC III, Adler SG et al. JAK1/JAK2 inhibition by baricitinib in diabetic kidney disease: results from a Phase 2 randomized controlled clinical trial. *Nephrol Dial Transplant* 2018;33:1950–9. <http://dx.doi.org/10.1093/ndt/gfx377>
46. Poyan Mehr A, Tran MT, Ralton KM et al. De novo NAD(+) biosynthetic impairment in acute kidney injury in humans. *Nat Med* 2018;24:1351–9. <http://dx.doi.org/10.1038/s41591-018-0138-z>
47. Liu KZ, Tian G, Ko AC et al. Detection of renal biomarkers in chronic kidney disease using microfluidics: progress, challenges and opportunities. *Biomed Microdevices* 2020;22:29. <http://dx.doi.org/10.1007/s10544-020-00484-6>
48. Niewczasz MA, Gohda T, Skupien J et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol* 2012;23:507–15. <http://dx.doi.org/10.1681/ASN.2011060627>
49. Rovin BH, Adler SG, Barratt J et al. Executive summary of the KDIGO 2021 guideline for the management of glomerular diseases. *Kidney Int* 2021;100:753–79. <http://dx.doi.org/10.1016/j.kint.2021.05.015>
50. Sethi S, Madden B, Casal Moura M et al. Hematopoietic stem cell transplant-membranous nephropathy is associated with protocadherin FAT1. *J Am Soc Nephrol* 2022;33:1033–44. <http://dx.doi.org/10.1681/ASN.2021111488>
51. Couser WG. Primary membranous nephropathy. *Clin J Am Soc Nephrol* 2017;12:983–97. <http://dx.doi.org/10.2215/CJN.11761116>
52. Tomas NM, Beck LH Jr, Meyer-Schwesinger C et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 2014;371:2277–87. <http://dx.doi.org/10.1056/NEJMoa1409354>
53. Watts AJB, Keller KH, Lerner G et al. Discovery of autoantibodies targeting nephrin in minimal change disease supports a novel autoimmune etiology. *J Am Soc Nephrol* 2022;33:238–52. <http://dx.doi.org/10.1681/ASN.2021060794>
54. Chindarkar NS, Chawla LS, Straseski JA et al. Reference intervals of urinary acute kidney injury (AKI) markers [IGFBP7][TIMP2] in apparently healthy subjects and chronic comorbid subjects without AKI. *Clin Chim Acta* 2016;452:32–7. <http://dx.doi.org/10.1016/j.cca.2015.10.029>
55. Wen Y, Parikh CR. Current concepts and advances in biomarkers of acute kidney injury. *Crit Rev Clin Lab Sci* 2021;58:354–68. <http://dx.doi.org/10.1080/10408363.2021.1879000>
56. Chorley BN, Ellinger-Ziegelbauer H, Tackett M et al. Urinary miRNA biomarkers of drug-induced kidney injury and their site specificity within the nephron. *Toxicol Sci* 2021;180:1–16. <http://dx.doi.org/10.1093/toxsci/kfaa181>
57. Nuzzo PV, Berchuck JE, Korthauer K et al. Detection of renal cell carcinoma using plasma and urine cell-free DNA methylomes. *Nat Med* 2020;26:1041–3. <http://dx.doi.org/10.1038/s41591-020-0933-1>
58. Verhoeven J, Baan CC, Peeters AMA et al. Circulating cell-free nucleosomes as biomarker for kidney transplant rejection: a pilot study. *Clin Epigenetics* 2021;13:32. <http://dx.doi.org/10.1186/s13148-020-00969-4>
59. Bloom RD, Bromberg JS, Poggio ED et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol* 2017;28:2221–32. <http://dx.doi.org/10.1681/ASN.2016091034>
60. Coimbra S, Rocha S, Nascimento H et al. Cell-free DNA as a marker for the outcome of end-stage renal disease patients on haemodialysis. *Clin Kidney J* 2021;14:1371–8. <http://dx.doi.org/10.1093/ckj/sfaa115>
61. Chang CC, Chiu PF, Wu CL et al. Urinary cell-free mitochondrial and nuclear deoxyribonucleic acid correlates with the prognosis of chronic kidney diseases. *BMC Nephrol* 2019;20:391. <http://dx.doi.org/10.1186/s12882-019-1549-x>
