Title

Alkaline phosphatase: a novel treatment target for cardiovascular disease in CKD.

Journal

Nature reviews. Nephrology, 13(7)

ISSN

1759-5061

Authors

Haarhaus, Mathias
Brandenburg, Vincent
Kalantar-Zadeh, Kamyar
et al.

Publication Date

2017-07-01

DOI

10.1038/nrneph.2017.60

Alkaline phosphatase: a novel treatment target for cardiovascular disease in CKD

Mathias Haarhaus[1,2], Vincent Brandenburg[3], Kamyar Kalantar-Zadeh[4,5], Peter Stenvinkel[1] and Per Magnusson[2]

Abstract | Cardiovascular disease is the main cause of early death in the settings of chronic kidney disease (CKD), type 2 diabetes mellitus (T2DM), and ageing. Cardiovascular events can be caused by an imbalance between promoters and inhibitors of mineralization, which leads to vascular calcification. This process is akin to skeletal mineralization, which is carefully regulated and in which isozymes of alkaline phosphatase (ALP) have a crucial role. Four genes encode ALP isozymes in humans. Intestinal, placental and germ cell ALPs are tissue-specific, whereas the tissue-nonspecific isozyme of ALP (TNALP) is present in several tissues, including bone, liver and kidney. TNALP has a pivotal role in bone calcification. Experimental overexpression of TNALP in the vasculature is sufficient to induce vascular calcification, cardiac hypertrophy and premature death, mimicking the cardiovascular phenotype often found in CKD and T2DM. Intestinal ALP contributes to the gut mucosal defence and intestinal and liver ALPs might contribute to the acute inflammatory response to endogenous or pathogenic stimuli. Here we review novel mechanisms that link ALP to vascular calcification, inflammation, and endothelial dysfunction in kidney and cardiovascular diseases. We also discuss new drugs that target ALP, which have the potential to improve cardiovascular outcomes without inhibiting skeletal mineralization.

Isozymes
Proteins encoded by separate genes with similar catalytic specificity but different primary structure.

Isoforms
Variations of a protein that arise from single nucleotide polymorphisms, differential splicing of mRNA, or post-translational modifications such as glycosylation.

Correspondence to M.H.
Mathias.loberg-haarhaus@sll.se

doi:10.1038/nrneph.2017.60
Published online 15 May 2017

Chronic kidney disease (CKD) is an important public health issue. The prevalence of CKD in the general populations of various countries is 10–12% and this proportion increases considerably with age[1,2]. Despite progress in the development of treatment strategies to improve outcomes, CKD is associated with high morbidity and mortality, mainly owing to cardiovascular complications[3–5]. CKD shares several pathophysiological processes with ageing and is a model of premature senescence[6,7]. Disorders of mineral metabolism have a central role in the pathophysiology of CKD, and their importance in the ageing process is increasingly recognized[6,7]. In addition, alterations of mineral metabolism are associated with increased risk of mortality and cardiovascular disease (CVD) in patients with CKD[8] and in the general population[9,10].

Tissue mineralization is tightly regulated by several stimulatory and inhibitory proteins such as alkaline phosphatase (ALP, also known as orthophosphoric-monoester phosphohydrolase, alkaline optimum). Both CKD and ageing are characterized by active dysregulation of the intricate balance between these proteins[6,7]. ALP stimulates mineralization mainly through modulation of the balance between inorganic phosphate (P_i) and inorganic pyrophosphate (PP_i) and also has a role in cardiovascular remodelling. In addition, ALP is a well-recognized biomarker of renal osteodystrophy and a biomarker and risk factor for increased mortality in patients with CKD[11] and in the general population[9].

In this Review we discuss the clinical relevance of ALP, and in particular that of bone-specific ALP (BALP) and intestinal-type ALP (IALP), in CVD and mortality, including their roles as putative targets for the treatment and prevention of cardiovascular complications in patients with CKD.

ALP biology: isozymes and isoforms

In this Review, we use the acronym ALP as a collective term for all isozymes and isoforms of ALP and when discussing studies that used unspecific methods for the determination of ALP. In humans, four ALP isozymes exist: alkaline phosphatase, tissue-nonspecific isozyme (TNALP, also known as TNSALP, TNAP, AP-TNAP, and alkaline phosphatase liver/bone/kidney); alkaline phosphatase, intestinal-type (IALP, also known as IAP); alkaline phosphatase, placental-type (PALP, also

Key points

- Circulating alkaline phosphatase (ALP) is a robust and independent predictor of all-cause mortality in the general population and in patients with chronic kidney disease (CKD)
- Tissue-nonspecific ALP (TNALP) is the most abundant ALP isozyme in the body, comprising >90% of circulating ALP; functional differences between bone ALP (BALP) and liver TNALP are the result of post-translational glycosylation
- BALP promotes tissue mineralization by inactivating calcification inhibitors and by supplying phosphate
- Liver ALP and intestinal ALP (IALP) contribute to the immune response through dephosphorylation of circulating endotoxins
- Modulation of ALP is a potential novel treatment strategy that might reduce vascular calcification and improve cardiovascular outcomes in patients with CKD or diabetes mellitus type 2

known as PLAP and PLAP-1); and alkaline phosphatase, placental-like (also known as germ cell ALP, GCAP, PLAP-like, and ALP-1) (TABLE 1). The expression of IALP, PALP, and germ cell ALP is restricted to specific tissues (duodenum, syncytiotrophoblast and testis, respectively), whereas TNALP is expressed in various tissues[12] (TABLE 1). The highest levels of TNALP are found in bone (BALP isoform), liver, and kidney[12]. In serum, BALP and liver TNALP are the most abundant isoforms, comprising more than 90% of total serum ALP activity in a 1:1 ratio[13]. These organ-specific isoforms of TNALP differ only in their levels of post-translational glycosylation[14], which affects their enzymatic activity[15,16]. BALP and liver TNALP have five putative *N*-glycosylation sites, which are all located around the catalytic site[17–19]. In addition, TNALP contains a magnesium ion and two zinc ions as cofactors near the active centre as well as calcium in a specific calcium-binding site[20]. Variations in the concentrations of these metals can also influence ALP activity[21–23].

All ALP isozymes function as ectoenzymes[24,25] and are attached to the outer cell membrane by a glycosylphosphatidylinositol (GPI) anchor[26,27]. Circulating levels of ALP activity reflect levels of ALP activity in tissues[28], but the exact mechanisms of ALP release into the general circulation remain unclear. Some studies suggested that GPI-specific phospholipase D converts membrane-anchored ALPs to the anchorless forms found in serum[28,29].

