Sclerostin in chronic kidney diseasemineral bone disorder : think first before you block it!

Figure 1: µ-CT scans of the femur metaphysis and diaphysis of 35-week-old sclerostin knockout ($SOST^{-/-}$) animals compared to wildtype (WT) littermates (Kaesler N, Verhulst A: data on file)

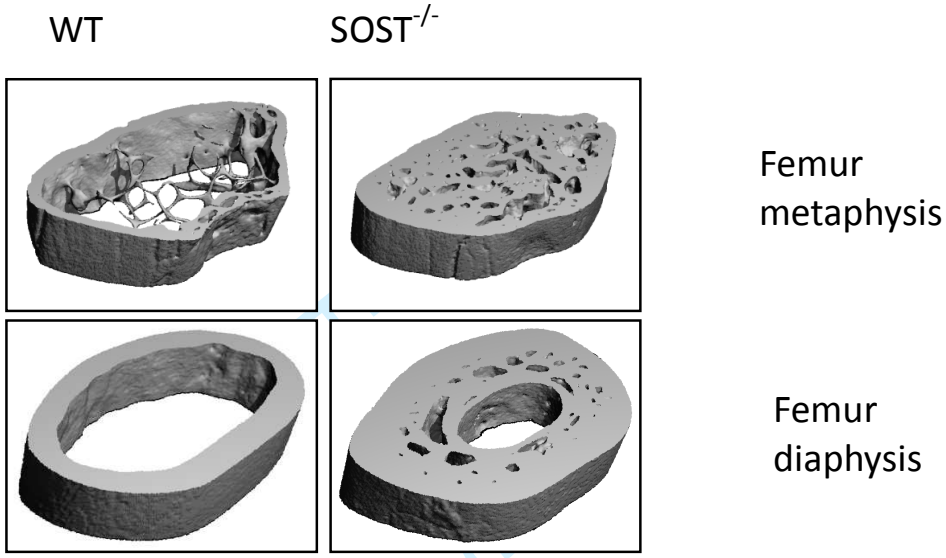

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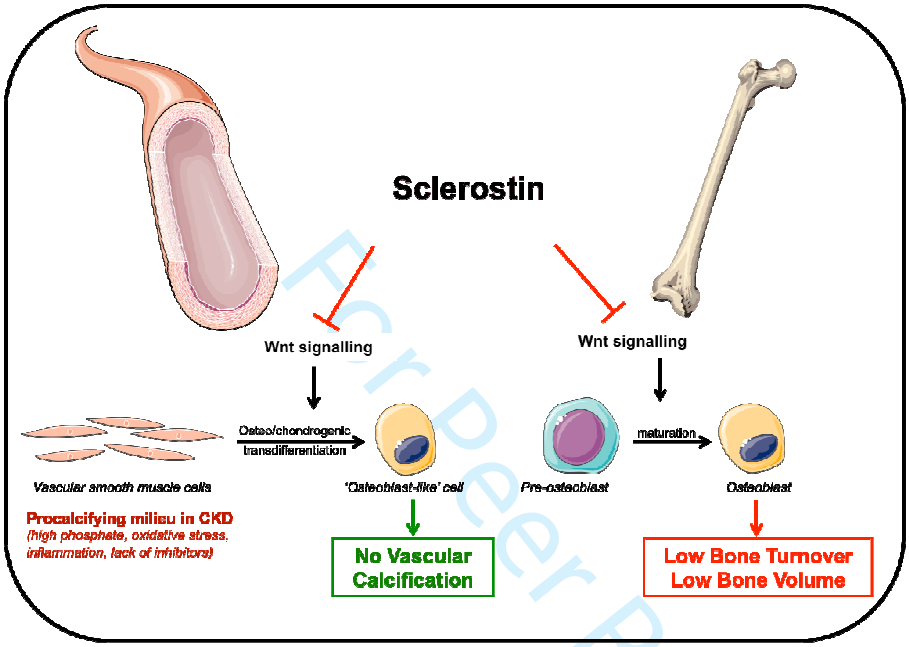
Figure 2: Activity of Wnt signalling, and specifically of sclerostin as a Wnt antagonist, is not limited to the bone compartment. Wnt signalling also influences the integrity of the arterial wall. Hence, blocking sclerostin will impact the vascular calcification processes. Theoretically, sclerostin should help to prevent vascular calcification, as shown in the left part of the figure.



Sclerostin in CKD-MBD: Think first before you block it!

Vincent M. Brandenburg [1,2], Anja Verhulst [3], Anne Babler [4],

Patrick C. D`Haese [3], Pieter Evenepoel [5], Nadine Kaesler [4],

[1] Department of Cardiology, University Hospital of the RWTH Aachen, Germany;
[2] Department of Cardiology, Rhein-Maas Klinikum, Würselen, Germany;
[3] Laboratory of Pathophysiology, Department of Biomedical Sciences, University of Antwerp, Wilrijk, Belgium;
[4] Department of Nephrology, University Hospital of the RWTH Aachen, Germany;
[5] Department of Immunology and Microbiology, Laboratory of Nephrology, KU Leuven and University Hospitals Leuven, Leuven, Belgium;