62. Merkle J, Daka A, Deppe AC et al. High levels of cell-free DNA accurately predict late acute kidney injury in patients after cardiac surgery. *PLoS One* 2019;14:e0218548. <http://dx.doi.org/10.1371/journal.pone.0218548>
63. Virzi GM, Clementi A, Milan Manani S et al. The role of cell-free plasma DNA in patients with cardiorenal syndrome type 1. *Cardiorenal Med* 2021;11:218–25. <http://dx.doi.org/10.1159/000518553>
64. Nagasubramanian S. The future of the artificial kidney. *Indian J Urol* 2021;37:310–7. [http://dx.doi.org/10.4103/iju.IJU\\_273\\_21](http://dx.doi.org/10.4103/iju.IJU_273_21)
65. Dolson GM. The wearable artificial kidney. *Methodist Debaque Cardiovasc J* 2020;16:324–5. <http://dx.doi.org/10.14797/mdcj-16-4-324>
66. Jacobsen SC, Stephen RL, Bulloch EC et al. A wearable artificial kidney: functional description of hardware and clinical results. *Proc Clin Dial Transplant Forum* 1975;5:65–71. <https://www.ncbi.nlm.nih.gov/pubmed/1232635>
67. Murisasco A, Baz M, Boobes Y et al. A continuous hemofiltration system using sorbents for hemofiltrate regeneration. *Clin Nephrol* 1986;26 Suppl 1:S53–7. <https://www.ncbi.nlm.nih.gov/pubmed/3829469>
68. Ronco C, Fecondini L. The Vicenza wearable artificial kidney for peritoneal dialysis (ViWAK PD). *Blood Purif* 2007;25:383–8. <http://dx.doi.org/10.1159/000107775>
69. Lee DB, Roberts M. A peritoneal-based automated wearable artificial kidney. *Clin Exp Nephrol* 2008;12:171–80. <http://dx.doi.org/10.1007/s10157-008-0050-9>
70. Topfer LA. Wearable Artificial Kidneys for End-Stage Kidney Disease. CADTH Issues in Emerging Health Technologies. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health. 2016.
71. Armignacco P, Garzotto F, Neri M et al. Wak engineering evolution. *Blood Purif* 2015;39:110–4. <http://dx.doi.org/10.1159/000368955>

72. Fissell WH, Roy S, Davenport A. Achieving more frequent and longer dialysis for the majority: wearable dialysis and implantable artificial kidney devices. *Kidney Int* 2013;**84**:256–64. <http://dx.doi.org/10.1038/ki.2012.466>
73. Gura V, Macy AS, Beizai M et al. Technical breakthroughs in the wearable artificial kidney (WAK). *Clin J Am Soc Nephrol* 2009;**4**:1441–8. <http://dx.doi.org/10.2215/CJN.02790409>
74. Gura V, Rivara MB, Bieber S et al. A wearable artificial kidney for patients with end-stage renal disease. *JCI Insight* 2016;**1**:e86397. <http://dx.doi.org/10.1172/jci.insight.86397>
75. Davenport A, Gura V, Ronco C et al. A wearable haemodialysis device for patients with end-stage renal failure: a pilot study. *Lancet* 2007;**370**:2005–10. [http://dx.doi.org/10.1016/S0140-6736\(07\)61864-9](http://dx.doi.org/10.1016/S0140-6736(07)61864-9)
76. Aristizabal AM, Caicedo LA, Martínez JM et al. Clinical xenotransplantation, a closer reality: literature review. *Cir Esp (Engl Ed)* 2017;**95**:62–72. <http://dx.doi.org/10.1016/j.ciresp.2016.12.008>
77. Najarian JS. Experimental xenotransplantation: a personal history. *Xenotransplantation* 2003;**10**:10–5. <http://dx.doi.org/10.1034/j.1399-3089.2003.01082.x>
78. Deschamps JY, Roux FA, Sai P et al. History of xenotransplantation. *Xenotransplantation* 2005;**12**:91–109. <http://dx.doi.org/10.1111/j.1399-3089.2004.00199.x>
79. Lu T, Yang B, Wang R et al. Xenotransplantation: current status in preclinical research. *Front Immunol* 2019;**10**:3060. <http://dx.doi.org/10.3389/fimmu.2019.03060>