Three different BALP isoforms, B/I (bone and intestinal), B1 and B2, can be separated and quantified in human serum by weak anion-exchange high-performance liquid chromatography[14,30]. Of note, the B/I fraction is not pure BALP isoform as it co-elutes with the small fraction of IALP isozyme present in the general circulation[14]. The B/I fraction includes ~70% BALP isoform activity and ~30% IALP isozyme activity, and comprises only 6% of the entire BALP activity in serum[28]. The three BALP isoforms (B/I, B1 and B2) are present in the serum and bone in healthy adults[31], children and adolescents[32], and in patients with various metabolic bone diseases[13,33,34]. In addition, a fourth BALP isoform, B1x, is present in extracts of human bone tissue[28,35] and exclusively in the serum of patients with different stages of CKD[31,36–38], in whom it is a marker of low bone turnover[38]. All BALP isoforms, including B1x, are also expressed in vascular smooth muscle cells (VSMCs), the calcification of which is associated with a strong increase in BALP activity level[39]. In particular, increased B1x, B/I and B2 activity could be associated with phosphate-induced vascular calcification[40]. In bone, the B1:B2 ratio differs between cortical sites (~1.7) and trabecular sites (~0.7)[35]. Intriguingly, this ratio was higher in serum samples from patients on dialysis than in those from healthy controls, which could be the result of higher cortical bone turnover in these patients, associated with hyperparathyroidism[31].

Syncytiotrophoblast
Epithelial layer that covers the highly vascular embryonic placental villi, which invades the wall of the uterus to establish nutrient circulation between the embryo and the mother.

ALP function

Bone and vascular calcification

Mineralizing cell types. Physiological bone and teeth mineralization is orchestrated by cells of mesenchymal origin, such as hypertrophic chondrocytes, odontoblasts and osteoblasts[41,42]. Vascular calcification is associated with osteochondrogenic transformation of vascular cells of mesenchymal origin, such as myocytes, pericytes or fibroblasts[43]. Macrophage-like cells with mineralizing potential have also been described in the vascular wall[44]; whether these cells are monocytic macrophages that migrated from the circulation or VSMCs that adopted a macrophage-like phenotype is unclear[45]. A 2016 genetic fate tracing study identified a novel mechanism of vascular calcification: adventitial Gli1$^+$ mesenchymal stem cell-like cells migrate into the media and neointima during atherosclerosis and arteriosclerosis in *ApoE*$^{-/-}$ mice with CKD[46]. Genetic ablation of Gli1$^+$ cells before the induction of kidney injury reduced the severity of vascular calcification, suggesting that Gli1$^+$ cells could be a major source of osteoblast-like cells during calcification in the media and intima.

Mechanisms of mineralization. Independent of the cell type involved, bone and vascular mineralization are associated with the release of matrix vesicles, which are a nidus of extracellular matrix mineralization[43] (FIG. 1). Two types of secreted matrix vesicles have been described in calcifying VSMCs: non-calcifying vesicles, which contain calcification inhibitors such as fetuin A, and matrix-bound vesicles with increased calcification potential, which are rich in membrane-bound TNALP[47].

BALP is responsible for the propagation of tissue mineralization and is expressed in osteoblasts, chondrocytes, and other mineralization competent cells, such as calcifying VSMCs[48]. Although calcifying human

Author addresses

[1]Division of Renal Medicine and Baxter Novum, Karolinska Institutet, Karolinska University Hospital, SE-14186 Stockholm, Sweden.
[2]Department of Clinical Chemistry and Department of Clinical and Experimental Medicine, Linköping University, SE-58185 Linköping, Sweden.
[3]Department of Cardiology and Intensive Care Medicine, RWTH University Hospital Aachen, Pauwelsstraße 30, D-52074 Aachen, Germany.
[4]Harold Simmons Center for Kidney Disease Research and Epidemiology and Division of Nephrology and Hypertension, University of California Irvine, School of Medicine, 101 The City Drive South, City Tower, Suite 400, Mail Code: 4088, Orange, California 92868, USA.
[5]Department of Epidemiology, UCLA Fielding School of Public Health, 1124 West Carson Street Suite C-1 Annex, Torrance, California 90502, USA.

Table 1 | **Human ALP isozymes and isoforms**

Gene	Protein	Isoforms*	Localization	Function	Accession number‡
ALPL	Alkaline phosphatase, tissue-nonspecific isozyme (TNALP)	Bone-specific TNALP: B/I, B1, B1x, and B2	Skeletal tissue, kidney, neutrophil granulocytes, developing nervous system, and other cell types such as vascular cells	Involved in skeletal mineralization, vitamin B_6 metabolism	NM_000478
		Liver-specific TNALP: L1, L2, and L3	Liver	Unknown	NM_000478
ALPI	Intestinal-type alkaline phosphatase (IALP)	Unknown	Mainly in duodenum	Involved in fat absorption and detoxification of LPS and free nucleotides	NM_001631
ALPP	Placental-type alkaline phosphatase (PALP)	Unknown	Syncytiotrophoblasts, various tumours	Unknown	NM_001632
ALPPL2	Alkaline phosphatase, placental-like (PLALP)§	Unknown	Testis, malignant trophoblasts, testicular cancer	Unknown	NM_031313

*Identified by high-performance liquid chromatography. ‡Accession numbers refer to the National Center for Biotechnology Information Nucleotide database. LPS, lipopolysaccharide. §Also known as germ cell alkaline phosphatase.

VSMCs exclusively express BALP *in vitro*[40], most studies in human and animal models do not specify the measured TNALP isoform, which does not exclude that other isoforms could be involved in vascular calcification. The highest concentration of BALP is found in matrix vesicles shed by VSMCs[49]. Loading of BALP onto matrix vesicles is an active process, which can be modulated by pro-calcific stimuli such as the inhibition of autophagy in a P_i-rich milieu[50]. Autophagy of damaged cellular components is a defence mechanism that protects VSMCs from P_i-induced oxidative stress. Inhibition of autophagy stimulates the release of pro-calcific matrix vesicles with increased ALP activity[50]. The protein-sorting peptide sortilin is involved in loading ALPs on matrix vesicles during vascular calcification in mice[51]. Interestingly, sortilin deficiency in mice reduces vascular calcification but does not affect bone mineralization[51].

The predominant role of BALP during mineralization is the inactivation of inhibitory polyphosphates[52], most importantly the main autocrine calcification inhibitor PP_i[53,54]. ALPs are also speculated to inactivate the calcification inhibitor osteopontin through dephosphorylation[55] and a 2017 study showed that human BALP can suppress the inhibitory effect of osteopontin on *in vitro* mineralization[56]. In addition, ALPs generate P_i, which is the substrate of mineralization, by hydrolysation of organic phosphate esters[57]. The P_i:PP_i ratio has become increasingly recognized as a central determinant of tissue mineralization as hydroxyapatite crystals form and grow within matrix vesicles and in the extracellular matrix when P_i and calcium concentrations surpass their solubility product, whereas PP_i hinders this process by attaching to the surface of hydroxyapatite crystals[58–60]. In the extracellular matrix, this balance is maintained by membrane-bound enzymes, mainly ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (E-NPP 1, also known as NPP1 and plasma-cell membrane glycoprotein PC-1) and BALP[48]. The expression of these enzymes is regulated by hormones that regulate mineral metabolism, such as fibroblast growth factor 23 (REF. 61) and 1,25-dihydroxyvitamin D[62]. A local disturbance of the P_i:PP_i ratio in the vascular wall can promote calcification in spite of normal serum phosphate levels[63]. Thus, the elevated ALP activity found in syndromes with accelerated vascular calcification and premature senescence such as CKD[64,65], diabetic arterial media calcification[66,67], arterial calcification due to 5′-nucleotidase (also known as CD73) deficiency[68], and Huntington-Gilford Progeria Syndrome[69], could contribute to a local pro-calcific environment in the vascular wall by decreasing PP_i concentrations. The importance of TNALP in inducing vascular wall calcification was convincingly shown in mice with genetically modified TNALP expression in vascular wall layers. TNALP overexpression in VSMCs[70] and in endothelial cells[71] both resulted in ectopic calcification.