Corresponding author

Professor Vincent M. Brandenburg
Vincent.Brandenburg@post.rwth-aachen.de

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Abstract

Canonical Wnt signalling activity is a major player in physiological and adaptive bone metabolism. Wnt signalling is regulated by soluble inhibitors, with sclerostin being the most widely studied. Sclerostin's main origin is the osteocyte and its major function is blockade of osteoblast differentiation and function. Therefore, sclerostin is a potent inhibitor of bone formation and mineralisation. Consequently, blocking sclerostin via human monoclonal antibodies (such as romosozumab) represents a promising perspective for treatment of (postmenopausal) osteoporosis. However, sclerostin's physiology and the effects of sclerostin monoclonal antibody treatment are not limited to the skeleton. Specifically the potential roles of sclerostin in chronic kidney disease (CKD) and associated pathologies covered by the term CKD-MBD (chronic kidney disease and mineral bone disorder), which also includes accelerated cardiovascular calcification, warrant specific attention. CKD-MBD is

a complex disease condition in which sclerostin antibodies may interfere at different levels and influence the multiform interplay of hyperparathyroidism, renal osteodystrophy and vascular calcification, but the clinical sequelae remain obscure. The present review summarizes the potential effects of sclerostin blockade in CKD-MBD. We will address and summarize the urgent research targets that are being identified and that need to be addressed before a valid risk-benefit ratio can be established in the clinical setting of CKD.

Physiology of sclerostin: What clinicians should know.

Wnt signalling encompasses several (at least three) different signalling pathways including the canonical Wnt/beta-catenin pathway [1;2]. The predominant function of the canonical Wnt pathway is stabilisation of beta-catenin by inhibiting the activity of the beta-catenin degradation complex [3]. The function of the beta-catenin degradation complex is to phosphorylate beta-catenin [4]. Phosphorylation renders beta-catenin susceptible to proteolysis, whereby it does not accumulate in the nucleus. Only unphosphorylated beta-catenin can translocate into the nucleus and modulate target gene transcription locally [4]. Secreted Wnt inhibitors are a group of proteins which interfere with the extracellular binding of Wnt ligands to the transmembrane receptor complex [3;5]. Amongst these, sclerostin, and to a lesser extent Dickkopf-related protein 1 [DKK-1]), have previously been studied intensively [6]. Sclerostin (22kDa) is a member of the cystatin knot family of proteins and is a product of the SOST gene [7]. It is measurable in human serum [8] and meanwhile also qualified as a therapeutic target in osteoporosis [9]. Sclerostin prevents beta-catenin nuclear translocation and subsequent gene expression. It is secreted almost exclusively by osteocytes, and to a lesser extent by other cell types including osteoclast precursors [10]. Hence, viable osteocytes regulate the proper functionality of the skeleton via sclerostin synthesis and release [6;10;11]. Sclerostin deficiency associates with high bone mass as impressively revealed by the murine knockout model [12] (**Figure 1**). Loss-of-function mutations were reported in patients with van Buchem disease, a disorder closely resembling sclerosteosis [13;14]. These human disorders coincide with the reduced activity of sclerostin. Sclerosteosis is caused by loss-of-function mutations in the *SOST* gene on chromosome 17q12-q21, which encodes sclerostin. In contrast, patients with van Buchem disease have a 52-kb homozygous noncoding deletion 35 kb downstream of the *SOST* gene, which is essential for the transcription of the gene in bone [13;14]. Life expectancy in sclerosteosis is reduced, with a large proportion of patients dying in early adulthood, mainly from complications of increased intracranial pressure [14].

Sclerostin overexpression revealed the expected opposite phenotype, i.e. low bone mass as a consequence of reduced bone formation [15]. Targeting sclerostin via monoclonal antibody treatment has the potential to become a cornerstone of osteoporosis therapy due to its potent osteoanabolic activity [9]. However, before introducing widespread therapeutic use in patients with CKD as well, it is crucial to consider the specific complex situation in these patients. Indeed, in CKD, the intricate skeletal (renal osteodystrophy), hormonal (hyperparathyroidism) and vascular (calcification) changes and sclerostin's role herein should be carefully weighed against each other.

Sclerostin antibodies and osteoporosis: it works!

The comprehensive insights into the osseous mode of action of sclerostin antibodies come from various animal models. Precinical studies in monkeys [16] revealed that the application of a humanized sclerostin monoclonal antibody increased the bone mineral content (BMC) and/or BMD at the femoral neck, radial metaphysis and tibial metaphysis. Bone histomorphometry showed marked dose-dependent increases in bone formation on trabecular, periosteal, endocortical, and intracortical surfaces, consistent with increased recruitment, activation, and/or survival of osteoblasts.

In young rats, application of sclerostin antibodies augmented cancellous and cortical bone mass and induced a strong increase in bone formation rate [17]. Accordingly, administration of sclerostin antibodies in 6-month-old rats that had undergone femoral osteotomy resulted in a significantly increased mineral apposition rate, mineralized surface and bone formation rate in trabecular bone of the distal femora [18]. It is noteworthy that romosozumab, a human monoclonal sclerostin antibody, has a dual mode of action in that in addition to its predominant influence upon bone formation, romosozumab also suppresses bone resorption; in human interventional trials, romosozumab application led to a sustained decrease in beta-CTX of up to 50% [9]. Application of romosozumab is an effective treatment to increase bone mineral density and reduce vertebral fracture risk in humans. In a previous 12-month Phase II trial, romosozumab treatment significantly increased bone mass in postmenopausal osteoporosis [19].

