Clinical Potential of Targeting Fibroblast Growth Factor-23 and αKlotho in the Treatment of Uremic Cardiomyopathy

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ABSTRACT: Chronic kidney disease is highly prevalent, affecting 10% to 15% of the adult population worldwide and is associated with increased cardiovascular morbidity and mortality. As chronic kidney disease worsens, a unique cardiovascular phenotype develops characterized by heart muscle disease, increased arterial stiffness, atherosclerosis, and hypertension. Cardiovascular risk is multifaceted, but most cardiovascular deaths in patients with advanced chronic kidney disease are caused by heart failure and sudden cardiac death. While the exact drivers of these deaths are unknown, they are believed to be caused by uremic cardiomyopathy: a specific pattern of myocardial hypertrophy, fibrosis, with both diastolic and systolic dysfunction. Although the pathogenesis of uremic cardiomyopathy is likely to be multifactorial, accumulating evidence suggests increased production of fibroblast growth factor-23 and αKlotho deficiency as potential major drivers of cardiac remodeling in patients with uremic cardiomyopathy. In this article we review the increasing understanding of the physiology and clinical aspects of uremic cardiomyopathy and the rapidly increasing knowledge of the biology of both fibroblast growth factor-23 and αKlotho. Finally, we discuss how dissection of these pathological processes is aiding the development of therapeutic options, including small molecules and antibodies, directly aimed at improving the cardiovascular outcomes of patients with chronic kidney disease and end-stage renal disease.

Key Words: αKlotho ■ cardiorenal syndrome ■ FGF23 ■ fibroblast growth factor ■ growth factor ■ kidney ■ treatment

Chronic kidney disease (CKD) and end-stage renal disease (ESRD) requiring dialysis are complex, chronic conditions with a combined prevalence of 10% to 15% of the adult population worldwide.1–3 Cardiovascular events and mortality increase exponentially with reduced estimated glomerular filtration rate (eGFR) independent of age, sex, and other risk factors.4–6 In the early stages of CKD, the risks of occlusive atheromatous disease are increased and account for the majority of cardiovascular events observed.7 Arterial atheroma remains an important modifiable pathophysiological process in CKD, as evidenced by trials in early CKD showing benefit from lipid-lowering therapies in modifying the risk of atherosclerotic events.8–10 However, the same treatments appear much less effective in patients with advanced stages of CKD, including ESRD.10–12 As CKD worsens, there is a shift from atherosclerotic complications to morbidity due to heart failure and sudden cardiac death (SCD).7,13,14 Atrial fibrillation (AF) is also common, detected in up to 41% of patients requiring hemodialysis.14 The pathophysiological basis of these events is a unique cardiovascular phenotype consisting primarily of the development of uremic cardiomyopathy with...
associated increased arterial stiffness and widespread atheroma.

The purpose of this article is to review the current state of the art on 2 newly postulated drivers of uremic cardiomyopathy, elevated circulating fibroblast growth factor-23 (FGF23) and reduced αKlotho, and discuss how recent insights into the pathophysiological processes has led to development of potential therapeutic options aimed at reducing the cardiovascular risk of patients with CKD/ESRD.

**UREMIC CARDIOMYOPATHY**

The term uremic cardiomyopathy arose in the 1980s with reports of common abnormalities in cardiac function and structure in patients with CKD/ESRD, including increased left ventricular (LV) mass and left ventricular hypertrophy (LVH); diastolic and systolic dysfunction; as well as profound myocardial fibrosis on histology.15–20 Uremic cardiomyopathy has been linked to conditions causing both heart failure and CKD, especially hypertension. Chronic stimulation of cardiac cells by renin, angiotensin, parathyroid hormone (PTH), cardiotoxic steroids, and other uremic toxins, has also been proposed.20 The severity of uremic cardiomyopathy as measured by LV mass is a powerful predictor of cardiovascular mortality probably as a result of the factors discussed below.

**Increased LV Mass and Hypertrophy**

Increased LV mass and LVH are common manifestations of uremic cardiomyopathy. Forty percent of patients with an eGFR <30 mL/min per 1.73 m² have LVH on echocardiography,21 increasing to ~80% in ESRD.22,23 In patients with CKD/ESRD, LVH is strongly associated with death; diastolic and systolic heart failure; and cardiac arrhythmias.22,23 However, LV mass is a continuous variable, with a graded relationship with adverse cardiovascular outcomes.24–28 It is also important to emphasize that cardiac structural changes occur early in the course of CKD, with a linear association between worsening renal function and a higher prevalence of LVH.29,30 While elevated blood pressure is an important determinant of LV mass,31 evidence from both animal and human studies supports the presence of mechanisms that are independent of pressure overload and hypertension in driving cardiac hypertrophy in CKD/ESRD.32–36