Parathyroid hormone (PTH) has a pivotal role in the regulation of bone turnover, but its involvement in vascular calcification is less clear than that of ALP[72]. Experimental studies suggest that increased levels of PTH are associated with vascular calcification[72]. However, in uraemic rats, hyperparathyroidism is only associated with vascular calcification in the presence of hyperphosphataemia[73]. Thus, PTH might not exert a direct effect on vascular cells. Instead, PTH-induced release of phosphate from bone, with subsequent hyperphosphataemia, could be responsible for the association between increased PTH and vascular calcification. Following the recommended target range for PTH levels does not improve the risk of cardiovascular events or mortality in patients with CKD[74,75]. A meta-analysis showed that higher serum phosphate levels, but not serum calcium or PTH levels, were associated with all-cause mortality and CVD[76], whereas in some clinical studies low PTH levels combined with high BALP levels were more strongly associated with mortality[11] and CVD[77] than high serum levels of PTH.

ALP activity in disease

Modulation of inflammation

Several associative studies on serum levels of components of mineral metabolism and inflammatory markers indicate a relationship between systemic inflammation and serum ALP activity[78–81] (FIG. 2a). In some

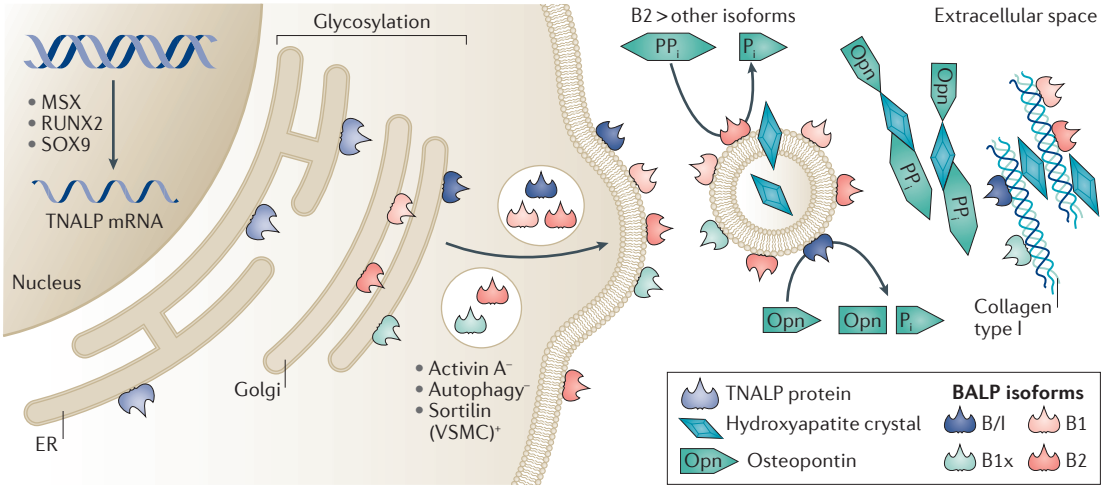

Figure 1 | **Role of bone-specific alkaline phosphatase (BALP) in tissue mineralization.** The expression of alkaline phosphatase, tissue-nonspecific isozyme (TNALP) is stimulated by RUNX2, MSX2 and SOX9. TNALP is post-translationally modified (*N*-linked and *O*-linked glycosylations) in the endoplasmatic reticulum (ER) and Golgi apparatus under the influence of unknown regulators. Glycosylation produces numerous structural modifications, which increase the functional diversity of TNALP. Four bone-specific isoforms of TNALP exist (B/I, B1x, B1, and B2). These BALP isoforms are transported to the cell membrane and attached to the outer layer, where they act as ectoenzymes. In mineralizing cells, more BALP isoforms are transported to specific sections of the cell membrane, which then are released and form matrix vesicles that are rich in membrane-bound BALP isoforms and Sortillin (in vascular mesenchymal stem cells (MSCs)) but not Activin A or autophagy markers. BALP inactivates the mineralization inhibitors inorganic pyrophosphate (PP_i) and osteopontin (by dephosphorylation) and thus substantially contributes to the generation of a pro-calcific extracellular milieu. BALP also binds directly to collagen type I, which forms a scaffold for the propagation of matrix mineralization. The functional role of collagen-bound BALP is not yet known. P_i, inorganic phosphate; VSMC, vascular smooth muscle cell.

observational studies levels of C-reactive protein (CRP) modulated the association of serum ALP levels with mortality or CVD in the general population[82–84].

Experimental studies in human osteoblasts and VSMCs suggested a direct association between the presence of mediators of inflammation in culture media and the induction of ALP activity in mineralizing cells[39,85–87]. For example, CRP decreased TNALP activity and mineralization in cultured osteoblasts, which was associated with decreased expression of the mineralizing transcription factor RUNX2[85], whereas TNF stimulated mineralization and ALP expression in osteoblasts independent of RUNX2 (REF. 86). TNF also stimulated BALP activity and calcium deposition in VSMCs[39]. Autocrine TNF signalling has an important role in VSMC calcification[87]. TNALP is not only expressed by mineralization competent cells and its expression in activated neutrophils might contribute to the inactivation of endotoxins in the circulation[88].

The role of purinergic signalling in the regulation of the immune response is also an evolving area of research. Extracellular ATP induces proinflammatory responses via P2Y receptors, whereas adenosine can have the opposite effect, conveyed by P1 receptors[89,90]. The conversion of proinflammatory ATP to anti-inflammatory adenosine is catalysed by membrane-bound and soluble ectonucleotidases, mainly 5′-nucleotidase and ectonucleoside triphosphate diphosphohydrolase 1 (also known as CD39), but also by ALPs[90]. Thus, increased ALP expression could be a cellular response to inflammatory stimuli. In this context, IALP[91], TNALP in vascular endothelial cells[92] and leucocytes[88], and potentially, liver isoforms of TNALP[93], could contribute to the increased levels of serum ALP activity found in acute sepsis and other acute inflammatory conditions (see below). A 2015 study proposed a potential involvement of purinergic signalling in diseases associated with chronic inflammation, such as atherosclerosis[94], suggesting a hypothetical role for ALPs as part of a vascular defence mechanism in CVD. However, the association of reduced nucleotide signalling via the P2Y receptor with increased arterial intimal and VSMC calcification in $P2Y_2R^{-/-}$ mice[95], as well as the accelerated arterial calcification seen in mice that overexpress TNALP in endothelial cells[71] and VSMCs[70], raise concerns about putative therapeutic interventions that aim to increase ALP activity for the long-term treatment of CVD.