These findings were confirmed and extended in a Phase III study - the FRAME trial [9] in which 6390 postmenopausal osteoporotic women received romosozumab or placebo. Active treatment significantly reduced the vertebral fracture risk after 12 months (risk ratio 0.27,

95% CI 0.16 – 0.47; p<0.001). However, romosozumab therapy was less clearly effective in risk reduction for non-vertebral fractures (0.75; 95% CI 0.53 – 1.05; p=0.10) [9].

Finally, the ARCH study confirmed fracture reducing properties of romosozumab in about 4000 postmenopausal women [20]. Over a period of 24 months, a 48% lower risk of new vertebral fractures was observed in the romosozumab-followed-by-alendronate group (6.2% [127 of 2046 patients]) than in the alendronate-followed-by-alendronate group (11.9% [243 of 2047 patients]) (P<0.001) [20]. In the ARCH study the risk of hip fractures was also lowered by 38% in the romosozumab group: (41 of 2046 patients (2.0%) versus 66 of 2047 patients (3.2%). Noteworthy, the ARCH trial raised safety concerns regarding the use of the sclerostin monoclonal antibody: during year 1, positively adjudicated serious cardiovascular adverse events were observed more often with romosozumab than with alendronate (50 of 2040 patients [2.5%] vs. 38 of 2014 patients [1.9%]) [20] – a finding which will undergo comprehensive review below. In summary, romosozumab represents a novel and effective osteoanabolic treatment strategy in human osteoporosis, at least with regard to postmenopausal fractures.

The potential role of sclerostin and sclerostin blockade in nephrology.

The above-mentioned physiological aspects and first results in postmenopausal osteoporosis studies turn sclerostin into an interesting research domain within the field of nephrology. Serum sclerostin measurements recently gained some interest in CKD as sclerostin levels varied with renal function [21] and serum levels were associated with a favourable outcome (the higher – the better) [22]. However, a substantial inter-variability has been reported between various sclerostin assays [23]. Noteworthy the association between serum sclerostin and outcome varies among different cohorts [22;24;25]. Based on these contradictory results from studies investigating the role of sclerostin as a prognostic biomarker and weak standardization of assays we currently cannot recommend measuring serum sclerostin in clinical routine.

The anabolic property renders sclerostin blockade particularly interesting for nephrologists who face the threat of adynamic bone disease in many of their patients [26]. Before applying it to CKD patients, however, we need to know more about sclerostin's involvement in renal

osteodystrophy; moreover, we need to examine what might happen with sclerostin antibody treatment in this particular clinical setting.

Sclerostin and renal osteodystrophy

Sclerostin is increasingly acknowledged as a modulator of renal osteodystrophy. The *jck* mouse model, which is a model of moderate progressive renal failure, nicely shows the time-course of CKD-MBD parameters as kidney dysfunction progresses [27]. Interestingly, osteocytic sclerostin expression and consecutive suppression of beta-catenin signalling in this mouse model occurs earlier (at week 5) than changes of PTH or FGF-23 (at week 10) The increased sclerostin expression also preceded cardiovascular and skeletal changes, typically seen in CKD-MBD and starting at about week 15 and week 9, respectively. The rise in sclerostin-positive osteocytes was transient and diminished in parallel with the severity of the developing hyperparathyroidism. These findings allow speculation that sclerostin is involved early in the development of renal osteodystrophy with some PTH-mediated counter-regulatory effects. We acknowledge that these findings, which are indicative of a subtle time-course of renal osteodystrophy changes, should be re-evaluated in other models of renal failure. Our understanding about the role of sclerostin in uremic bone disease grew substantially with experiments about the development of renal osteodystrophy in the absence of this early rise in sclerostin. Two working groups examined the renal osteodystrophy phenotype of sclerostin-deficient mice that had undergone 5/6th nephrectomy [28;29]. One CKD model revealed a low [28], the other one a pronounced renal hyperparathyroidism [29]. Overall, the sclerostin deficiency was characterized by high cortical thickness, lower cortical porosity, lower bone marrow area, and particularly, high bone volume as detected by µCT compared to wildtype (WT) mice. Both groups came to a similar conclusion upon analysis of the skeletal phenotype: The development of renal osteodystrophy was masked by the overwhelming phenotype of the homozygous sclerostin deficiency [28;29]. It is currently unknown which phenotype the opposite genetic model, i.e. overexpression of skeletal sclerostin, might exhibit in the setting of CKD and what might be the effect on the development of renal osteodystrophy.

Data regarding sclerostin's involvement in human renal osteodystrophy are limited: a study in which 60 adult dialysis patients underwent a bone biopsy after tetracycline labelling revealed a statistically significant negative correlation between serum sclerostin and PTH [30].

