CKJ REVIEW

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

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ABSTRACT

Chronic kidney disease (CKD) will become the fifth global case 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 counties 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^2 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
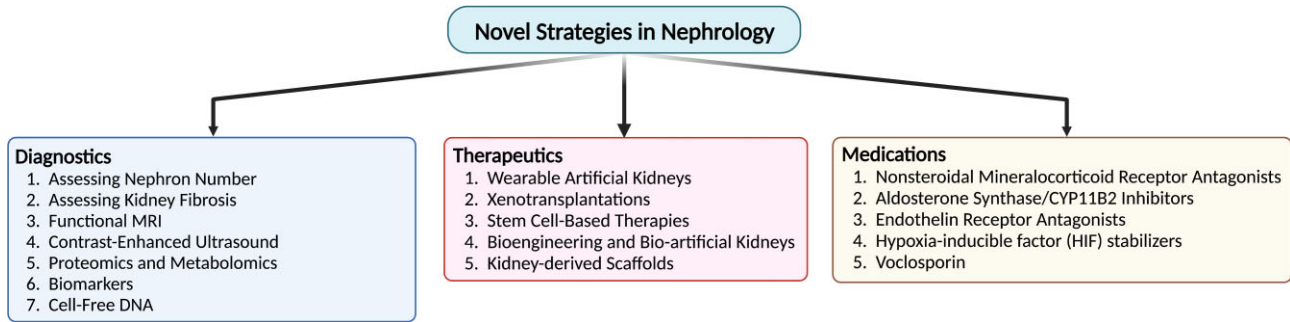
Figure 1: General overview of the novel strategies in nephrology. (Diagnostics will be further elaborated in detail in Fig. 2.)

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 suboptimal, 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,
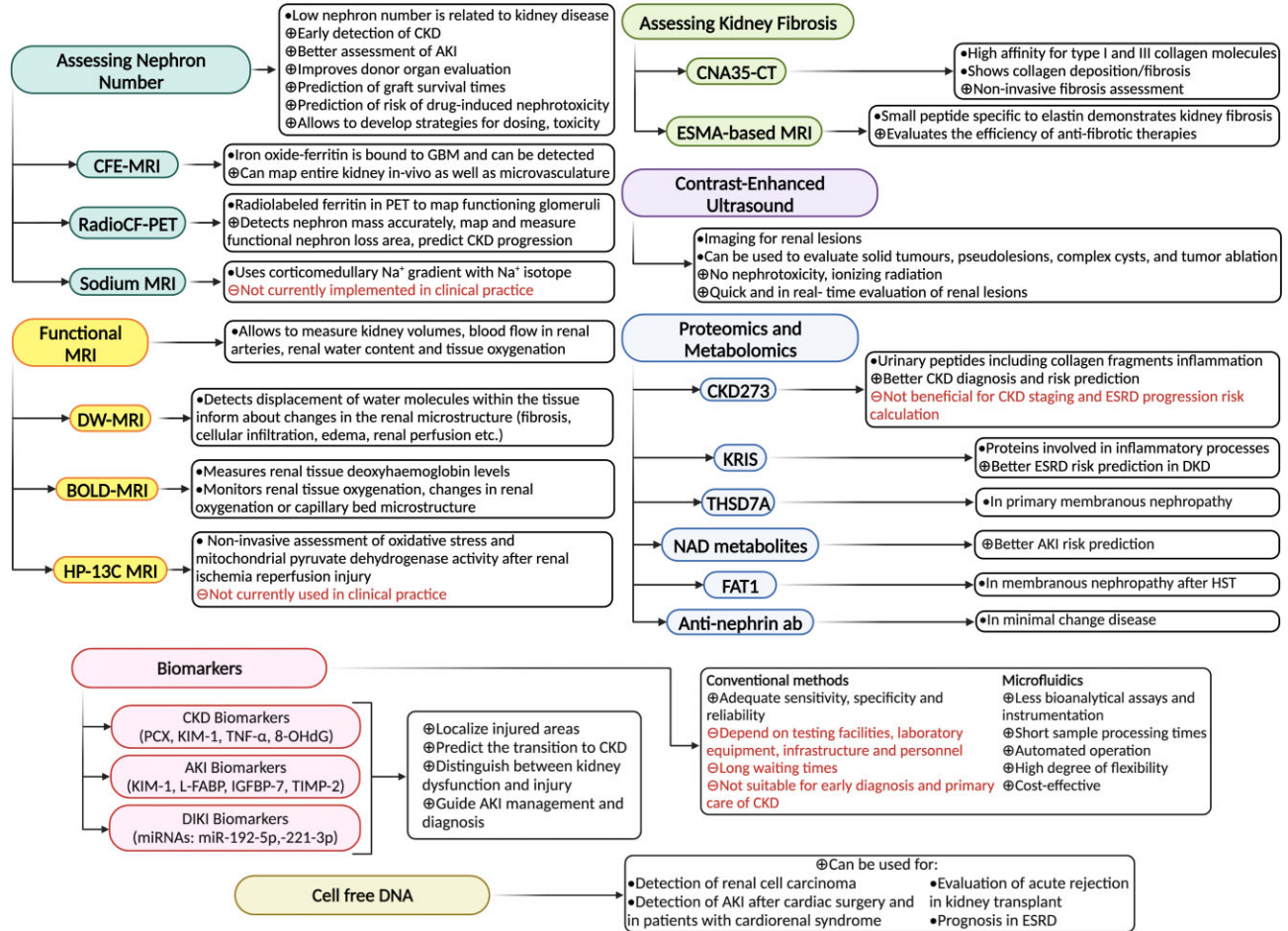
Novel Diagnostic Methods