**Diastolic and Systolic Dysfunction**

Diastolic dysfunction is highly prevalent in patients with CKD, with over two thirds affected in CKD stages 2 to 4 and up to 85% in ESRD.37 Diastolic dysfunction is strongly associated with increased LV mass and LVH,7 as well as myocardial fibrosis,20,38 and correlates with increased mortality.37,38 Furthermore, the presence of diastolic dysfunction is considered to be a major cause for the frequent presentation of hemodialysis patients with pulmonary edema or intradialytic hypotension, despite only minor changes in fluid status.7,38

Overt LV systolic dysfunction, as manifested by reduced ejection fraction, is uncommon in predialysis CKD with a reported prevalence of 8% and no association with eGFR.17,30 However, several studies using echocardiography have shown changes in LV deformation in early stages of CKD, indicating the presence of subnormal LV systolic function.39–41 In ESRD, LV systolic dysfunction is very common, with a reported prevalence 10 to 30 times greater than in the general population.42,43

**Myocardial Fibrosis**

It has been suggested that increased interstitial myocardial fibrosis may be the unifying pathophysiological process underlying uremic cardiomyopathy.44 In the 1990s, a postmortem study found that myocardial fibrosis was present in 91% of CKD/ESRD patients without significant flow-limiting coronary lesions. The severity of fibrosis was related to the length of time on dialysis, but independent of hypertension, blood pressure, diabetes mellitus, or anemia.15 Over a decade later, Aoki et al.45 performed endocardial biopsies in 40 ESRD patients with reduced LV ejection fraction without coronary artery disease. The predominant pathologic findings were extensive interstitial fibrosis and cardiomyocyte hypertrophy and disarray.

Studying myocardial fibrosis in CKD/ESRD has been challenging given that myocardial biopsies are not without risk, especially in multimorbid patients and therefore are not always clinically and ethically justified.45,46 Late gadolinium enhancement cardiac magnetic resonance imaging, a validated noninvasive method, allows in vivo quantification of myocardial fibrosis in conditions such as myocardial infarction,47 dilated48 and hypertrophic cardiomyopathies.49 This technique has also been used to characterize myocardial tissue in patients with ESRD demonstrating midwall patterns of late gadolinium enhancement consistent with replacement myocardial

**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AF</td>
<td>atrial fibrillation</td>
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<tr>
<td>ESRD</td>
<td>end-stage renal disease</td>
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<tr>
<td>FGF23</td>
<td>fibroblast growth factor 23</td>
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<tr>
<td>LV</td>
<td>left ventricular</td>
</tr>
<tr>
<td>LVH</td>
<td>left ventricular hypertrophy</td>
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<tr>
<td>SCD</td>
<td>sudden cardiac death</td>
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</table>

**AF** atrial fibrillation; **ESRD** end-stage renal disease; **FGF23** fibroblast growth factor 23; **LV** left ventricular; **LVH** left ventricular hypertrophy; **SCD** sudden cardiac death.
fibrosis not associated with large vessel coronary artery disease.\textsuperscript{17} Noncontrast myocardial native T1 relaxation time, or T1 mapping, has emerged as a novel viable technique to quantify diffuse interstitial myocardial fibrosis in CKD/ESRD\textsuperscript{60} correlating with histological interstitial fibrosis in a number of disease states, including cardiomyopathy and valvular disease.\textsuperscript{51} Indeed, native T1 times are increased in early CKD,\textsuperscript{19} increasing with worsening CKD stages\textsuperscript{52} and correlates with increased LV mass.\textsuperscript{8,53} Native T1 mapping offers an exciting opportunity to investigate novel mechanisms of cardiac fibrosis (eg, FGF23-mediated changes), in patients with CKD and in animal models.

FIBROBLAST GROWTH FACTOR-23 AND \textalpha\textsubscript{KLOTHO}

The hormone FGF23, first discovered in 2000, is a circulating growth factor secreted by osteocytes whose main physiological role is to increase urinary phosphate excretion.\textsuperscript{54,55} The 4 mammalian FGF receptors (FGFR1-4) are membrane-bound receptor tyrosine kinases.\textsuperscript{56,57} FGFR1 is suggested to be the primary FGF23 receptor in target organs—the kidneys and parathyroid glands.\textsuperscript{58–61} Crystallography studies clearly demonstrate that the presence of \textalpha\textsubscript{Klotho} is required for the efficient binding of FGF23 to FGFR1.\textsuperscript{62} \textalpha\textsubscript{Klotho} is a cell-surface protein, mainly expressed in the kidneys and parathyroid glands.\textsuperscript{63–65} In addition to the membrane-associated full-length protein, the ectodomain of \textalpha\textsubscript{Klotho} can exist in a soluble form.\textsuperscript{66–69} In the presence of membrane-bound \textalpha\textsubscript{Klotho} or soluble \textalpha\textsubscript{Klotho}, FGF23 can activate Fibroblast Growth Factor Receptor Substrate-2 (FRS2\textalpha)/Ras/Mitogen-Activated Protein Kinase signaling (Figure 1).\textsuperscript{92,70,71} Soluble \textalpha\textsubscript{Klotho}, therefore, may act as a circulating FGF23 coreceptor in cells that do not express \textalpha\textsubscript{Klotho}. Such a mechanism has been reported in osteoblasts.\textsuperscript{72} However, its role in the heart is yet to be fully characterized as neither cardiomyocytes nor cardiac fibroblasts express \textalpha\textsubscript{Klotho}.\textsuperscript{55} It is possible that FGF23 might act on these cells with circulating \textalpha\textsubscript{Klotho} as a cofactor. Treatment of cardiac myofibroblasts with full-length \textalpha\textsubscript{Klotho} resulted in upregulated proliferation and ERK phosphorylation, which was suppressed by FGFR1 antagonism.\textsuperscript{73} This suggests the presence of FGFR1 in cardiac myofibroblasts for which soluble Klotho acts as a circulating co-receptor, although the authors did not comment on endogenous FGFR1/FGF23 expression.