Most importantly, sclerostin showed pronounced negative associations with parameters of bone turnover, pointing towards a role of increased sclerostin levels in the development of adynamic bone disease [30]. Another bone biopsy study was done in patients with various levels of CKD [31]. The authors quantified both serum and bone sclerostin and found a weak correlation between the two. They confirmed a potential association between skeletal expression of sclerostin and turnover, since patients with high turnover had lower bone sclerostin expression than those with low bone turnover [31]. Interstingly, the bone expression of sclerostin quantified by immunohistochemistry varied significantly between different stages of CKD and revealed its peak in CKD stage 2 and 3. In all CKD stages it was higher than in healthy controls [31]. Noteworthy, sclerostin is not a lone warrior: Other CKD-MBD mediators and Wnt signalling inhibitors act in concert in this setting, i.e. directly inhibit osteoblastic Wnt activity and promote skeletal resistance to anabolic stimuli such as FGF23 [32] or klotho [33]. However, putting forward the hypothesis that the development of adynamic bone disease in humans is (in part) mediated by a state of overactivity or sclerostin and similar mediators, thereby counterbalancing other osteoanabolic effectors, is currently still highly speculative. It is, however, noteworthy that PTH and sclerostin interact strongly with each other on a physiological basis. Hence, the PTH-sclerostin "balance" is a suitable target in modulating the development of renal osteodystrophy.

Sclerostin and PTH and their physiological interplay: they need each other.

PTH downregulates sclerostin expression in osteocytes, and this interaction represents an important aspect of how PTH stimulates bone metabolism [34]. There is substantial experimental evidence available that a balanced cross-talk between PTH and sclerostin is relevant for bone physiology. In healthy conditions, the anabolic activity of PTH, to a certain extent, is mediated by suppressing the anti-anabolic activity of sclerostin. PTH administration rapidly reduces sclerostin mRNA as well as protein synthesis in osteocytes [34;35]. Kramer and co-workers convincingly showed skeletal PTH actions to rely upon sclerostin physiology [12]. They investigated the skeletal effects of intermittent PTH administration in mouse models with sclerostin overexpression and also with sclerostin deficiency. Six-month-old genetically engineered mice of both types underwent a two-month treatment period with 1-34 PTH. Both sclerostin-deficient as well as sclerostin-overexpressing mice revealed the expected skeletal phenotype, i.e. high bone mass in the former and severe osteopenia in

the latter. In both mouse models, the response to intermittent PTH treatment in terms of stimulation of bone metabolism was significantly diminished. Therefore, the authors came to the conclusion that suppression of sclerostin in osteocytes is necessary to mediate anabolic responses to PTH [12]. As discussed above, uraemia may impel sclerostin expression and this chronic stimulation may turn sclerostin irresponsive to PTH, and this might be one important factor in skeletal uraemia-associated PTH resistance. Alternatively, high sclerostin levels may be the consequence of PTH resistance in the osteocytes. Additional research is needed to clarify this chicken-and-egg issue.

In addition, sclerostin is thought to participate in several biochemical feedback loops, as evidenced by the fact that sclerostin-deficient mice reveal alterations in a number of classical biochemical CKD-MBD-related parameters [36]. While serum calcium and PTH levels were not different between sclerostin knockout and wildtype mice with normal renal function, FGF-23 levels were about 2.5 times lower in sclerostin-deficient mice compared to their wildtypes, and vice versa, 1,25-dihydroxyvitamin D and serum phosphate levels were significantly elevated. Sclerostin also directly alters vitamin D synthesis in proximal tubular cells [36].

Taken together, the discovery of sclerostin sheds novel light on the long-standing discussion about skeletal PTH resistance in CKD and also on the pathophysiology of adynamic bone. From a therapeutic perspective, romosozumab appears to be an attractive option for haemodialysis patients, in particular because of the intermittent monthly application strategy which might easily help overcome issues related to non-adherence in this patient cohort due to their particularly high pill burden. However, are we ready to apply romosozumab in severe CKD?

Sclerostin antibodies in the setting of underlying renal osteodystrophy: a new hope?

In patients with normal renal function or only mild impaired renal function, such as those participating in the Phase II and Phase III romosozumab trials mentioned above, application of the antibody was associated with a decrease in serum calcium and increase in PTH levels. Such a finding can be interpreted as a reflection of stimulated bone anabolism or reduced bone resorption respectively resulting in increased calcium incorporation into and/or decreased calcium efflux out of the bone, both resulting in consecutive PTH stimulation.

Noteworthy, in the FRAME trial [9] patients with less than 40 ng/mL 25-vitamin D levels at baseline, received 50 000 to 60 000 IU of vitamin D, thus preventing incident hypocalcaemia. Due to lack of data, the magnitude and clinical meaning of such biochemical changes are unknown for patients with CKD III or IV or those on haemodialysis – a group of patients already prone to hypocalcaemia and secondary HPT. Hence, continuous attention is warranted.

As the effects of sclerostin antibody treatment in humans with osteoporosis plus severe CKD stage III, IV and V have not been investigated so far, fracture risk reduction or cellular effects are yet undetermined in this population. However, the prospects behind romosozumab in renal osteodystrophy are enticing because such a treatment may hypothetically combine two modes of action: osteoanabolism plus a decrease in PTH resistance. Conditions associated with supra-physiological sclerostin activity may impede PTH-mediated bone anabolism. Adynamic bone disease is a subtype of renal osteodystrophy characterised by a substantially reduced bone formation rate, impaired remodelling activity and reduced osteoblastic and osteoclastic activity [37]. In uraemia, high sclerostin levels may exacerbate PTH resistance, which could cause and/or aggravate adynamic bone disease [26]. Therefore, blocking sclerostin is a valuable research target in treatment of low-turnover renal osteodystrophy and might help resuscitate cellular activity [10].