IALP, inflammation and the metabolic syndrome

IALP is expressed and secreted by intestinal epithelial cells (especially in the duodenum), but is also biologically active in the serum[91]. The expression of IALP is regulated by various nutritional and inflammatory factors[96]. The effects of IALP activity in inflammatory conditions and in the metabolic syndrome is an emerging field of clinical research as this ALP isozyme has important functions in gut mucosal defence. IALP maintains intestinal homeostasis by inactivating bacterial endotoxins such as lipopolysaccharide (LPS), regulating intestinal lipid absorption, eliminating toxic nucleotides and determining the composition of the intestinal microbiome[91] (FIG. 2b).

Metabolic syndrome
Array of conditions — raised blood pressure, dyslipidaemia (raised triglycerides and lowered HDL cholesterol), raised fasting glucose, and increased waist circumference — that increase the risk of cardiovascular disease and type 2 diabetes mellitus.

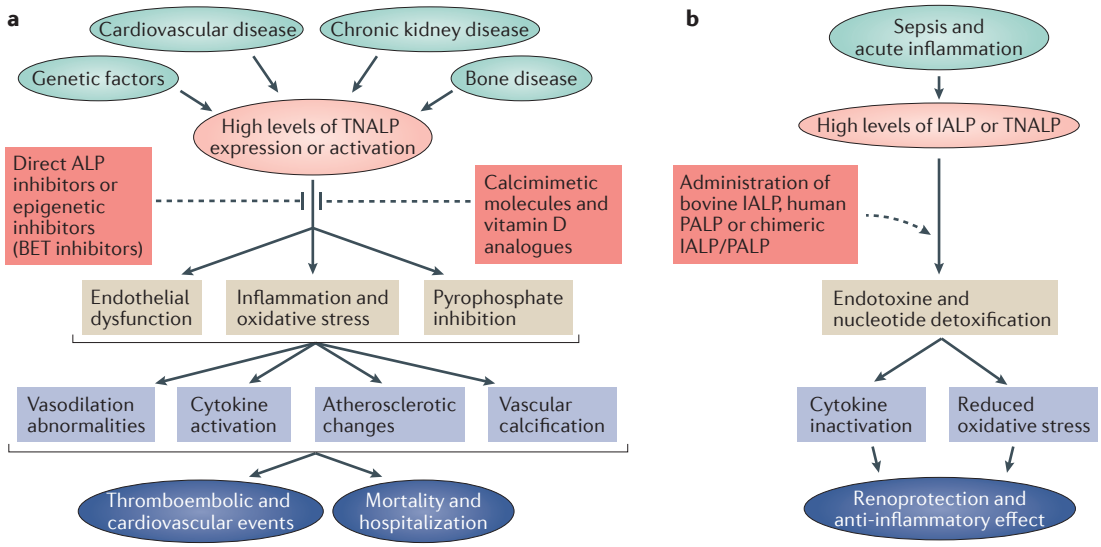

Figure 2 | **Pathological consequences of high alkaline phosphatase (ALP) levels. a** | Cardiovascular dysfunction, chronic kidney disease, genetic factors and bone disease result in increased circulating expression of alkaline phosphatase, tissue-nonspecific isozyme (TNALP)[Au:OK?]. This increasd expression of TNALP is associated with increased mortality and cardiovascular events via mechanisms that involve vascular calcification, endothelial dysfunction, and inflammation. Interventional strategies to reduce TNALP activity include direct ALP inhibitors; epigenetic inhibitors of TNALP expression such as bromodomain and extra-terminal motif (BET) inhibitors; calcimimetic molecules; and vitamin D analogues. **b** | Elevation of the levels of intestinal-type alkaline phosphatase (IALP) and TNALP owing to sepsis and/or acute inflammation can lead to endotoxin and nucleotide detoxification, cytokine inactivation and reduced oxidative stress, which protect the kidney from inflammatory damage. Similarly, systemic administration of IALP, placental-type alkaline phosphatase (PALP) or chimeric IALP/PALP can exert nephroprotective and anti-inflammatory effects in sepsis and after cardiac surgery.

Studies of the isozyme composition of serum ALP revealed that levels of IALP activity are considerably increased in patients with CKD[97–101]. CKD might thus be a suitable model for further clinical and experimental studies on the biological significance of IALP. Given the important role of bacterial endotoxins[102], intestinal dysbiosis and barrier dysfunction[103] in uraemic inflammation and associated morbidity and mortality, studies of the function of IALP in mucosal defence of the uraemic gut and in metabolic alterations certainly deserve more attention.

Patients with CKD have profound alterations in the gut environment, such as shifts in microbial composition and increased blood levels of gut microorganism-derived metabolites[104]. Interestingly, faeces of IALP-knockout mice contain fewer and less diverse bacteria than those of wild-type mice; these effects are reversed by IALP supplementation[105]. Patients with CKD can also suffer from antibiotic-associated diarrhoea caused by infection with *Clostridium difficile*, which is a major clinical problem[106]. IALP administration prevented antibiotic-induced susceptibility to enteric pathogens in mice[107].

Chronic endotoxaemia, systemic low-grade inflammation, gut dysbiosis and local intestinal inflammation are important contributors of the metabolic syndrome, which is a commonly associated with CKD[108]. IALP-deficient mice had increased circulating levels of endotoxins, central obesity, dyslipidemia and insulin resistance[109], suggesting that IALP could have a central role in the development of the metabolic syndrome. Oral supplementation with IALP could prevent and reverse the development of several symptoms of the metabolic syndrome in these animals. A 2015 study showed that patients with type 2 diabetes mellitus (T2DM) have lower faecal levels of IALP activity than do healthy controls, and that higher faecal IALP activity might be protective against T2DM, independent of obesity[110].

Endothelial dysfunction

The importance of endothelial ALP for vascular health is increasingly recognized. TNALP is highly expressed in the endothelial cells of cardiac[111] and brain microvessels and contributes to the function of the blood–brain barrier (BBB) by regulating transcellular transport and organization of the cytoskeleton[112]. Similar to senescence-induced TNALP expression and calcification in VSMCs[113], vascular endothelial cell and pericyte senescence contributes to BBB breakdown[114]. To what extent this process involves TNALP in brain microvessels has yet to be determined.

ALP might also participate in the anti-inflammatory function of endothelial barriers[115]. According to this hypothesis, increased release of membrane-bound endothelial ALP into the circulation caused by increased activity of phospholipase C or D could result in decreased cellular ALP levels and thereby contribute to BBB dysfunction in neurodegenerative diseases such as Alzheimer disease, owing to disturbed transcellular transport. Whether ALP is involved in dysfunction of the endothelial barrier, disturbs BBB function in CKD, or causes arterial stiffness or proteinuria in the kidney and leads to CKD progression has yet to be elucidated.

Increased levels of serum ALP are also associated with endothelial dysfunction in hypertensive individuals without CKD[116]. Experimental data suggest that the induction of vascular ALP activity by oxidative stress and the consequent promotion of vascular calcification is a possible underlying mechanism[117–120]. The increase in the mortality risk associated with high levels of TNALP caused by oxidative stress (assessed by γ-glutamyltransferase levels) in patients with CKD gives some clinical support to this hypothesis[121]. In addition, overexpression of endothelial TNALP is sufficient to induce arterial medial calcification and vascular stiffening in mice[71], underscoring the importance of endothelial ALP activity in vascular disease.