FGF23 can also exert cellular effects via \textalpha\textsubscript{Klotho}-independent mechanisms.\textsuperscript{74} FGF23 has been shown to stimulate phospholipase C\textgamma (PLC\textgamma)/calcineurin/nuclear factor of activated T-cells (NFAT) via FGFR4 in cells that lack \textalpha\textsubscript{Klotho} (Figure 1).\textsuperscript{74–77} Such increases in PLC\textgamma/calcineurin/NFAT signaling appear to be important in pathological, as opposed to physiological, cardiac hypertrophy.\textsuperscript{76,79} Clearly further mechanistic studies are warranted to delineate mechanisms that can be targeted therapeutically in patients with elevated FGF23 levels.

FGF23, \textalpha\textsubscript{KLOTHO}, AND KIDNEY DISEASE

One of the first clinically detectable signs of CKD is an elevation in serum FGF23, probably in response to increased extracellular phosphate, although the details of the stimulus and its detection are still unclear, with levels rising steeply as kidney function worsens.\textsuperscript{54,80} (Figure 2). Indeed, elevations are observed as early as eGFR 75 mL/min per 1.73 m\textsuperscript{2}, long before increased concentrations in PTH or phosphate are observed.\textsuperscript{80} Circulating FGF23 levels are 2- to 5-fold above the normal range in early/intermediate CKD, but can reach levels of 1000-fold above normal in ESRD.\textsuperscript{80–82} Increased FGF23 levels are also found in heart failure\textsuperscript{83–88} and AF,\textsuperscript{89–93} and are associated with all-cause and cardiovascular mortality in patients with and without CKD.\textsuperscript{87,88,94–99} Ongoing research therefore explores FGF23 as both a potential biomarker\textsuperscript{100} and a causative factor for cardiac mecanoelectrical dysfunction. However, effective quantification of circulating FGF23 is not currently standardized. Several FGF23 assay kits utilize differing detection techniques, epitope binding regions, analytical ranges and measurement units, making direct comparisons challenging.\textsuperscript{101}

The kidney is the principal source of circulating soluble \textalpha\textsubscript{Klotho}.\textsuperscript{69,102} Its levels are downregulated in the presence of albuminuria,\textsuperscript{103} inflammation,\textsuperscript{104} and with the progression of CKD. \textalpha\textsubscript{Klotho} levels start to decline in CKD stage 2 and precede the elevation of FGF23, PTH, and serum phosphate.\textsuperscript{105} Low levels of circulating \textalpha\textsubscript{Klotho} are associated with increased cardiovascular events and mortality in patients with CKD/ESRD.\textsuperscript{106–109} It is, therefore, conceivable that some of the adverse physiological effects that have been attributed to increased FGF23 may be either caused by, or compounded by, lower \textalpha\textsubscript{Klotho} (Figure 2). These mechanistic complexities require further investigation and need to be considered when developing FGF23/\textalpha\textsubscript{Klotho}-directed therapies.