Newman and co-workers investigated the effects of sclerostin antibody treatment in Cy/+ rats which resemble polycystic kidney disease [38]. Anti-sclerostin antibodies were applied in two different experimental settings – either in Cy/+ with uncontrolled hyperparathyroidism or in Cy/+ mice on a high calcium diet and with consecutively low PTH levels. Their data point towards relevant interactions between the status of underlying hyperparathyroidism and effects of anti-sclerostin antibody application. Sclerostin antibodies were effective in enhancing bone mass and ameliorating mechanical properties only if hyperparathyroidism was treated sufficiently (by high calcium intake) [38]. Only in the low-PTH group did sclerostin-antibody treatment reveal remarkable changes in renal osteodystrophy as evidenced by an increased cortical thickness and bone volume as well as an increase in bone quality/strength (as measured by ultimate load and energy to failure) [38].

Data from Moe and coworkers [39] point towards a comparable direction – i.e. the skeletal effects of sclerostin and sclerostin antibodies are different in renal failure versus healthy conditions and depend specifically upon the degree of renal hyperparathyroidism. In chronic

renal failure rats, the researchers titrated renal hyperparathyroidism via calcium administration towards different levels of PTH and measured phosphorylated β-catenin by western blot from total bone extracts. In the CKD animals, basal expression was 0.39 ± 0.18. In the CKD animals treated with anti-sclerostin Ab, the expression was 0.52 ± 0.28 in the high PTH group and 0.19 ± 0.17 in the low PTH group, with differences being significant between the two treated groups of p = 0.008). Phosphorylated β-catenin expression represents degradation, and hence these data indicate a positive effect of the anti-sclerostin antibodies basically in the low PTH group, consistent with their bone volume findings.

In summary, the application of sclerostin antibodies will certainly have an impact upon the degree and nature of renal osteodystrophy. Our current knowledge on sclerostin antibody treatment allows speculation about a potential amelioration of low bone turnover, and therefore, a future interventional trial targeting adynamic bone disease should be encouraged. However, any enthusiasm about increases in physiological mineralisation or calcification induced by sclerostin antibodies should be weighed against the fact that similar processes are involved in cardiovascular calcification processes, which are another hallmark of CKD-MBD. Does sclerostin block ectopic mineralization processes as well? And most importantly, will this ectopic calcification "explode" with sclerostin blockade?

Sclerostin and cardiovascular disease: What about vascular calcification?

Wnt signalling and its alterations are not limited to the skeleton play a role in human atherosclerosis and [40;41]. **Figure 2** depicts the double role of sclerostin in the vascular wall and the bone compartment. Noteworthy, ectopic vascular calcification and physiological bone formation share similarities in terms of the involved cellular processes [42].

Especially the derangements in the Wnt signalling pathway and in the soluble Wnt inhibitors contribute to the development of uraemia-associated combined bone and vascular disease [43]. In consequence, we need to be cautious and not too optimistic regarding the likely absence of any potential cardiovascular side effects when blocking sclerostin activity [44]. In fact, the above-mentioned ARCH trial underlined the need to create additional data regarding the cardiovascular safety of romosozumab [20]. The clinical relevance of the higher incidence of serious adverse cardiovascular events in romosozumab treated patients (2.5%) versus those receiving alendrontate (1.9%) is currently unknown, but they fuel the hypothesis that sclerostin and accordingly its antibody play a role in the cardiovascular

system. To the best of our knowledge, there are no data regarding the cardiovascular status in human genetic diseases due to reduced sclerostin activity, such as sclerosteosis or van Buchem`s disease [45].

A specific model of vascular calcification has been shown to act via Wnt signalling, i.e. vitamin K antagonist treatment. Vitamin K antagonists, such as warfarin, are suspected to trigger vascular calcification [46;47]. Beazley and co-workers showed that warfarin activates beta-catenin signalling in vascular smooth muscle cells (VSMCs) *in vitro* by (i) increasing the amount of total beta catenin protein, (ii) by upregulating its nuclear translocation and (iii) by stimulating transcription of beta catenin target genes [48].

Very recently, it was nicely shown that sclerostin is necessary to preserve or strengthen vascular health. An interesting experimental set-up to investigate sclerostin's role in atherosclerosis was elaborated by Krishna et al. [49]. The authors used ApoE-null mice, which develop atherosclerosis and aortic aneurysms with infusion of angiotensin II (AngII) [49]. In this study the putative protective role of sclerostin was examined via two different experimental approaches; i.e. transgenic overexpression and recombinant mouse sclerostin injection. In this way the authors were able to demonstrate that sclerostin protects AngII-infused ApoE-null mice from atherosclerosis and inflammation, aortic matrix degradation, as well as macrophage infiltration.