80. Kim SC, Mathews DV, Breeden CP et al. Long-term survival of pig-to-rhesus macaque renal xenografts is dependent on CD4 T cell depletion. *Am J Transplant* 2019;**19**:2174–85. <http://dx.doi.org/10.1111/ajt.15329>
81. Porrett PM, Orandi BJ, Kumar V et al. First clinical-grade porcine kidney xenotransplant using a human decedent model. *Am J Transplant* 2022;**22**:1037–53. <http://dx.doi.org/10.1111/ajt.16930>
82. Montgomery RA, Stern JM, Lonze BE et al. Results of two cases of pig-to-human kidney xenotransplantation. *N Engl J Med* 2022;**386**:1889–98. <http://dx.doi.org/10.1056/NEJMoa2120238>
83. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–76. <https://doi.org/10.1016/j.cell.2006.07.024>
84. Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–72. <https://doi.org/10.1016/j.cell.2007.11.019>
85. Wu J, Platero-Luengo A, Sakurai M et al. Interspecies chimerism with mammalian pluripotent stem cells. *Cell* 2017;**168**:473–86 e415. <http://dx.doi.org/10.1016/j.cell.2016.12.036>
86. Pan B, Fan G. Stem cell-based treatment of kidney diseases. *Exp Biol Med (Maywood)* 2020;**245**:902–10. <http://dx.doi.org/10.1177/1535370220915901>
87. Wong CY, Tan EL, Cheong SK. In vitro differentiation of mesenchymal stem cells into mesangial cells when co-cultured with injured mesangial cells. *Cell Biol Int* 2014;**38**:497–501. <http://dx.doi.org/10.1002/cbin.10231>
88. Zhang A, Wang H, Wang B et al. Exogenous miR-26a suppresses muscle wasting and renal fibrosis in obstructive kidney disease. *FASEB J* 2019;**33**:13590–601. <http://dx.doi.org/10.1096/fj.201900884R>
89. Guo Y, Bao S, Guo W et al. Bone marrow mesenchymal stem cell-derived exosomes alleviate high phosphorus-induced vascular smooth muscle cells calcification by modifying microRNA profiles. *Funct Integr Genomics* 2019;**19**:633–43. <http://dx.doi.org/10.1007/s10142-019-00669-0>
90. Wang B, Jia H, Zhang B et al. Pre-incubation with hucMSC-exosomes prevents cisplatin-induced nephrotoxicity by activating autophagy. *Stem Cell Res Ther* 2017;**8**:75. <http://dx.doi.org/10.1186/s13287-016-0463-4>
91. Jia H, Liu W, Zhang B et al. HucMSC exosomes-delivered 14-3-3 $\zeta$  enhanced autophagy via modulation of ATG16L in preventing cisplatin-induced acute kidney injury. *Am J Transl Res* 2018;**10**:101–13. <https://www.ncbi.nlm.nih.gov/pubmed/29422997>
92. Jin J, Shi Y, Gong J et al. Exosome secreted from adipose-derived stem cells attenuates diabetic nephropathy by promoting autophagy flux and inhibiting apoptosis in podocyte. *Stem Cell Res Ther* 2019;**10**:95. <http://dx.doi.org/10.1186/s13287-019-1177-1>
93. Yin F, Yan J, Zhao Y et al. Bone marrow mesenchymal stem cells repair Cr (VI)-injured kidney by regulating mitochondria-mediated apoptosis and mitophagy mediated via the MAPK signaling pathway. *Ecotoxicol Environ Saf* 2019;**176**:234–41. <http://dx.doi.org/10.1016/j.ecoenv.2019.03.093>
94. Rashed LA, Elattar S, Eltablawy N et al. Mesenchymal stem cells pretreated with melatonin ameliorate kidney functions in a rat model of diabetic nephropathy. *Biochem Cell Biol* 2018;**96**:564–71. <http://dx.doi.org/10.1139/bcb-2017-0230>
95. Li B, Leung JCK, Chan LYY et al. Amelioration of endoplasmic reticulum stress by mesenchymal stem cells via hepatocyte growth factor/c-Met signaling in obesity-associated kidney injury. *Stem Cells Transl Med* 2019;**8**:898–910. <http://dx.doi.org/10.1002/sctm.18-0265>