FGF23, \textalpha\textsubscript{KLOTHO}, AND LEFT VENTRICULAR MASS/HYPERTROPHY

The heart has been shown to respond to FGF23, increasing LV mass independently of blood pressure,
promoting cardiac fibrosis and reducing LV systolic function in animal models.\textsuperscript{74,75,77,110,111} Elevations of cardiac FGFR4 and enhanced PLC\textsubscript{γ1}/calcineurin/NFAT signaling have been observed in both animal models of CKD and in patients with CKD/ESRD.\textsuperscript{75,112–114} Several studies have shown that repetitive administration of FGF23 in wild-type mice, either intravenous or intraperitoneal, induced cardiac hypertrophy within 5 days.\textsuperscript{74,110,111} The signaling actions of FGF23 on the heart are still not fully characterized. However, several independent experimental approaches demonstrate the involvement of the αKlotho-independent FGFR4-PLC\textsubscript{γ1}/calcineurin/NFAT signaling pathway in cardiomyocytes.\textsuperscript{74,75,77} On the other hand, independent studies demonstrate that αKlotho may be cardioprotective and that subnormal levels may be required for FGF23 to induce LVH.\textsuperscript{115–118} Although many of the studies indicate that elevation of FGF23 and reduction in αKlotho are involved in the development of LVH, recent studies by Leithheit-Nestler and Slavic have not recapitulated these findings.\textsuperscript{119,120} Chronic FGF23 overexpression (via myocardial gene transfer), or genetic ablation of FGF23 or αKlotho on the background of transverse aortic constriction did not affect cardiac function or morphology.\textsuperscript{119,120}

**FIBROBLAST GROWTH FACTOR-23, αKLOTHO, AND MYOCARDIAL FIBROSIS**

Every third to fourth cell in the heart is a fibroblast. Fibroblasts produce the extracellular matrix in the heart and act as regulators of the cardiac interstitium.\textsuperscript{121,122} In the injured myocardium, inflammation and
mechanical stress promote activation of fibroblasts to myofibroblasts, leading to maladaptive deposition of extensively cross-linked extracellular matrix, which drives increased stiffness and impaired mechanoelectrical coupling of cardiomyocytes. This loss of cardiomyocyte coupling not only leads to attenuated cardiac function, but also provides a substrate for arrhythmias.123–126 Although recent studies have shown that FGF23 can activate cardiac fibroblasts, neither the underlying mechanism127,128 nor its role in the development of cardiac fibrosis are fully defined.127,129 As cardiac fibroblasts do not express αKlotho,74,102,113,114 the alternative pathway of αKlotho-independent FGF23 signaling through FGFR4-PLCγ/calcineurin/NFAT is likely to play a role (Figure 1). Future studies are required to examine which FGF receptors are expressed in cardiac fibroblasts, whether FGF23 contributes to cardiac fibrosis, and whether these mechanisms are dependent on αKlotho.

**FGF23, αKLOTHO, AND CARDIAC ARRHYTHMIAS**

Patients with CKD/ESRD are at increased risk of a wide spectrum of cardiac arrhythmias, including supraventricular tachycardias, particularly AF, and potentially lethal ventricular arrhythmias.14,130,131 All 3 components of uremic cardiomyopathy (increased LV mass/LVH; diastolic and systolic dysfunction; and especially myocardial fibrosis) are associated with arrhythmogenesis.131,132 While emerging evidence from implantable loop recorder studies is beginning to implicate bradyarrhythmias as the major cause of SCD in ESRD, rather than the previously assumed tachyarrhythmias, the precise causes of SCD in ESRD are the subject of investigation.14 αKlotho has been found in sinoatrial node pacemaker cells in mice133 and αKlotho-deficient animals exhibit sinoatrial node dysfunction, and higher rates of bradyarrhythmias and SCD.133
FGF23 disrupts intracellular calcium cycling within the cardiomyocyte, which is an important risk factor for arrhythmogenesis. Administration of FGF23 to rat ventricular cardiomyocytes caused calmodulin-dependent protein kinase II-dependent aberrant intracellular calcium, resulting in in vitro and in vivo arrhythmogenicity. Administration of recombinant αKlotho or a pan-FGFR blocker prevented contractile dysfunction and reduced pro-arrhythmogenic activity.

Large observational studies in patients with CKD or AF and in the general population have found an association between elevated FGF23 and increased risk of developing AF. High FGF23 and low αKlotho levels are associated with periods of AF in patients with paroxysmal or persistent AF. Increased expression of FGF23, FGFR4 mRNA, and FGFR4 protein in the right atrial appendages of patients with AF has been reported and positively correlate with atrial collagen fraction. Collectively these data/studies suggest that FGF23/FGFR4 may play a role in promoting AF through atrial fibrosis.

**REVERSING OR PREVENTING UREMIC CARDIOMYOPATHY BY TARGETING THE FGF23 AND αKLOTHO AXIS**

Several therapies exist that directly or indirectly target FGF23, αKlotho, and abnormalities in bone metabolism. These are reviewed below and summarized in Table.

**Targeting Phosphate Levels in the Body**

Studies in healthy subjects have shown that circulating FGF23 levels are associated with dietary phosphate intake levels, and can be further increased by acute phosphate loading. This can be reduced, in the short-term, by aggressive reduction of dietary phosphate absorption and restriction. Overall, in relatively short-term studies, noncalcium-based phosphate binders lower FGF23 in patients with CKD/ESRD, whereas calcium-based binders do not. Calcium is thought to be a secondary stimulus for FGF23 synthesis, but lowering intestinal phosphate absorption with dietary change, phosphate binders, nicotinamide, tenapanor, or combination therapy produces only modest decreases in FGF23 that do not appear to be sustained in the long term. Whether this is because of increased total intestinal phosphate absorption by active phosphate transport, high pill burden, or intolerability of the medications is unknown.