Proteinuria

In patients with type 1 diabetes mellitus (T1DM), an increase in circulating levels of ALP is associated with glomerular hyperfiltration, proteinuria and CKD progression in the early stages of diabetic nephropathy[122,123]. These studies did not characterize the origin of the increased levels of serum ALP. Although the association was independent of blood pressure, a contribution of oxidative stress levels, inflammation or vascular calcification (as discussed above) cannot be ruled out, even in the early stages of the disease. In addition, increased levels of urinary TNALP, presumably derived from tubular cells, have long been suggested to be a marker of kidney disease[124]. TNALP released from tubular cells damaged by glomerular hyperfiltration[125,126] could contribute to increased levels of urinary ALP in patients in the early stages of diabetic nephropathy. Furthermore, glomerular endothelial and mesangial cells[127], epithelial cells in the Bowman capsule[128], and cells of the juxtraglomerular apparatus[129] express ALP in physiological conditions (albeit at lower levels than do tubular cells); release of ALP from these cells could further elevate urinary ALP levels in patients with glomerular hyperfiltration. The biological function of ALP in the kidney is unknown and to what extent renal isoforms of TNALP contribute to circulating ALP activities in the early stages of diabetic nephropathy is unclear; however, kidney TNALP seems to be linked to PP_i metabolism and LPS detoxification[127]. Of note, patients with hypophosphatasia owing to loss-of-function mutations in *ALPL*, which encodes TNALP[130], do not usually develop kidney disease.

Cardiovascular disease

General population. In a 2014 meta-analysis, Li and colleagues investigated the involvement of ALP in CVD in non-CKD populations[9]. They found that ALP levels were independently associated with CVD death; however, the association between ALP levels and non-fatal CVD events or coronary heart disease became nonsignificant after full adjustment for confounders, including inflammation. In a second meta-analysis, also published in 2014, baseline levels of ALP were independently associated with prospective CVD risk in four studies[131]. Although inflammation was accounted for, the researchers did not specify the direct effect of inflammation on the association between ALP levels and different types of CVD. In 2015, an independent association was reported between serum ALP levels and CVD during a 10-year prospective follow-up of 6,974 participants aged 28–75 years without pre-existing CVD[82]. This association was weak but significant, even after adjustment for CRP levels. ALP levels were not associated with stroke and the association with ischaemic heart disease disappeared after several adjustments. Increased total ALP levels were associated with acute myocardial infarction (AMI) in a case–control study of patients who had experienced a first AMI[132], and further characterization revealed that BALP was the main source of the increased total ALP activity. In another prospective study of patients with coronary heart disease undergoing coronary stent implantation, high levels of total ALP activity predicted all-cause mortality, AMI and stent thrombosis[104]. The latter was speculated to be an effect of reduced endothelialisation in calcified coronary arteries as ALP levels were independently associated with coronary calcification in this study. Although the combination of elevated CRP and ALP levels increased hazard ratios for all-cause mortality, the correlation between ALP and CRP levels was small, and the association between ALP and cardiovascular events was not influenced by CRP levels[133]. Thus, although evidence of an association between ALP levels and CVD incidence exists, associations can vary depending on the population studied and the type of CVD. Inflammation modulated the association of ALP levels with CVD incidence in some, but not all studies, suggesting that ALP could be used as a novel predictor of cardiovascular mortality and morbidity in the non-CKD population.

Patients with CKD. Vascular calcification is a widespread feature of the uraemic phenotype and is frequently coupled to premature CVD and increased mortality[2]. We have identified high serum ALP levels as a strong risk factor for coronary calcification in patients on maintenance haemodialysis, independent of inflammation and traditional cardiovascular risk factors[134]. In a prospective study of 135 patients with stage 1–5 CKD not on dialysis, cardiovascular events were positively associated with circulating BALP levels and negatively associated with circulating levels of tartrate-resistant acid phosphatase isoform type 5b (TRAP 5b, also known as TRACP and TR-AP)[77]; however, no association between cardiovascular events and PTH levels was observed. TRAP 5b is mainly expressed by osteoclasts, which drive bone resorption, and is expressed at low levels outside the bone. As non-bone-derived TRAP 5b is unlikely to contribute substantially to circulating levels, low serum levels of TRAP 5b indicate reduced bone turnover[77]. BALP expression, on the other hand, can be upregulated in calcifying arteries, as discussed above. Thus, the researchers suggested that the increased levels of circulating BALP in patients with CKD were derived from calcified arteries rather than from bone. Several reports have provided direct evidence for an association between low bone turnover and accelerated vascular calcification[135,136]. Decreased bone turnover reduces the capacity to incorporate ingested calcium

Hypophosphatasia
Autosomal dominant or autosomal recessive rare metabolic disease with an extraordinary range of severity caused by loss-of-function mutations within *ALPL*, which encodes TNALP.

into bone, thereby providing a possible substrate for vascular calcification[137,138]. In mild CKD, high circulating levels of BALP are also independently associated with carotid enlargement, an early sign of arterial disease[139]. Further evidence supporting a role of ALP in uraemic vascular calcification was provided by a study in which upregulation of ALP activity in the vascular wall by experimental uraemia was associated with reduced levels of circulating PP_i[140], which is in accordance with previous descriptions of reduced PP_i levels in patients with CKD[141]. Interestingly, experimental treatment with systemically administered PP_i can inhibit uraemic vascular calcification[142]. The different enzymatic activities of BALP isoforms towards PP_i and other substrates[16] suggest functional differences among isoforms in the multifaceted process of vascular calcification.

ALP and mortality

General population. The focus on ALP as an emerging risk factor for mortality in different populations is novel. A 2014 meta-analysis investigated the association between total levels of ALP and mortality in the general population[131]. In this study, patients in the highest tertile of serum ALP levels experienced a 38% higher risk of all-cause mortality than patients in the lowest tertile. Li *et al.* also found a positive correlation between ALP and all-cause and cardiovascular mortality independent of kidney function and pre-existing CVD (relative risk of all-cause mortality 1.57 for patients with ALP levels >90 U/l, compared with patients with ALP levels <70 U/l)[9]. A 2009 study showed that increased levels of ALP and phosphate are independent risk factors for all-cause and cardiovascular mortality in the general population[143]. Individuals with a combination of increased ALP and phosphate levels had the highest mortality risk. In a large cohort study, high ALP levels were associated with CRP levels and mortality in the general North-American population, independent of liver function[83]. These associations were not found for BALP levels and the researchers speculated that the associations of ALP levels with CRP levels and mortality were caused by a TNALP isoform produced by activated neutrophils. However, this study was flawed by the use of different methods for the determination of serum BALP activities and the change of age limits for inclusion during the recruitment period. Furthermore, the researchers speculated that the study lacked power owing to low mortality rates. A different study reported that total levels of ALP were associated with all-cause mortality independent of classical cardiovascular risk factors and CRP levels[133]. Taken together, these studies identify ALP as an independent predictor of mortality in the general population. CRP levels might have a modulatory effect on the association of ALP activity with mortality in CVD, but in the majority of studies the association of ALP with mortality was independent of inflammation.