96. Song IH, Jung KJ, Lee TJ et al. Mesenchymal stem cells attenuate adriamycin-induced nephropathy by diminishing oxidative stress and inflammation via downregulation of the NF- $\kappa$ B. *Nephrology (Carlton)* 2018;**23**:483–92. <http://dx.doi.org/10.1111/nep.13047>
97. Zhang JB, Wang XQ, Lu GL et al. Adipose-derived mesenchymal stem cells therapy for acute kidney injury induced by ischemia-reperfusion in a rat model. *Clin Exp Pharmacol Physiol* 2017;**44**:1232–40. <http://dx.doi.org/10.1111/1440-1681.12811>
98. Zhang R, Yin L, Zhang B et al. Resveratrol improves human umbilical cord-derived mesenchymal stem cells repair for cisplatin-induced acute kidney injury. *Cell Death Dis* 2018;**9**:965. <http://dx.doi.org/10.1038/s41419-018-0959-1>
99. Jiao X, Cai J, Yu X et al. Paracrine activation of the Wnt/ $\beta$ -Catenin pathway by bone marrow stem cell attenuates cisplatin-induced kidney injury. *Cell Physiol Biochem* 2017;**44**:1980–94. <http://dx.doi.org/10.1159/000485904>
100. Tian SF, Jiang ZZ, Liu YM et al. Human urine-derived stem cells contribute to the repair of ischemic acute kidney injury in rats. *Mol Med Rep* 2017;**16**:5541–8. <http://dx.doi.org/10.3892/mmr.2017.7240>
101. Geng X, Hong Q, Wang W et al. Biological membrane-packed mesenchymal stem cells treat acute kidney disease by ameliorating mitochondrial-related apoptosis. *Sci Rep* 2017;**7**:41136.
102. Shao Z, Meng X, Meng F. Efficacy and safety of mesenchymal stem cell in Chinese patients with chronic renal failure: a pilot study in Shandong province, China. *Pak J Pharm Sci* 2021;**34**:1227–31. <https://www.ncbi.nlm.nih.gov/pubmed/34602393>

103. Morizane R, Lam AQ, Freedman BS et al. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat Biotechnol* 2015;33:1193–200. <http://dx.doi.org/10.1038/nbt.3392>
104. Takasato M, Er PX, Becroft M et al. Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat Cell Biol* 2014;16:118–26. <http://dx.doi.org/10.1038/ncb2894>
105. Morizane R, Bonventre JV. Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells. *Nat Protoc* 2017;12:195–207. <http://dx.doi.org/10.1038/nprot.2016.170>
106. Wang C, Rabadan Ros R, Martinez-Redondo P et al. In vivo partial reprogramming of myofibers promotes muscle regeneration by remodeling the stem cell niche. *Nat Commun* 2021;12:3094. <http://dx.doi.org/10.1038/s41467-021-23353-z>
107. MacKay SM, Funke AJ, Buffington DA et al. Tissue engineering of a bioartificial renal tubule. *ASAIO J* 1998;44:179–83. <http://dx.doi.org/10.1097/00002480-199805000-00011>
108. Peired AJ, Mazzinghi B, De Chiara L et al. Bioengineering strategies for nephrologists: kidney was not built in a day. *Expert Opin Biol Ther* 2020;20:467–80. <http://dx.doi.org/10.1080/14712598.2020.1709439>
109. Humes HD, Weitzel WF, Bartlett RH et al. Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure. *Kidney Int* 2004;66:1578–88. <http://dx.doi.org/10.1111/j.1523-1755.2004.00923.x>
110. Humes HD, Fissell WH, Weitzel WF et al. Metabolic replacement of kidney function in uremic animals with a bioartificial kidney containing human cells. *Am J Kidney Dis* 2002;39:1078–87. <http://dx.doi.org/10.1053/ajkd.2002.32792>
111. Tumlin J, Wali R, Williams W et al. Efficacy and safety of renal tubule cell therapy for acute renal failure. *J Am Soc Nephrol* 2008;19:1034–40. <http://dx.doi.org/10.1681/ASN.2007080895>