**Targeting Vitamin D**

There is strong experimental data supporting vitamin D as a potential treatment for FGF23-mediated uremic cardiomyopathy. Calcitriol, the synthetic analogue of vitamin D₃, blocks FGF23-induced activation of FGFR4 and cardiomyocyte growth. Increases in FGF23 expression, FGFR4-induced calcineurin/NFAT signaling, and LVH in 5/6 nephrectomized rats are reduced by calcitriol. Vitamin D also increases αKlotho expression. Observational studies demonstrate a survival advantage of vitamin D therapy in patients with CKD/ESRD despite raising calcium and phosphate levels. However, in a randomized, placebo-controlled study in patients with CKD stages 4 to 5, paricalcitol (activated vitamin D₃ analogue) treatment did not reduce LV mass. Taken together, these data suggest combining vitamin D receptor activation with FGF23/FGFR4 signaling blockade could have beneficial synergistic actions on uremic cardiomyopathy.

**Targeting Parathyroid Hormone**

In patients on dialysis, the clinically available allosteric modulators (calcimimetics) of the calcium-sensing receptor, cinacalcet and etelcalcetide, are used to treat hyperparathyroidism and consistently lower circulating FGF23. In secondary analyses of the large and well-designed EVOLVE (Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events) trial, a >30% reduction in FGF23 in patients randomized to cinacalcet was associated with a reduction in cardiovascular mortality, SCD, and admissions for heart failure. The findings were amplified in those with a >50% reduction in FGF23.

In CKD patients not requiring dialysis, randomized-controlled trials of cinacalcet have reported significant reductions in FGF23, but also poor suppression of PTH as well as high rates of hypocalcemia and hyperphosphatemia. These actions are thought to negate many of the clinical benefits of calcimimetics and these agents are not licensed for use in patients with non-end-stage CKD. Nevertheless, cinacalcet remains a promising therapeutic option for the treatment of uremic cardiomyopathy in ESRD.

**Other Indirect Targets**

Intensified dialysis treatment, renal transplantation, reduced inflammation, and treatment of iron deficiency all reduce circulating FGF23 levels. Angiotensin-receptor antagonists, statins, peroxisome proliferator-activated receptor gamma agonists, and exercise all increase αKlotho expression. The clear indications for these treatments remain and may well continue to give further insights into the pathophysiology of FGF23, but it is unlikely that these interventions will be used to directly target FGF23 and αKlotho.