Recent research indicates that in human aortic valve tissue from haemodialysis patients with microscopic as well as macroscopic calcification, a significant local sclerostin mRNA upregulation is detectable which is absent in aortic valve tissue from haemodialysis patients without calcification [50]. Thus, it is not uraemia *per se*, but the calcification process itself which is seemingly responsible for local sclerostin expression in the vascular system.

Experimental *in vitro* data from Zhu and co-workers confirm sclerostin expression in calcifying VSMCs [51]. *In vitro,* VSMCs express osteocytic markers when grown in a pro-calcific environment, which is indicative of an osteoblastic to osteocytic transition (terminal transdifferentiation) [51]. Accordingly, the same authors found *in vivo* expression of sclerostin in calcified mouse aortas. The occurrence of sclerostin in calcified aortic valve tissue is not only limited to haemodialysis patients, but occurs in patients with dominant calcific aortic stenosis as well [52]. Moreover, ectopic sclerostin production and deposition was also detectable in skin specimens from dialysis patients with calciphylaxis [53], while no such local

sclerostin was found in control skin specimens from counterparts without cutaneous calcification.

These and other previous experimental data indicate that Wnt signalling actively participates in atherosclerosis and vascular calcification [40;49]. A missense mutation in LRP6, which encodes a co-receptor for sclerostin in the Wnt signalling pathway, was shown to be associated with autosomal dominant, early coronary artery disease [41]. Consequently, the particular role of sclerostin specifically in uraemic vascular disease is an interesting, novel, yet-to-be-investigated field [3]. However, these results are not without contradiction. Calcified epigastric artery specimens obtained at the time of renal transplantation were without relevant sclerostin mRNA and protein [54]. Interpretation of these heterogeneous and partly conflicting data however, should take into account the anatomical structures (aortic valve, coronary artery, large elastic arteries) and heterogeneity of the study populations (particularly dialysis versus non-dialysis cohorts).

With all these data in mind, the decisive question at this point in the discussion is: what happens when sclerostin activity is antagonized in the uraemic vascular wall, given the fact that local Wnt signalling is active in this setting [53]? The final answer is pending!

We contend strongly for controlled human studies in which patients with combined bone and arteriosclerotic disease are investigated in the setting of sclerostin blockade with a thorough work-up for vascular and skeletal effects.

Sclerostin blockade in CKD-MBD: the good, the bad, the ugly?

Romosozumab is a potent osteoanabolic agent that promises to enrich our armamentarium in the treatment of osteoporosis. Nevertheless, future clinical practice inevitably will also have to deal with the fact that romosozumab-treated patients may suffer from or develop CKD-MBD – at least CKD IIIb patients. The two areas of interest identified in the discussion above in terms of sclerostin inhibition in the realm of CKD-MBD that should undergo further careful evaluation are as follows:

1) Application of romosozumab in the treatment of renal osteodystrophy and particularly adynamic bone disease is appealing. It is presumably an over-simplification to attribute the driving force behind the development of adynamic bone solely to sclerostin overactivity. Nevertheless, blocking sclerostin opens

fascinating prospects in terms of ameliorating PTH responsiveness as well as augmenting the bone metabolism by increasing the bone formation rate. Such a study could be adequately performed in the same order of magnitude as the previous BONAFIDE trial [55]. The BONAFIDE trial was a multicentre, single-arm study characterizing the skeletal response to cinacalcet in adult dialysis patients with plasma parathyroid hormone (PTH) levels of 300 pg/ml or more, serum calcium of 8.4 mg/dl or more, bone-specific alkaline phosphatase over 20.9 ng/ml and biopsy-proven high-turnover bone disease. Of 110 enrolled patients, 77 underwent a second bone biopsy with quantitative histomorphometry after 6-12 months of cinacalcet treatment.

2) Assuming that sclerostin's role in the vascular wall is similar to its physiological role in bone (i.e. decreasing mineralisation), sclerostin blockade might actually stimulate mineralisation, hence promoting vascular calcification. This should serve as a warning signal. Adding to the complexity of this theoretical threat, we acknowledge that the interaction between sclerostin and vascular wall calcification will depend on many additional factors such as background diseases (particularly the type of bone disease) as well as the degree, maturity and distribution of (ectopic) calcification. The nephrology society should soon initiate interventional trials in patients prone to vascular calcification and investigate thoroughly the long-term cardiovascular effects of sclerostin antibodies in this setting.

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Reference List

1. Kestler HA, Kuhl M. From individual Wnt pathways towards a Wnt signalling network. *Philos.Trans.R.Soc.Lond B Biol.Sci.* 2008; 363: 1333-1347

2. Kim W, Kim M, Jho EH. Wnt/beta-catenin signalling: from plasma membrane to nucleus. *Biochem.J* 2013; 450: 9-21

3. Brandenburg VM, D'Haese P, Deck A *et al*. From skeletal to cardiovascular disease in 12 steps-the evolution of sclerostin as a major player in CKD-MBD. *Pediatr.Nephrol.* 2016; 31: 195-206

4. Monroe DG, McGee-Lawrence ME, Oursler MJ, Westendorf JJ. Update on Wnt signaling in bone cell biology and bone disease. *Gene* 2012; 492: 1-18