112. Buffington DA, Pino CJ, Chen L et al. Bioartificial renal epithelial cell system (BRECS): a compact, cryopreservable extracorporeal renal replacement device. *Cell Med* 2012;4:33–43. <http://dx.doi.org/10.3727/215517912X653328>
113. Pino CJ, Westover AJ, Buffington DA et al. Bioengineered renal cell therapy device for clinical translation. *ASAIO J* 2017;63:305–15. <http://dx.doi.org/10.1097/MAT.0000000000000485>
114. Westover AJ, Buffington DA, Johnston KA et al. A bioartificial renal epithelial cell system conveys survival advantage in a porcine model of septic shock. *J Tissue Eng Regen Med* 2017;11:649–57. <http://dx.doi.org/10.1002/term.1961>
115. Johnston KA, Westover AJ, Rojas-Pena A et al. Development of a wearable bioartificial kidney using the bioartificial renal epithelial cell system (BRECS). *J Tissue Eng Regen Med* 2017;11:3048–55. <http://dx.doi.org/10.1002/term.2206>
116. Fissell WH, Roy S. The implantable artificial kidney. *Semin Dial* 2009;22:665–70. <http://dx.doi.org/10.1111/j.1525-139X.2009.00662.x>
117. Rabb H, Lee K, Parikh CR. Beyond kidney dialysis and transplantation: what's on the horizon? *J Clin Invest* 2022;132:e159308. <http://dx.doi.org/10.1172/JCI159308>
118. Kensing C, Karp S, Kant R et al. First implantation of silicon nanopore membrane hemofilters. *ASAIO J* 2016;62:491–5. <http://dx.doi.org/10.1097/MAT.0000000000000367>
119. Figliuzzi M, Remuzzi G, Remuzzi A. Renal bioengineering with scaffolds generated from rat and pig kidneys. *Nephron Exp Nephrol* 2014;126:113. <http://dx.doi.org/10.1159/000360683>
120. Figliuzzi M, Bonandrini B, Remuzzi A. Decellularized kidney matrix as functional material for whole organ tissue engineering. *J Appl Biomater Funct Mater* 2017;15:e326–33. <https://www.ncbi.nlm.nih.gov/pubmed/29131298>
121. Hussein KH, Saleh T, Ahmed E et al. Biocompatibility and hemocompatibility of efficiently decellularized whole porcine kidney for tissue engineering. *J Biomed Mater Res A* 2018;106:2034–47. <http://dx.doi.org/10.1002/jbm.a.36407>
122. Song JJ, Guyette JP, Gilpin SE et al. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. *Nat Med* 2013;19:646–51. <http://dx.doi.org/10.1038/nm.3154>
123. Ross EA, Williams MJ, Hamazaki T et al. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. *J Am Soc Nephrol* 2009;20:2338–47. <http://dx.doi.org/10.1681/ASN.2008111196>
124. Bonandrini B, Figliuzzi M, Papadimou E et al. Recellularization of well-preserved acellular kidney scaffold using embryonic stem cells. *Tissue Eng Part A* 2014;20:1486–98. <http://dx.doi.org/10.1089/ten.tea.2013.0269>
125. Ortiz A, Ferro CJ, Balafa O et al. Cardiovascular Medicine working group of the European Renal Association—European Dialysis and Transplant Association (ERA-EDTA) and the Hypertension and Kidney working group of the European Society of Hypertension (ESH). Mineralocorticoid receptor antagonists for nephroprotection and cardioprotection in patients with diabetes mellitus and chronic kidney disease. *Nephrol Dial Transplant* 2021;fgab167. <http://dx.doi.org/10.1093/ndt/fgab167>
126. Pitt B, Filippatos G, Agarwal R et al. Cardiovascular events with finerenone in kidney disease and type 2 diabetes. *N Engl J Med* 2021;385:2252–63. <http://dx.doi.org/10.1056/NEJMoa2110956>
127. Bakris GL, Agarwal R, Anker SD et al. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. *N Engl J Med* 2020;383:2219–29. <http://dx.doi.org/10.1056/NEJMoa2025845>