**DIRECTLY TARGETING FGF23**

The mechanism(s) regulating FGF23 synthesis are poorly understood and no “phosphate-sensor” has...
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<tr>
<th>Treatment</th>
<th>Study</th>
<th>Species</th>
<th>CKD Status</th>
<th>Outcome</th>
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<tr>
<td>Targeting phosphate</td>
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<tr>
<td>Dietary phosphate restriction</td>
<td>Burnett et al&lt;sup&gt;141&lt;/sup&gt;</td>
<td>Human</td>
<td>No renal impairment</td>
<td>Reduction in serum FGF23</td>
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<tr>
<td></td>
<td>Antoniucci et al&lt;sup&gt;142&lt;/sup&gt;</td>
<td>Human</td>
<td>No renal impairment</td>
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<td></td>
<td>Moe et al&lt;sup&gt;144&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3B to 4</td>
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<td></td>
<td>Di Iorio et al&lt;sup&gt;145&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3A to 4</td>
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<tr>
<td></td>
<td>Signist et al&lt;sup&gt;146&lt;/sup&gt;</td>
<td>Human</td>
<td>No CKD &amp; Stage 3A to 4</td>
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<td></td>
<td>Rodriguez-Ortiz et al&lt;sup&gt;147&lt;/sup&gt;</td>
<td>Rat</td>
<td>5/6 Nx</td>
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<tr>
<td>Calcium-sparing phosphate binders (e.g., sevelamer)</td>
<td>Oliveira et al&lt;sup&gt;148&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3A to 4</td>
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<tr>
<td></td>
<td>Block et al&lt;sup&gt;149&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3B to 4</td>
<td></td>
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<td></td>
<td>Chue et al&lt;sup&gt;150&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3</td>
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<td></td>
<td>Rodelo-Haad et al&lt;sup&gt;151&lt;/sup&gt;</td>
<td>Human</td>
<td>ESRD on HD</td>
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<td></td>
<td>Sprague et al&lt;sup&gt;152&lt;/sup&gt;</td>
<td>Human</td>
<td>ESRD on HD/PD</td>
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<tr>
<td>Nicotinamide</td>
<td>Shahbazian et al&lt;sup&gt;153&lt;/sup&gt;</td>
<td>Human</td>
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<td>Tenapanor</td>
<td>Block et al&lt;sup&gt;154&lt;/sup&gt;</td>
<td>Human</td>
<td>ESRD on HD</td>
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<td></td>
<td>Labonte et al&lt;sup&gt;155&lt;/sup&gt;</td>
<td>Rat</td>
<td>5/6 Nx</td>
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<tr>
<td>Combination therapy with lanthanum and nicotinamide</td>
<td>Ix et al&lt;sup&gt;156&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3B to 4</td>
<td>No sustained reduction in serum FGF23</td>
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<tr>
<td>Targeting Vitamin D</td>
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<tr>
<td>Calcitriol</td>
<td>Leifheit-Nestler et al&lt;sup&gt;157&lt;/sup&gt;</td>
<td>Rat</td>
<td>5/6 Nx</td>
<td>Reduction in LVH, cardiac FGF23 &amp; FGFR4 expression, and NFAT/calcineurin activation</td>
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<tr>
<td></td>
<td>Leifheit-Nestler et al&lt;sup&gt;157&lt;/sup&gt;</td>
<td>Rat (NRVM)</td>
<td>n/a</td>
<td>In vitro reduction in FGF23-induced cardiomyocyte hypertrophy</td>
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<td>Calcitriol &amp; paricalcitol</td>
<td>Lau et al&lt;sup&gt;158&lt;/sup&gt;</td>
<td>Mice</td>
<td>Partial renal ablation, phosphate loaded</td>
<td>Increase in serum αKlotho. No effect on renal/parathyroid αKlotho expression</td>
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<tr>
<td>Paricalcitol</td>
<td>Ritter et al&lt;sup&gt;159&lt;/sup&gt;</td>
<td>Rat</td>
<td>5/6 Nx</td>
<td>Preservation of renal αKlotho, and increase in parathyroid αKlotho expression in uremia</td>
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<td>Targeting parathyroid hormone</td>
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<td>Cinacalcet</td>
<td>Moe et al&lt;sup&gt;160&lt;/sup&gt;</td>
<td>Human</td>
<td>ESRD on HD</td>
<td>Reduction in serum FGF23, cardiovascular death, SCD, and heart failure</td>
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<td></td>
<td>Charytan et al&lt;sup&gt;161&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3A to 4</td>
<td>Reduction in FGF23 and PTH</td>
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<tr>
<td></td>
<td>Chonchol et al&lt;sup&gt;162&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3A to 4</td>
<td>Reduction in FGF23 and PTH; increase in hypocalcemia</td>
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<tr>
<td>Other indirect targets</td>
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<tr>
<td>Intensified (daily) hemodialysis</td>
<td>Zaritsky et al&lt;sup&gt;163&lt;/sup&gt;</td>
<td>Human</td>
<td>ESRD on HD</td>
<td>Reduction in FGF23 vs conventional hemodialysis</td>
</tr>
<tr>
<td>Renal transplantation</td>
<td>Barros et al&lt;sup&gt;164&lt;/sup&gt;</td>
<td>Human</td>
<td>ESRD (4x on HD)</td>
<td>Reduction in FGF23 and phosphate</td>
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<tr>
<td>Treatment of iron deficiency (e.g., ferric citrate)</td>
<td>Block et al&lt;sup&gt;165,166&lt;/sup&gt;</td>
<td>Human</td>
<td>CKD Stage 3A to 5</td>
<td>Reduction in serum FGF32</td>
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<tr>
<td>Inhibition of inflammation (e.g., NFκB inhibitor)</td>
<td>Rodriguez-Ortiz et al&lt;sup&gt;147&lt;/sup&gt;</td>
<td>Rat</td>
<td>No renal impairment</td>
<td>Attenuation of LPS-induced FGF23 elevation</td>
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<tr>
<td>ATII receptor blockade</td>
<td>Yoon et al&lt;sup&gt;167&lt;/sup&gt;</td>
<td>Mice</td>
<td>CsA-induced renal injury</td>
<td>Increase in renal αKlotho expression</td>
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<td>Statins (e.g., atorvastatin, pitavastatin)</td>
<td>Narumiya et al&lt;sup&gt;168&lt;/sup&gt;</td>
<td>Mouse (IMCD3)</td>
<td>n/a</td>
<td>In vitro upregulation of αKlotho mRNA expression</td>
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<td>PPARγ agonist (e.g., pioglitazone)</td>
<td>Yang et al&lt;sup&gt;169&lt;/sup&gt;</td>
<td>Rat</td>
<td>No renal impairment</td>
<td>Increase in renal αKlotho expression</td>
</tr>
<tr>
<td>Exercise</td>
<td>Matsubara et al&lt;sup&gt;170&lt;/sup&gt;</td>
<td>Human</td>
<td>No renal impairment</td>
<td>Increase in serum/plasma αKlotho</td>
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<td>Tan et al&lt;sup&gt;171&lt;/sup&gt;</td>
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(Continued)
yet been found in mammals.\textsuperscript{186,187} Animal data have recently suggested that sodium-phosphate cotransporter PiT2 found in bone might regulate phosphate-dependent FGF23 synthesis and that targeting PiT2 could potentially reduce FGF23 synthesis.\textsuperscript{187} The development of novel small molecules against PiT2 or the yet-to-be characterized PiT2–FGF23 pathway would give a proof of principle approach in animals for blocking FGF23 synthesis.