5. Ke HZ, Richards WG, Li X, Ominsky MS. Sclerostin and Dickkopf-1 as therapeutic targets in bone diseases. *Endocr.Rev.* 2012; 33: 747-783

6. Vervloet MG, Massy ZA, Brandenburg VM *et al*. Bone: a new endocrine organ at the heart of chronic kidney disease and mineral and bone disorders. *Lancet Diabetes Endocrinol.* 2014; 2: 427-436

7. Winkler DG, Sutherland MK, Geoghegan JC *et al*. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. *EMBO J* 2003; 22: 6267-6276

8. Mause SF, Deck A, Hennies M *et al*. Validation of commercially available ELISAs for the detection of circulating sclerostin in hemodialysis patients. *Discoveries.(Craiova.)* 2016; 4: e55

9. Cosman F, Crittenden DB, Adachi JD *et al*. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. *N.Engl.J Med.* 2016; 375: 1532-1543

10. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. *Nat.Med.* 2013; 19: 179-192

11. Bonewald LF. The amazing osteocyte. *J Bone Miner.Res.* 2011; 26: 229-238

12. Kramer I, Loots GG, Studer A, Keller H, Kneissel M. Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. *J Bone Miner.Res.* 2010; 25: 178-189

13. Balemans W, Van Hul W. Identification of the disease-causing gene in sclerosteosis--discovery of a novel bone anabolic target? *J Musculoskelet.Neuronal.Interact.* 2004; 4: 139-142

14. van Lierop AH, Appelman-Dijkstra NM, Papapoulos SE. Sclerostin deficiency in humans. *Bone* 2017; 96: 51-62

15. Yorgan TA, Peters S, Jeschke A *et al*. The Anti-Osteoanabolic Function of Sclerostin Is Blunted in Mice Carrying a High Bone Mass Mutation of Lrp5. *J Bone Miner.Res.* 2015; 30: 1175-1183

16. Ominsky MS, Vlasseros F, Jolette J *et al*. Two doses of sclerostin antibody in cynomolgus monkeys increases bone formation, bone mineral density, and bone strength. *J Bone Miner.Res.* 2010; 25: 948-959

17. Ominsky MS, Brown DL, Van G *et al*. Differential temporal effects of sclerostin antibody and parathyroid hormone on cancellous and cortical bone and quantitative differences in effects on the osteoblast lineage in young intact rats. *Bone* 2015; 81: 380-391

18. Suen PK, Zhu TY, Chow DH *et al*. Sclerostin Antibody Treatment Increases Bone Formation, Bone Mass, and Bone Strength of Intact Bones in Adult Male Rats. *Sci.Rep.* 2015; 5: 15632

19. McClung MR, Grauer A, Boonen S *et al*. Romosozumab in postmenopausal women with low bone mineral density. *N.Engl.J Med.* 2014; 370: 412-420

20. Saag KG, Petersen J, Brandi ML *et al*. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. *N.Engl.J Med.* 2017; 377: 1417-1427

21. Pelletier S, Dubourg L, Carlier MC, Hadj-Aissa A, Fouque D. The relation between renal function and serum sclerostin in adult patients with CKD. *Clin.J Am.Soc.Nephrol.* 2013; 8: 819-823

22. Drechsler C, Evenepoel P, Vervloet MG *et al*. High levels of circulating sclerostin are associated with better cardiovascular survival in incident dialysis patients: results from the NECOSAD study. *Nephrol.Dial.Transplant.* 2014; 30: 288-293

23. Delanaye P, Paquot F, Bouquegneau A *et al*. Sclerostin and chronic kidney disease: the assay impacts what we (thought to) know. *Nephrol.Dial.Transplant.* 2017;

24. Lips L, de Roij van Zuijdewijn CLM, Ter Wee PM *et al*. Serum sclerostin: relation with mortality and impact of hemodiafiltration. *Nephrol.Dial.Transplant.* 2017; 32: 1217-1223

25. Gong L, Zheng D, Yuan J *et al*. Elevated levels of serum sclerostin are linked to adverse cardiovascular outcomes in peritoneal dialysis patients. *Int.Urol.Nephrol.* 2018;

26. Massy Z, Drueke T. Adynamic bone disease is a predominant bone pattern in early stages of chronic kidney disease. *J Nephrol.* 2017; 30: 629-634

27. Sabbagh Y, Graciolli FG, O'Brien S *et al*. Repression of osteocyte Wnt/beta-catenin signaling is an early event in the progression of renal osteodystrophy. *J Bone Miner.Res.* 2012; 27: 1757-1772

28. Cejka D, Parada-Rodriguez D, Pichler S *et al*. Only minor differences in renal osteodystrophy features between wild-type and sclerostin knockout mice with chronic kidney disease. *Kidney Int.* 2016; 90: 828-834

29. Kaesler N, Verhulst A, De Mare A *et al*. Sclerostin deficiency modifies the development of CKD-MBD in mice. *Bone* 2017; 107: 115-123

30. Cejka D, Herberth J, Branscum AJ *et al*. Sclerostin and Dickkopf-1 in renal osteodystrophy. *Clin.J Am.Soc.Nephrol.* 2011; 6: 877-882

31. Graciolli FG, Neves KR, Barreto F *et al*. The complexity of chronic kidney disease-mineral and bone disorder across stages of chronic kidney disease. *Kidney Int.* 2017; 91: 1436-1446