Patients with CKD. ALP and, more specifically, BALP have been recognized as biomarkers of renal osteodystrophy for decades[75]. Additionally, numerous cross-sectional and longitudinal studies have shown correlations between high ALP levels and mortality in patients with CKD[121,144–167] (TABLES 2–4). Notably, these associations were independent of several confounders, including phosphate, calcium, PTH, vitamin D treatment, protein energy wasting, liver function and inflammation (TABLES 2–4). In prevalent and incident patients on dialysis, ALP levels <100 U/l were not associated with any survival benefit[145,158]. In our opinion, this finding could indicate that low circulating ALP levels are associated with low bone turnover (adynamic bone), which is a risk factor for vascular calcification[135] and mortality[138]. In a longitudinal study, the association between increasing levels of ALP over time and mortality depended on baseline ALP levels in patients on incident dialysis[158]. Patients with baseline ALP levels >120 U/l had increased mortality, independent of ALP trends over time. In patients with baseline ALP levels of 80–120 U/l increasing ALP levels over time were associated with increased mortality; stable or decreasing ALP levels did not affect mortality. In patients with ALP <80 U/l, neither increasing nor decreasing trends were associated with mortality. Our data indicate that ALP levels <120 U/l confer a survival benefit[144,145,148,151,159,167]; however, randomized prospective trials are needed to evaluate target ALP levels for the prevention of cardiovascular events and all-cause mortality.

We showed that pre-transplantation levels of ALP, but not PTH, predicted mortality after kidney transplantation in a large cohort of patients undergoing dialysis[159]. A prospective study of prevalent kidney transplant recipients showed that γ-glutamyltransferase and ALP levels, but not BALP levels, were independently associated with mortality[160]. The researchers speculated that these associations might be related to vascular oxidative stress, as indicated by the presence of the γ-glutamyltransferase circulating marker. Oxidative stress is associated with CVD and can induce the expression of ALP in vascular cells (see above). This study did not correct for liver function tests; however, separate analyses of these tests did not show any association between liver function and mortality. In addition, total levels of ALP are associated with mortality[118,119,127,138] and CKD progression[118] in pre-dialysis patients with stage 3–4 CKD.

Taken together, these studies show that ALP levels greater than or equal to the upper reference interval limit were associated with high mortality despite the use of various routine laboratory methods for the determination of serum ALP activity. Serum ALP and BALP activities are reported in a variety of units and a wide range of numerical values, which could be due to the fact that reaction conditions[30] (substrate type and concentration; buffer type, concentration, and pH; reaction temperature) influence ALP activity levels differently among ALP isozymes and isoforms[168,169]. This methodological variability might explain why expert committees have not yet endorsed definite target levels for ALP[170]. Introduce future target levels for ALP in relation to the upper reference interval limit instead of fixed values in order to harmonize treatment strategies globally might be preferable.

Table 2 | **Studies of the association of total ALP and BALP levels with risk of all-cause mortality in patients with CKD undergoing dialysis**

Study	Population (*n*)	Dialysis type	Study duration	All-cause mortality risk	Adjustments	Refs
Torino *et al.* (2016)	PROGREDIRE (992)	Prevalent HD	Median 3 years	HR 1.06 for each 50 U/l increase in ALP activity compared to baseline per increasing quintiles of baseline GGT activity	Age, gender, current smoking, DM, cholesterol, SBP, antihypertensive treatment, CV comorbidities, CRP, BMI, albumin, dialysis vintage, Hb, ALT, AST, hepatitis B and C infection, alcohol consumption, and pre-existing liver disease	121
Scialla *et al.* (2016) (	CHOICE (466)	Prevalent HD	Median 3.4 years	HR 1.66 for baseline ALP activity ≥113.6 U/l	Age, sex, race, education, smoking, BMI, comorbidity, including DM and atherosclerotic disease, and albumin and Hb levels	166
Zhu *et al.* (2016)	Single centre (1,091)	Prevalent HD	5 years	• HR 1.6 for time-dependent ALP levels ≥104 U/l • Non-significant HR for different levels of baseline ALP	Age, sex, dialysis vintage, etiology of renal failure, ESA usage, vitamin D usage, antihypertensive treatment, iron usage, PTH, Ca, PO_4, and parathyroidectomy status	165
Soohoo *et al.* (2016)	Da Vita (102,754)	Incident HD	Median 1.3 years	• HR 1.24–1.4 for baseline ALP activity ≥120 U/l • HR 0.9–0.95 for baseline ALP activity <80 U/l, independent of changes in ALP activity during first 6 months	Entry calendar quarter, age, sex, race, ethnicity, comorbidities, primary insurance, vascular access, underlying disease, dialysis dose, and malnutrition-inflammation complex syndrome	158
Lin *et al.* (2015)	TWRDS (94,983)	Prevalent HD	Study period 7 years, individual observational period not reported	• HRs increased for baseline ALP activity ≥100 U/l • Highest HR for baseline ALP activity ≥120 U/l and PTH levels <50 pg/ml	Age, sex, DM, haematocrit, albumin, Ca and PO_4 levels, and dialysis dose	164
Chua *et al.* (2014)	UREA5 (983)	Incident PD (27.6%) and HD (72.4%)	1 year	OR 1.7 for baseline ALP activity >80 U/l	Age, sex, ethnicity, underlying disease, comorbidity, LVEF, planned RRT, no permanent vascular access, Hb, albumin, urea, GFR, PTH, Ca, PO4, Ca×PO_4 product, and urate levels	163
Beige *et al.* (2014)	Three dialysis units (407)	Prevalent PD (4.3%) and HD (95.7%)	5 years	OR 2.7 for baseline ALP activity ≥77.5 IU/L	Age, dialysis vintage and dose, DM, and levels of PTH, Ca, and PO_4	157
Maruyama *et al.* (2014)	JRDR (158,277)	Prevalent HD	1 year	• OR 1.46 for highest (≥308 U/l) versus lowest (≤183 U/l) quartile of baseline ALP activity • OR 1.01 per 10 U/l increase in baseline ALP activity	Age, sex, dialysis vintage, BMI, underlying disease, comorbid disease, medication and levels of albumin, urea, creatinine, CRP, Ca, PO_4, magnesium, and PTH	153
Liu *et al.* (2014)	Single centre (1,021)	Incident PD	Median 2.6 years	• HR 1.04 for each 10 U/l increase of baseline ALP activity • HR 1.7 for baseline ALP activity ≥82 U/l versus ≤52 U/l	Age, sex, 24-h urine output, SBP, comorbidity, Hb, albumin, ALT, AST, phosphate binder use, PO_4, Ca, PTH, and dialysate calcium levels	152
Rhee *et al.* (2014)	Da Vita (108,567)	Prevalent PD (8.5%) and HD (91.5%)	Median 2.7 years	• Increasing HRs with increasing baseline ALP activity • HR 1.91 (PD) and 1.62 (HD) for ALP activity ≥210 U/l versus 70–90 U/l	Entry calendar quarter, age, sex, race or ethnicity, DM, CV comorbidity, smoking, dialysis vintage, primary insurance, marital status, BMI, levels of ferritin, albumin, TIBC, bicarbonate, creatinine, WBC, lymphocyte %, normalized protein catabolic rate, Ca, PO_4, Hb, and ESA use	151
Chang *et al.* (2014)	Enfield Medical (9,514)	Prevalent HD	5 years	Increasing HRs by baseline ALP activity quintiles (HR 1.57 for ALP ≥150 U/l versus <60 U/l)	Age, DM, sex, dialysis vintage and dose, normalized protein catabolic rate, levels of albumin, urea, creatinine, triglyceride, cholesterol, glucose, ferritin, haematocrit, ALT, PTH, Ca, and PO_4	162
Lertdumrongluk *et al.* (2013)	Da Vita (102,149)	Prevalent HD	2.3 years (median)	• Increasing HRs with increasing baseline ALP activity • HRs for ALP activity ≥160 U/l versus <80 U/l 1.59 for age <45 years and 1.21 for age ≥75 years	Sex, race, ethnicity, DM, comorbidities, tobacco smoking, dialysis vintage, marital status, primary insurance, types of vascular access, dialysis dose, activated vitamin D agents, serum levels of albumin Ca, PO_4, and PTH	155