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-α: tumor necrosis factor-α; 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; DIKI: 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 CKD73 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 that 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-α (TNF-α), 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

Wearable Artificial Kidney (WAK)
- Dialysis device that allows patients to have mobility and get dialysis while performing their day-to-day routines
⊕ Allows mobilization and continuous toxin clearance
⊕ Good hemodynamic stability
⊕ Weighs less than 5 kg
⊕ Reusable dialysate solutions without needing large amount of water
⊕ Higher satisfaction and quality of life
⊖ Variations in blood and dialysate flow rates and toxin clearance
⊖ Clotting risk at the vascular access

Xenotransplantation
- Transplantation of kidney from another species, mostly pigs
⊕ No sign for hyperacute rejection and stable urine output for a short period of time
⊖ Thrombotic microangiopathy
⊖ Problems to excrete creatinine in physiological level
⊖ Limited studies, outcomes and evidence

Stem Cell-Based Therapy
- Mesenchymal stem cells differentiate into mesangial cells
- Upregulation of autophagy, anti-apoptotic, paracrine signaling and anti-inflammatory properties are useful for kidney diseases
⊕ Prevents vascular calcification, fibrosis, CKD
⊕ Beneficial in nephropathies, nephrotoxicities, AKIs, oxidative stress, rhabdomyolysis, renal artery stenosis, cardiorenal syndrome

Bioenginering-Bioartificial Kidney

Renal Assist Device (RAD)
- Membrane hemofilter and a bioreactor of renal tubule cells to mimic healthy kidney
⊕ Improves hemodialysis for acute uremia
⊕ Demonstrates metabolic and endocrinologic activity
⊕ Decreases G-CSF and interleukin levels
⊕ Improves survival rates
⊖ Hard to find cell sourcing
⊖ Expensive
⊖ Storage requirements and distribution problems

Implantable Bioartificial Kidney (iBK)
- Contains a silicon membrane filter and a bioreactor made of renal tubular epithelial cells
⊕ Hybrid mechanism with glomerulus like filtration and engineered renal tubular cells that provides improved toxin clearance, cardiovascular stability and electrolyte balance
⊕ Wastes can be removed into bladder, less need for electrical pumps
⊖ Clotting risk of the blood filter

Glomerulus/tubule-on-a-chip
- Microfluidic cell culture platform can mimic living human organs
⊕ Used for disease modeling, drug screening assays, regenerative medicine
⊕ Better understanding of glomerular and tubular functions