128. Filippatos G, Anker SD, Agarwal R et al. Finerenone reduces risk of incident heart failure in patients with chronic kidney disease and type 2 diabetes: analyses from the FIGARO-DKD trial. *Circulation* 2022;145:437–47. <http://dx.doi.org/10.1161/CIRCULATIONAHA.121.057983>
129. Agarwal R, Filippatos G, Pitt B et al. Cardiovascular and kidney outcomes with finerenone in patients with type 2 diabetes and chronic kidney disease: the FIDELITY pooled analysis. *Eur Heart J* 2022;43:474–84. <http://dx.doi.org/10.1093/eurheartj/ehab777>
130. Filippatos G, Bakris GL, Pitt B et al. Finerenone reduces new-onset atrial fibrillation in patients with chronic kidney disease and type 2 diabetes. *J Am Coll Cardiol* 2021;78:142–52. <http://dx.doi.org/10.1016/j.jacc.2021.04.079>
131. [https://www.ema.europa.eu/en/documents/product-information/kerendia-epar-product-information\\_enpdf](https://www.ema.europa.eu/en/documents/product-information/kerendia-epar-product-information_enpdf) (5 August 2022, date last accessed).
132. Iijima T, Katoh M, Takedomi K et al. Discovery of apararenone (MT-3995) as a highly selective, potent, and novel nonsteroidal mineralocorticoid receptor antagonist. *J Med Chem* 2022;65:8127–43. <http://dx.doi.org/10.1021/acs.jmedchem.2c00402>



133. Azizi M, Amar L, Menard J. Aldosterone synthase inhibition in humans. *Nephrol Dial Transplant* 2013;**28**:36–43. <http://dx.doi.org/10.1093/ndt/gfs388>
134. Lenzini L, Zanotti G, Bonchio M et al. Aldosterone synthase inhibitors for cardiovascular diseases: a comprehensive review of preclinical, clinical and in silico data. *Pharmacol Res* 2021;**163**:105332. <http://dx.doi.org/10.1016/j.phrs.2020.105332>
135. Wang HZ, Tian JB, Yang KH. Efficacy and safety of LCI699 for hypertension: a meta-analysis of randomized controlled trials and systematic review. *Eur Rev Med Pharmacol Sci* 2015;**19**:296–304. <https://www.ncbi.nlm.nih.gov/pubmed/25683946>
136. Bogman K, Schwab D, Delporte ML et al. Preclinical and early clinical profile of a highly selective and potent oral inhibitor of aldosterone synthase (CYP11B2). *Hypertension* 2017;**69**:189–96. <http://dx.doi.org/10.1161/HYPERTENSIONAHA.116.07716>
137. Minnaard-Huiban M, Emmen JM, Roumen L et al. Furozole reverses cardiac fibrosis in spontaneously hypertensive heart failure rats: discordant enantioselectivity versus reduction of plasma aldosterone. *Endocrinology* 2008;**149**:28–31. <http://dx.doi.org/10.1210/en.2007-0584>
138. Kohan DE, Lambers Heerspink HJ, Coll B et al. Predictors of atrasentan-associated fluid retention and change in albuminuria in patients with diabetic nephropathy. *Clin J Am Soc Nephrol* 2015;**10**:1568–74. <http://dx.doi.org/10.2215/CJN.00570115>
139. Koomen JV, Stevens J, Bakris G et al. Individual atrasentan exposure is associated with Long-term kidney and heart failure outcomes in patients with type 2 diabetes and chronic kidney disease. *Clin Pharmacol Ther* 2021;**109**:1631–8. <http://dx.doi.org/10.1002/cpt.2143>
140. Heerspink HJL, Parving HH, Andress DL et al. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): a double-blind, randomised, placebo-controlled trial. *Lancet* 2019;**393**:1937–47. [http://dx.doi.org/10.1016/S0140-6736\(19\)30772-X](http://dx.doi.org/10.1016/S0140-6736(19)30772-X)
