Introduction
CKD is a significant health care burden that afflicts 10% of the world population (1). Progression of CKD includes tubular epithelial cell injury, renal inflammation, and fibrosis (2,3). There has been extensive focus on phosphate regulation by parathyroid hormone (PTH) and the hormone fibroblast growth factor 23 (FGF23) during the progression of CKD (3,4). Urinary excretion of phosphate increases during the progression of CKD to maintain proper plasma phosphate levels (3,4). Unfortunately, the enhanced nephron phosphate excretion can be detrimental to epithelial cells, resulting in further kidney damage (2–4). The research findings in the Kidney360 article by Stubbs et al. (5) provides convincing evidence that increased osteopontin in early CKD critically maintains phosphate solubility to facilitate clearance of mineral aggregates.

Phosphate, Calcium-Phosphate Nanocrystals, and CKD
Phosphate levels are regulated through kidney and intestinal phosphate transport. Intestinal epithelium transports dietary phosphate to the bloodstream, and phosphate is excreted from the body by the kidney. Bone phosphate uptake and release is another important place for regulating serum phosphate levels. PTH is a key regulator of the intestinal, bone, and kidney sodium-coupled phosphate transporters to maintain proper phosphate levels. Regrettably, hyperphosphatemia occurs during the progression of CKD, which has detrimental consequences on soft tissue calcification and cardiovascular function.

Intensive research has focused on the regulation of phosphate homeostasis during CKD (4,6). The contributions of PTH and vitamin D to phosphate and calcium homeostasis have been extensively evaluated (4,6). More recently, critical roles for soluble αKlotho and FGF23 to modulate intestinal phosphate absorption, urinary phosphate excretion, and phosphate distribution into bone during CKD have been determined (3,6). Elevated levels of FGF23 occur in CKD, and FGF23 and αKlotho regulate sodium-dependent phosphate transport across the epithelial cell apical membrane (3,6). Intriguingly, increased FGF23 levels act to combat hyperphosphatemia in CKD; however, the increased FGF23 and urinary phosphate excretion is associated with increased risk of kidney function decline (3,6). This is because high concentrations of tubular phosphate promote calcium-phosphate nanocrystal formation that drives tubular injury and kidney fibrosis (3,4,6). The novel finding of Stubbs et al. (5) in this Kidney360 article is that these calcium-phosphate nanocrystals induce osteopontin to solubilize the high tubular concentrations of phosphate and prevent further nanocrystal formation.

Evidence for Osteopontin to Maintain Phosphate Solubility
Osteopontin is a phosphoprotein that functions as a proinflammatory cytokine to promote cell-mediated immune responses (7,8). In addition, osteopontin has beneficial actions, such as biomineralization and wound healing (8). There is significant evidence that osteopontin is involved in heart attacks, atherosclerosis, diabetes, chronic inflammatory diseases, and CKD (7,8). Chronic elevations in osteopontin predict poor prognosis of a major adverse cardiovascular event, independent of other cardiovascular risk factors (8).

Kidney osteopontin is localized to the thick ascending limb of the loops of Henle and distal nephrons in the steady state (7). On the other hand, in pathologic conditions, osteopontin is upregulated and thought to play a critical role in interstitial fibrosis associated with proximal tubules (7). In this regard, genetic osteopontin deficiency decreases angiotensin II–induced inflammation, oxidative stress, and kidney fibrosis (9). Factors that upregulate kidney osteopontin include hormones, cytokines, hypoxia, and hyperglycemia (7). Increased plasma osteopontin levels are associated with the development of microalbuminuria and negatively correlated with GFR in patients with diabetes (10). Interestingly, in the current Kidney360 article, Stubbs et al. (5) demonstrate very early osteopontin upregulation in CKD mouse models and present evidence for osteopontin to solubilize tubular phosphate, resulting in decreased calcium-phosphate crystal formation.

The experimental findings of Stubbs et al. (5) provide ample evidence for this critical role of osteopontin using several experimental approaches. Initial
experiments demonstrated that tubular osteopontin expression is increased in CKD mouse models. Kidney immunohistochemistry identified increased osteopontin expression in cystic kidney disease (pcy/pcy), tubular toxic (aristolochic acid), and GN (Col4a3/−/−) CKD mice models. These studies demonstrated that osteopontin expression was not restricted to the distal tubule in CKD. The increase in kidney tubular osteopontin (Spp1) gene expression and urinary osteopontin was detected before elevations in BUN, PTH, or FGF23 in a Col4a3/−/− Alport syndrome mouse model. Additional evidence for induction of kidney Spp1 gene expression in association with reduced functional nephron numbers was observed in unilaterally nephrectomized mice. The increase in osteopontin observed in unilaterally nephrectomized mice was evident in cortical and medullary tubular segments.