Table 2 (cont.) | **Studies of the association of total ALP and BALP levels with risk of all-cause mortality in patients with CKD undergoing dialysis**

Study	Population (*n*)	Dialysis type	Study duration	All-cause mortality risk	Adjustments	Refs
Fein *et al.* (2013)	Single centre (90)	Prevalent HD	Median 2.6 years	RR 6.0 for baseline ALP activity >120 U/l, RR 1.02 for each unit increase of baseline ALP activity	Age, race, sex, DM, hypertension, dialysis vintage, and albumin, corrected calcium, intact PTH, urea, creatinine, Hb, iron, AST, and WBC levels	154
Kobayashi *et al.* (2012)	Shirasagi (196)	Prevalent HD in male patients (female patients not included in the study)	5 years	HR 8.3 for baseline BALP activity >20.9 U/l	Age, dialysis vintage, CV comorbidity, SBP, DM, levels of PO4, albumin, creatinine, CRP, and vitamin D intake	171
Drechsler *et al.* (2011)	NECOSAD (800)	Incident HD plus 1 year of HD	4 years	Increasing HRs by tertiles of baseline BALP activity (5.7 (6 months) and 1.3 (4 years) for BALP activity >18 U/l versus ≤12 U/l)	Age, gender, dialysis modality, primary kidney disease, DM, CV disease, Khan comorbidity index, SBP, BMI, and levels of albumin, Ca, PO_4, PTH, and 25(OH) vitamin D	11
Beddhu *et al.* (2010)	HEMO (1,827)	Prevalent HD	Mean 6.6 years	• HR 1.2 for baseline ALP activity ≥97 U/l • HR 1.5 for time-dependent ALP activity ≥97 U/l	Demographics, randomized dialysis dose, flux interventions, clinical center, dialysis vintage, type of vascular access, comorbidity, BMI, and levels of hematocrit, albumin, AST, ALT, Ca, PO_4, and PTH	149
Blayney *et al.* (2008)	DOPPS I+II (14,643)	Prevalent HD	Mean 1.6 years	Increased HR with baseline ALP activity ≥0.65 × ULN or time-varying ALP activity ≥0.9 × ULN	Stratified by region and adjusted for facility-clustering effects, baseline case-mix, comorbidity and baseline or time-varying laboratory values	146
Regidor *et al.* (2008)	Da Vita (73,960)	Prevalent HD	3 years	• HR 1.25 for baseline ALP activity ≥120 U/l • A rise in ALP activity by 10 U/l during first 6 months was incrementally associated with increased HR during the subsequent 2.5 years	Entry calendar quarter, age, gender, race, ethnicity, comorbidity, smoking status, dialysis vintage, primary insurance, marital status, standardized mortality ratio of the dialysis clinic during entry quarter, dialysis dose, dialysis catheter, residual renal function during entry quarter, BMI, vitamin D analogues, daily protein intake, levels of albumin, AST, TIBC, ferritin, creatinine, PO_4, Ca, PTH, bicarbonate, WBC, lymphocyte (%), and Hb	145
Kalantar-Zadeh *et al.* (2006)	Da Vita (69,819)	Prevalent HD	2 years	Increased HR with baseline ALP activity ≥90 U/l and time-dependent ALP activity ≥110 U/l	Ca, PO_4, Ca×PO_4 product, PTH, entry calendar quarter, age, gender, race and ethnicity, DM, dialysis vintage, primary insurance, marital status, standardized mortality ratio of the dialysis clinic during entry quarter, dialysis dose, dialysate Ca, vitamin D analogs, and indicators of nutritional status and inflammation	144
Foley *et al.* (1996)	Multi-centre (423)	Incident PD (40%) and HD (60%)	Mean 3.4 years	RR 1.55 for baseline ALP activity >120 U/l	Age, DM, ischaemic heart disease, smoking status, cholesterol, mean arterial blood pressure, albumin, Hb, Ca and PO_4 levels	161

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BALP, bone-specific alkaline phosphatase; Ca, calcium; CKD, chronic kidney disease; CRP, C-reactive protein; CV, cardiovascular; DM, diabetes mellitus; ESA, erythropoiesis stimulating agents; GFR, glomerular filtration rate; GGT, γ-glutamyltransferase; Hb, haemoglobin; HD, haemodialysis; HR, hazard ratio; LVEF, left ventricular ejection fraction; OR, odds ratio; PD, peritoneal dialysis; PO_4, phosphate; PTH, parathyroid hormone; RR, relative risk; RRT, renal replacement therapy; SBP, systolic blood pressure; TIBC, total iron binding capacity; ULN, upper-limit of normal; WBC, white blood cell count.

BALP and mortality

The use of BALP levels in clinical practice is less common than that of total ALP levels and data on the association of BALP levels with mortality in the general population are sparse. As mentioned above, a large North American observational study could not establish an association between BALP levels and mortality in the general population or in patients with mild CKD[83]; however, this study had several shortcomings and its findings are unconfirmed. Other studies reported an independent association between BALP levels and mortality in patients with more advanced stages of CKD[11,171]. A stronger association between short-term mortality and BALP levels, compared to total ALP levels, was described

Table 3 | **Studies of the association of total ALP levels with all-cause mortality risk in patients with CKD not on dialysis**