Kidney-derived Scaffold
- To generate a whole-kidney scaffold with a 3D geometry and vasculature by decellularization and recellularization processes
⊕ Can form a whole-kidney if seeded with stem cells or other appropriate cells
⊖ Number of donor cells is limited
⊖ Not ready for clinical use, needs improvements in decellularization and recellularization methods and functionality
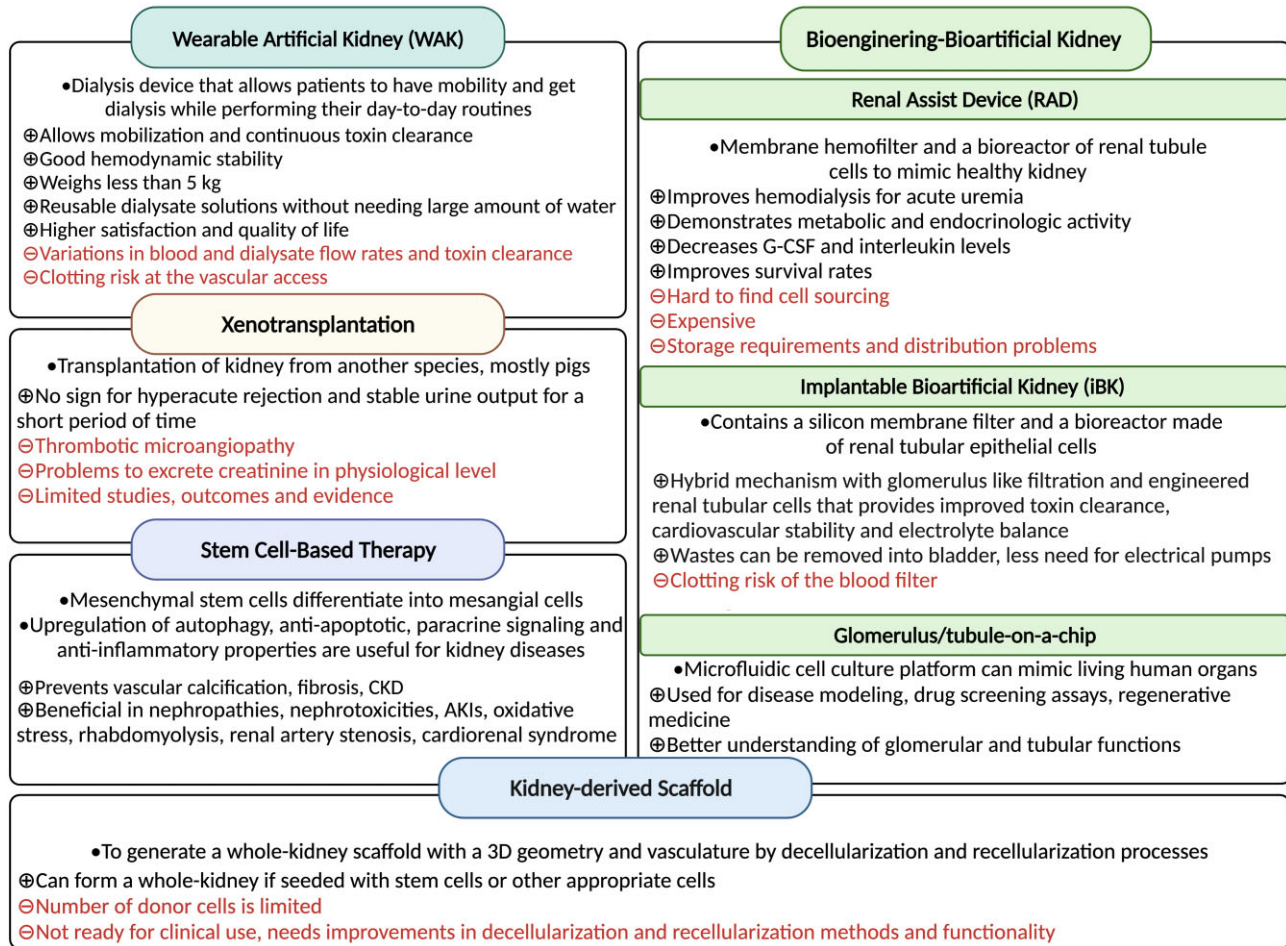
Figure 3: Novel therapeutic alternatives in nephrology. G-CSF: granulocyte colony stimulating factor. plus sign: advantages; minus sign: disadvantages/limitations (indicated in red color).

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 ± 24 mL/min and a mean dialysate flow rate of 43 ± 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 ± 11.7 mL/min and mean dialysate flow of 47.1 ± 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 xenozoonosis, 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 of xenotransplantation has been questioned for human subjects without any clinical or preclinical 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^2 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^2 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	• Better cardiovascular outcomes: • Decreases hospitalization for HF, MI, stroke and death risk • Reduces CKD progression • Declines proteinuria
LCI699, fadrozole, RO6836191	Aldosterone synthase/CYP11B2 inhibitors	• Lead to reduction in plasma aldosterone concentration along with an increase in plasma renin and deoxycortisol concentration • Beneficial in the treatment of primary hypertension
Sparsentan, sitaxentan, zibotentan	Endothelin receptor antagonists	• Can be used to decrease proteinuria and kidney progression in CKD, FSGS, patients with scleroderma
Daprodustat	HIF prolyl hydroxylase inhibitor (HIF stabilizer)	• Similar efficiency and cardiovascular event risk with darbepoetin alfa or epoetin alfa in anemia treatment • Can be administrated orally
Voclosporin	Calcineurin inhibitor	• Voclosporin with MMF and low-dose steroids shows better kidney outcomes versus MMF and low-dose steroids

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 to 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.

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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.

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