Indiscriminate FGF23 neutralization with monoclonal antibodies has been shown to worsen hyperphosphatemia, and increase vascular calcification and mortality in rat models of CKD.\textsuperscript{188,189} Use of anti–FGF23 monoclonal antibodies such as burosumab, currently approved for the treatment of x-linked hypophosphatemia, causes severe side effects in patients with CKD by decreasing phosphaturia.\textsuperscript{190} Analogous to the use of calcimimetics, total blockade of FGF23 may theoretically be of benefit in ESRD. From a clinical therapeutic and drug development perspective, the ideal target would be the FGFR responsible for the adverse cardiac effects of FGF23 and not FGFR1, which is critical for maintaining normal phosphate levels. Indiscriminate blockade of FGFRs, although shown to be effective at preventing the development of,\textsuperscript{191} and reversing LVH in rodents,\textsuperscript{74} results in cardiac toxicity, hyperphosphatemia, and ectopic calcium deposition.\textsuperscript{192} Targeting cardiac FGFR4, especially

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study</th>
<th>Species</th>
<th>CKD Status</th>
<th>Outcome</th>
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</thead>
<tbody>
<tr>
<td>Directly targeting FGF23</td>
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<td>FGF23 neutralizing antibodies</td>
<td>Hasegawa et al\textsuperscript{189}</td>
<td>Rat</td>
<td>Anti-GBM nephritis</td>
<td>Increase in PTH; increase in vitamin D, calcium and phosphate</td>
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<tr>
<td></td>
<td>Shalhoub et al\textsuperscript{186}</td>
<td>Rat</td>
<td>5/6 Nx</td>
<td>In addition to above, increase in mortality &amp; aortic calcification</td>
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<td>FGFR antagonists</td>
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<td>FGFRI antibody</td>
<td>Grabner et al\textsuperscript{175}</td>
<td>Rat</td>
<td>5/6 Nx</td>
<td>Attenuation of LVH</td>
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<td>In vitro inhibition of FGF23-induced cardiac myocyte hypertrophy</td>
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<td>Pan-FGFR antibody</td>
<td>Faul et al\textsuperscript{174}</td>
<td>Rat</td>
<td>5/6 Nx</td>
<td>Attenuation of LVH</td>
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<td></td>
<td>Di Marco et al\textsuperscript{177}</td>
<td>Rat</td>
<td>5/6 Nx</td>
<td>Reduction in LV mass and fibrosis; improvement in ejection fraction</td>
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<tr>
<td></td>
<td>Yanochko et al\textsuperscript{192}</td>
<td>Rat</td>
<td>No renal impairment</td>
<td>Cardiac toxicity, hyperphosphatemia and ectopic calcification</td>
</tr>
<tr>
<td>Sodium-phosphate co-transporter PiT2 knockout</td>
<td>Bon et al\textsuperscript{197}</td>
<td>Mice</td>
<td>No renal impairment</td>
<td>PIT2 regulates FGF23 synthesis; potential target for therapeutics</td>
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| Directly targeting αKlotho    |                                |         |                  |                                                              |
| Intravenous αKlotho transgene | Xie et al\textsuperscript{198} | Mice    | 5/6 Nx; heterozygous Klotho | Attenuation of cardiac hypertrophy and fibrosis |
| Recombinant αKlotho           | Hu et al\textsuperscript{196} | Mice    | Uni-nephrectomy + contralateral IR injury | Preservation of cardiac function, reduced hypertrophy and fibrosis; attenuation of renal fibrosis |
|                               | Yang et al\textsuperscript{178} | Mice    | 5/6 Nx           | Inhibition of LVH and reduction in myocardial reactive oxygen species production |
|                               | Yu et al\textsuperscript{200} | Mice    | No renal impairment | Attenuation of angiotensin II-induced cardiac hypertrophy, fibrosis, and dysfunction |
|                               | Suassuna et al\textsuperscript{193} | Rat     | 5/6 Nx           | Reduction of uremic cardiac remodeling (hypertrophy and fibrosis) |
| Small molecule αKlotho modulators | King et al\textsuperscript{201} | Human (HEK293) | n/a          | In vitro elevation of αKlotho protein expression |