32. Carrillo-Lopez N, Panizo S, Alonso-Montes C *et al*. Direct inhibition of osteoblastic Wnt pathway by fibroblast growth factor 23 contributes to bone loss in chronic kidney disease. *Kidney Int.* 2016; 90: 77-89

33. Komaba H, Kaludjerovic J, Hu DZ *et al*. Klotho expression in osteocytes regulates bone metabolism and controls bone formation. *Kidney Int.* 2017; 92: 599-611

34. Bellido T, Saini V, Pajevic PD. Effects of PTH on osteocyte function. *Bone* 2013; 54: 250-257

35. Keller H, Kneissel M. SOST is a target gene for PTH in bone. *Bone* 2005; 37: 148-158

36. Ryan ZC, Ketha H, McNulty MS *et al*. Sclerostin alters serum vitamin D metabolite and fibroblast growth factor 23 concentrations and the urinary excretion of calcium. *Proc.Natl.Acad.Sci.U.S.A* 2013; 110: 6199-6204

37. Cannata-Andia JB, Rodriguez GM, Gomez AC. Osteoporosis and adynamic bone in chronic kidney disease. *J Nephrol.* 2013; 26: 73-80

38. Newman CL, Chen NX, Smith E *et al*. Compromised vertebral structural and mechanical properties associated with progressive kidney disease and the effects of traditional pharmacological interventions. *Bone* 2015; 77: 50-56

39. Moe SM, Chen NX, Newman CL *et al*. Anti-sclerostin antibody treatment in a rat model of progressive renal osteodystrophy. *J Bone Miner.Res.* 2015; 30: 499-509

40. Marinou K, Christodoulides C, Antoniades C, Koutsilieris M. Wnt signaling in cardiovascular physiology. *Trends Endocrinol.Metab* 2012; 23: 628-636

41. Mani A, Radhakrishnan J, Wang H *et al*. LRP6 mutation in a family with early coronary disease and metabolic risk factors. *Science* 2007; 315: 1278-1282

42. Hruska KA, Sugatani T, Agapova O, Fang Y. The chronic kidney disease - Mineral bone disorder (CKD-MBD): Advances in pathophysiology. *Bone* 2017; 100: 80-86

43. Fang Y, Ginsberg C, Seifert M *et al*. CKD-Induced Wingless/Integration1 Inhibitors and Phosphorus Cause the CKD-Mineral and Bone Disorder. *J Am.Soc.Nephrol.* 2014; 25: 1760-1773

44. Evenepoel P, D'Haese P, Brandenburg V. Romosozumab in postmenopausal women with osteopenia. *N.Engl.J Med.* 2014; 370: 1664

45. Balemans W, Patel N, Ebeling M *et al*. Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. *J Med.Genet.* 2002; 39: 91-97

46. Cozzolino M, Brandenburg V. Warfarin: to use or not to use in chronic kidney disease patients? *J Nephrol.* 2010; 23: 648-652

47. Kruger T, Oelenberg S, Kaesler N *et al*. Warfarin induces cardiovascular damage in mice. *Arterioscler.Thromb.Vasc.Biol.* 2013; 33: 2618-2624

48. Beazley KE, Deasey S, Lima F, Nurminskaya MV. Transglutaminase 2-mediated activation of beta-catenin signaling has a critical role in warfarin-induced vascular calcification. *Arterioscler.Thromb.Vasc.Biol.* 2012; 32: 123-130

49. Krishna SM, Seto SW, Jose RJ *et al*. Wnt Signaling Pathway Inhibitor Sclerostin Inhibits Angiotensin II-Induced Aortic Aneurysm and Atherosclerosis. *Arterioscler.Thromb.Vasc.Biol.* 2017; 37: 553-566

50. Brandenburg VM, Kramann R, Koos R *et al*. Relationship between sclerostin and cardiovascular calcification in hemodialysis patients: a cross-sectional study. *BMC.Nephrol.* 2013; 14: 219

51. Zhu D, Mackenzie NC, Millan JL, Farquharson C, MacRae VE. The appearance and modulation of osteocyte marker expression during calcification of vascular smooth muscle cells. *PLoS.One.* 2011; 6: e19595

52. Koos R, Brandenburg V, Mahnken AH *et al*. Sclerostin as a potential novel biomarker for aortic valve calcification: an in-vivo and ex-vivo study. *J Heart Valve Dis* 2013; 22: 317-325

53. Kramann R, Brandenburg VM, Schurgers LJ *et al*. Novel insights into osteogenesis and matrix remodelling associated with calcific uraemic arteriolopathy. *Nephrol.Dial.Transplant.* 2013; 28: 856-868

54. Qureshi AR, Olauson H, Witasp A *et al*. Increased circulating sclerostin levels in end-stage renal disease predict biopsy-verified vascular medial calcification and coronary artery calcification. *Kidney Int.* 2015; 88: 1356-1364

55. Behets GJ, Spasovski G, Sterling LR *et al*. Bone histomorphometry before and after long-term treatment with cinacalcet in dialysis patients with secondary hyperparathyroidism. *Kidney Int.* 2015; 87: 846-856