The mechanisms by which osteopontin expression increased in tubular segments was determined in two phosphaturic mouse models and in two types of cultured renal tubular epithelial cells. Phosphaturic mouse models included the phosphate-wasting NaPi2a−/− mice and Hyp mice that exhibit excess urinary phosphate excretion due to high serum FGF23 levels. These phosphaturic mouse models demonstrated increased kidney Spp1 gene expression and osteopontin protein expression. Experiments using mouse (IMCD-3) or human (RTCE) collecting duct cells demonstrated that calcium-phosphate nanocrystals stimulated Spp1 gene expression. Taken together, these findings demonstrate that increased tubular phosphate or calcium-phosphate nanocrystals directly induce Spp1 gene expression and the generation of osteopontin.

Another main finding by Stubbs et al. (5) was that osteopontin (Spp1) genetic deficiency resulted in severe nephrocalcinosis in CKD mice. For these studies, CKD was induced by an adenine-containing diet or injection of aristolochic acid. Kidney microcomputed tomography scans revealed extensive mineral deposition in CKD mice that was greatly increased in Spp1−/− CKD mice. Interestingly, there was no difference in inflammation noted between the wild-type and Spp1−/− CKD mice. There was also no difference in serum calcium and phosphate or BUN. Additional studies evaluated neutralization of the osteopontin mineral-binding (ASARM) peptide sequence on nephrocalcinosis in Hyp mice. Neutralization of the osteopontin phosphorylated poly-aspartate region (ASARM) prevents the ability of osteopontin to bind to hydroxyapatite (calcium phosphate) to enhance mineral solubility. Significant nephrocalcinosis was found in Hyp phosphaturic mice administered the ASARM inhibitor (SPR4). Taken together, these findings demonstrate that osteopontin serves a critical role of opposing mineral deposition and nephrocalcinosis in CKD (Figure 1).

**Conclusion**

Osteopontin is an intracellular protein and soluble excreted cytokine that regulates tissue remodeling and immune infiltration in the kidney (7,8). It has positive actions, such as wound healing, whereas increased cardiovascular risk and kidney injury have been associated with increases in osteopontin levels (7,8). The findings of Stubbs et al. (5) provide a new critical role to increase phosphate solubility in early CKD. These findings have potential implications for early detection of CKD progression and therapeutics for preventing CKD progression.

A critical finding by Stubbs et al. (5) was that osteopontin levels increased before increases in BUN. Could elevations in osteopontin levels predict the onset of CKD? On the other hand, an inability to increase kidney osteopontin levels in response to tubular phosphate levels or calcium-phosphate nanocrystals could lead to rapid progression of CKD. Interestingly, inactivating mutations in SLC34A1 and SLC34A3, which directly reduce renal phosphate reabsorption and increase tubular phosphate levels, cause nephrocalcinosis and kidney stones (11). Are there mutations in Spp1 that lead to decreased osteopontin levels in response to elevations in tubular phosphate? Osteopontin gene
polymorphic variants have been associated with the pathogenesis and progression of cancer and autoimmune, neurodegenerative, and cardiovascular diseases (7). Lower urinary osteopontin levels have also been demonstrated in patients with kidney stones (12). It would be predicted that genetic variants that decrease osteopontin could result in rapid progression of CKD. On the therapeutic side, ASARM peptides can significantly enhance mineral solubility (13). What is unknown is if administration of ASARM peptides to enhance mineral solubility could slow CKD progression. Excitedly, information on osteopontin regulation could affect treatment strategies in patients with CKD.

Disclosures
J.D. Imig reports serving in an advisory or leadership role for the American Heart Association, American Physiological Society, and Biochemical Society; having patents or royalties with the Medical College of Georgia and Medical College of Wisconsin; being employed by the Medical College of Wisconsin; having ownership interest in MetaSyn Therapeutics and Nephega; and receiving research funding from the National Institutes of Health (NIH).

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Author Contributions
J.D. Imig conceptualized the study, wrote the original draft, and reviewed and edited the manuscript.

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