141. Ortiz A, Fernandez-Fernandez B. Atrasentan: the difficult task of integrating endothelin a receptor antagonists into current treatment paradigm for diabetic kidney disease. *Clin J Am Soc Nephrol* 2021;**16**:1775–8. <http://dx.doi.org/10.2215/CJN.13601021>
142. Trachtman H, Nelson P, Adler S et al. DUET: a phase 2 study evaluating the efficacy and safety of sparsentan in patients with FSGS. *J Am Soc Nephrol* 2018;**29**:2745–54. <http://dx.doi.org/10.1681/ASN.2018010091>
143. Dhaun N, MacIntyre IM, Kerr D et al. Selective endothelin-A receptor antagonism reduces proteinuria, blood pressure, and arterial stiffness in chronic proteinuric kidney disease. *Hypertension* 2011;**57**:772–9. <http://dx.doi.org/10.1161/HYPERTENSIONAHA.110.167486>
144. Ohnishi A, Orita Y, Okahara R et al. Potent aquaretic agent. A novel nonpeptide selective vasopressin 2 antagonist (OPC-31260) in men. *J Clin Invest* 1993;**92**:2653–9. <http://dx.doi.org/10.1172/JCI116881>
145. Nelson JB. Endothelin receptor antagonists. *World J Urol* 2005;**23**:19–27. <http://dx.doi.org/10.1007/s00345-004-0478-9>
146. Widlitz AC, Barst RJ, Horn EM. Sitaxsentan: a novel endothelin-A receptor antagonist for pulmonary arterial hypertension. *Expert Rev Cardiovasc Ther* 2005;**3**:985–91. <http://dx.doi.org/10.1586/14779072.3.6.985>
147. Barratt J, Sulowicz W, Schomig M et al. Efficacy and cardiovascular safety of roxadustat in dialysis-dependent chronic kidney disease: pooled analysis of four phase 3 studies. *Adv Ther* 2021;**38**:5345–60. <http://dx.doi.org/10.1007/s12325-021-01903-7>
148. Singh AK, Carroll K, Perkovic V et al. Daprodustat for the treatment of anemia in patients undergoing dialysis. *N Engl J Med* 2021;**385**:2325–35. <http://dx.doi.org/10.1056/NEJMoa2113379>
149. Singh AK, Carroll K, McMurray JJV et al. Daprodustat for the treatment of anemia in patients not undergoing dialysis. *N Engl J Med* 2021;**385**:2313–24. <http://dx.doi.org/10.1056/NEJMoa2113380>
150. Akizawa T, Nangaku M, Yonekawa T et al. Efficacy and safety of daprodustat compared with darbepoetin alfa in Japanese hemodialysis patients with anemia: a randomized, double-blind, phase 3 trial. *Clin J Am Soc Nephrol* 2020;**15**:1155–65. <http://dx.doi.org/10.2215/CJN.16011219>
151. Kanai H, Nangaku M, Nagai R et al. Efficacy and safety of daprodustat in Japanese peritoneal dialysis patients. *Ther Apher Dial* 2021;**25**:979–87. <http://dx.doi.org/10.1111/1744-9987.13686>
152. Tsubakihara Y, Akizawa T, Nangaku M et al. A 24-Week anemia correction study of daprodustat in Japanese dialysis patients. *Ther Apher Dial* 2020;**24**:108–14. <http://dx.doi.org/10.1111/1744-9987.12962>
153. Bailey CK, Caltabiano S, Cobitz AR et al. A randomized, 29-day, dose-ranging, efficacy and safety study of daprodustat, administered three times weekly in patients with anemia on hemodialysis. *BMC Nephrol* 2019;**20**:372. <http://dx.doi.org/10.1186/s12882-019-1547-z>
154. Nangaku M, Hamano T, Akizawa T et al. Daprodustat compared with epoetin beta pegol for anemia in Japanese patients not on dialysis: a 52-Week randomized open-label phase 3 trial. *Am J Nephrol* 2021;**52**:26–35. <http://dx.doi.org/10.1159/000513103>
155. Rovin BH, Teng YKO, Ginzler EM et al. Efficacy and safety of voclosporin versus placebo for lupus nephritis (AURORA 1): a double-blind, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2021;**397**:2070–80. <http://dx.doi.org/10.1016/j.kint.2018.08.025>