5/6 Nx indicates 5/6 nephrectomized; anti-GBM, anti-glomerular basement membrane; ATII, angiotensin II; CKD, chronic kidney disease; CM, cardiomyocyte; CsA, cyclosporine A; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FGF23, fibroblast growth factor-23; FGFR, fibroblast growth factor receptor; HD, hemodialysis; HEK293, human embryonic kidney 293 cells; IR, ischemia-reperfusion; LPS, lipopolysaccharide; LV, left ventricle; LVH, left ventricular hypertrophy; n/a, not applicable; NFAT, nuclear factor of activated T-cells; NRVM, neonatal rat ventricular myocytes; PD, peritoneal dialysis; PPARγ, peroxisome proliferator-activated receptor γ; PTH, parathyroid hormone; and SCD, sudden cardiac death.
its αKlotho-independent activation of downstream signaling pathways, represents an exciting possibility. Indeed, FGFR4-blocking antibodies have been shown to inhibit FGF23-induced hypertrophy of isolated rat cardiomyocytes in vitro, and attenuated LVH in a 5/6 nephrectomy rat model of CKD.75 Currently, very little is known about the specific FGFRs mediating the actions of FGF23 in nonmyocyte cardiac cells including fibroblasts, or whether blocking FGFR4 prevents or reverses cardiac fibrosis.128 Several anti-FGF small molecule tyrosine kinase inhibitors and FGFR-analogues currently in development are mainly for use in oncology.193 Development of these agents specifically for the treatment of uremic cardiomyopathy is, therefore, a real possibility.

DIRECTLY TARGETING αKLOTHO

In animal studies, administration of αKlotho protein has been shown to be effective in protecting against progression of CKD.194–197 Intravenous administration of a transgene-encoding soluble αKlotho reduces LVH in αKlotho-deficient mice.198 Recombinant αKlotho also attenuates cardiac remodeling, fibrosis,195,199 reactive oxygen species production, and LVH117 induced by CKD in mice. In another study, αKlotho improved cardiac function and reduced hypertrophy and fibrosis in a mouse model of hypertension, although decreasing FGF23 expression.200 However, it remains unclear whether αKlotho is cardioprotective in the absence of increased FGF23.

Elevated FGF23 and decreased circulating αKlotho are observed in both aging and in CKD,116,117,198 leading to speculation that both CKD and the age-related decline in this and other physiological functions are caused in part by increased FGF23 and decreased αKlotho.55 If true, this would assume soluble αKlotho acts as an inhibitor of αKlotho-independent actions of FGF23. Potential mechanisms include inhibiting FGF23/FGFR4 signaling by either binding first to FGFR4 or via an initial interaction with FGF23 (decoy receptor). An alternative mechanism involves FGFR3 and FGFR4 forming a complex in the presence of soluble αKlotho but activating FRS2α/Ras/mitogen-activated protein inase rather than PLCγ/calcineurin/NFAT signaling (Figure 1).55 Therefore, development of αKlotho-mimetics, through the development of protein–protein inhibitors, provides another potential therapeutic option. Trials of such agents to prevent progression of CKD are expected to start in the next couple of years.

To date, small molecule αKlotho modulators have been identified from a high-throughput screen of 150,000 compounds with those showing most promise being αKlotho transcription activators. Furthermore, extracellular signal-regulated kinase phosphorylation in FGFR-transfected cells increased, demonstrating an effect on FGF23 signaling.201 The recently discovered crystal structure of αKlotho:FGF23:FGFR1 in a 1:1:1 relationship has provided new insight into this dynamic interplay of factors and may reveal new therapeutic options.62 Although clearly at an early stage of exploration, the identification of new small molecules demonstrates the potential of drugs acting via αKlotho.

CONCLUSIONS

While patients with early stages of CKD are at increased risk of atherosclerotic complications, later stages of kidney disease are associated with heart failure and sudden death caused by uremic cardiomyopathy. Significant progress has been made over the past 2 decades in our understanding of, and ability to study the pathological basis of uremic cardiomyopathy using native T1 mapping. There are clear clinical data illustrating an association of increased FGF23 and reduced αKlotho with uremic cardiomyopathy in patients with CKD, and in heart failure and AF in subjects without known CKD. However, whether FGF23 has a truly causal relationship in uremic cardiomyopathy remains controversial. Characterization of the receptors and molecular pathways by which FGF23 might mediate LVH, cardiac fibrosis, and arrhythmias will help to identify therapeutic targets. Further work is required to identify the interplay between FGF23, cardiomyocytes and fibroblasts, and the effects of these interactions on the subsequent cardiac remodeling to reveal the molecular and cellular targets of FGF23 and αKlotho. Improved understanding is likely to enable the development of novel therapeutic interventions capable of effectively reducing the excess cardiovascular risk associated with CKD/ESRD, and perhaps even the risk of AF and heart failure in patients without CKD.

ARTICLE INFORMATION

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Targeting FGF23, αKlotho in Uremic Cardiomyopathy
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