Study	Population (*n*)	Sex, CKD stage	Study duration	All-cause mortality risk	Adjustments	Refs
Sumida *et al.* (2017)	TC–CKD (17,732)	Male (98%), stage 5	Median 2 years	HR 1.43 for averaged 6 month pre-dialysis ALP activity <66.0 U/l versus ≥111.1 U/l	Age, sex, race, ethnicity, marital status, CV comorbidity, dementia, lung disease, DM, liver disease, malignancy, Charlson Comorbidity Index, vitamin D analogs, phosphate binders, ACEI, ARB, statins, bicarbonate, ESA, eGFR, albumin averaged over the 6-month pre-dialysis period, and type of vascular access	167
Taliercio *et al.* (2013)	Cleveland Clinic, (28,678)	Not reported, stages 3–4	Median 2.2 years	• Increasing HRs with increasing quartiles of baseline ALP activity • HR 1.68 for baseline ALP activity ≥102 U/l versus <66 U/l	Age, sex, ethnicity, smoking, DM, CV comorbidity, hyperlipidaemia, malignancy, BMI, and levels of ACEI, ARB, statins, serum albumin, Hb, bicarbonate, GFR, AST, ALT, bilirubin, and Ca	156
Kovesdy *et al.* (2010)	Single centre (1,259)	Male, stages 1–5	Median 3.5 years	HR 1.17 for each 50 U/l increase of time-averaged ALP activity	Time period of inclusion, age, race, comorbidities, SBP, BMI, smoking, medication, levels of GFR, albumin, bicarbonate, AST, ALT, Ca, PO_4, Hb, WBC, lymphocytes (%) and 24-h urinary protein excretion	148
Beddhu *et al.* (2009)	AASK (1,094)	Not reported, stages 3–4	Mean 4.6 years	HR 1.3 for baseline ALP activity ≥80 U/l, HR 1.55 for each doubling of baseline ALP activity	Age, sex, CV comorbidity, SBP, BMI, smoking, GFR, proteinuria, and levels of AST, albumin, LDH, total bilirubin, GGT, Ca, and PO_4	147

ACEI, angiotensin converting enzyme inhibitor; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARB angiotensin II receptor blocker; AST, aspartate aminotransferase; Ca, calcium; CKD, chronic kidney disease; CV, cardiovascular; DM, diabetes mellitus; ESA, erythropoiesis stimulating agents; eGFR, estimated glomerular filtration rate; GGT, γ-glutamyltransferase; Hb, haemoglobin; HR, hazard ratio; LDH, lactate dehydrogenase; PO4, phosphate; RR, relative risk; SBP, systolic blood pressure; WBC, white blood cell count.

in 800 patients undergoing dialysis[11]. This study also found a somewhat weaker association between BALP and long-term mortality. Patients with low PTH and BALP levels had an increased risk of short-term mortality and signs of protein–energy wasting, compared to patients with low PTH and high BALP levels[11]. In male Japanese patients on dialysis, BALP levels were independently associated with all-cause mortality at 70 months[171]. No such association was found for the levels of PTH or other metabolic bone markers. In addition, patients had a lower biological variability in BALP levels than in the levels of PTH or other metabolic bone markers[171], which was consistent with findings from other studies[172,173]. High levels of total ALP and BALP were associated with high mortality in patients undergoing dialysis[157]; however, the association of BALP levels with mortality was no longer significant when total ALP levels was omitted from the Cox regression analysis. On the other hand, the lowest individual BALP levels during a 3-year period were associated with improved survival. A stronger association of ALP or BALP levels with short-term mortality than with long-term mortality in patients with CKD has been shown in several studies[11,149], which suggests underlying pathophysiologic processes with high progression rates. BALP is a promising predictor of mortality, especially in patients with CKD. The development of novel analytic methods based on levels of BALP isoforms will further improve the diagnostic accuracy of BALP levels in bone disease and CVD.

Treatment strategies targeting ALP

Established treatment regimens for mineral and bone disorders in CKD, such as administration of vitamin D receptor activators, cinacalcet or parathyroidectomy, target serum levels of PTH, calcium and phosphate[170]. Although these strategies can also influence ALP levels (FIG. 2a), no specific target levels of ALP have yet been proposed and no treatment strategy directed at lowering ALP levels has been established. ALP levels are a superior target than PTH levels for the treatment of disturbed bone turnover in CKD[75,174,175]. In addition, serum ALP and BALP levels predict cardiovascular complications and mortality and these enzymes are essential regulators of ectopic mineralization. These findings point towards a role of TNALP and, more specifically BALP, in the pathophysiology of CVD. Efforts are, therefore, underway to characterize ALP levels as a promising treatment target for the prevention of these severe complications. Such novel therapies have the potential to reduce the contribution of extra-skeletally generated ALP to increased cardiovascular risk in CKD.

Inhibition of ALP activity

TNALP has several inhibitor binding sites[176] and this knowledge has fuelled the development of several pharmacological inhibitors[70,177–181]. Most therapeutic strategies aimed at inhibiting ectopic mineralization are flawed by the risk of undesirable inhibition of skeletal mineralization. A novel direct ALP inhibitor, SBI-425, effectively inhibits vascular calcification in animal models at doses that do not alter bone mineralization[70]. This agent is an interesting compound for further development and application in human studies.

Modulation of ALP expression

A different approach to prevent cardiovascular complications in patients with renal diseases is the epigenetic modulation of TNALP expression. The novel bromodomain and extra-terminal motif inhibitor RVX-208 (also known as apabetalone) modulates the epigenetic regulation of several genes[182]. This inhibitor, which was developed for the treatment of atherosclerosis, increases circulating levels of apolipoprotein A-I and high-density lipoprotein[183]. Interestingly, two phase II trials showed

Table 4 | **Studies of the association of total ALP levels with all-cause mortality risk in renal transplant recipients with CKD**

Study	Population (*n*)	Transplantation status	Study duration	All-cause mortality risk	Comment	Refs
Molnar *et al.* (2012)	Da Vita, SRTR (11,776)	Incident	Median 2.3 years	HRs 1.64 for time-averaged pre-transplantation ALP activity ≥160 U/l and 1.49 for ALP activity 120 to <160 U/l versus ALP activity 80 ≤120 U/l	Adjusted for age, sex, recipient race, ethnicity, DM, dialysis vintage and dose, primary insurance, marital status, standardized mortality ratio of the dialysis clinic, HD catheter, residual renal function, comorbidities, BMI, nPNA, TIBC, ferritin, Ca, PO_4, PTH, bicarbonate, WBC, lymphocyte (%), albumin, donor type, donor age, donor sex, panel reactive antibody titre, number of HLA mismatches, cold ischaemia time, delayed graft function, and extended donor criteria	159
Zelle *et al.* (2010)	Single centre (602)	Prevalent	Median 5.3 years	HR 1.3 for each SD increase of baseline ALP activity	Adjusted for age, sex, GFR, urinary protein excretion, DM, components of metabolic syndrome, and CV risk factors	160

ALP, alkaline phosphatase; Ca, calcium; CKD, chronic kidney disease; CV, cardiovascular; DM, diabetes mellitus; GFR, glomerular filtration rate; HD, haemodialysis; HLA, human leukocyte antigen; nPNA, normalized protein nitrogen appearance; PO4, phosphate; PTH, parathyroid hormone; TIBC, total iron binding capacity; WBC, white blood cell count.

that apabetalone administration reduced circulating levels of ALP, which was associated with a marked reduction of major cardiovascular events[184,185]. The mechanism by which apabetalone modulates ALP activity is still unclear. A large phase III study of this compound for the prevention of cardiovascular complications in T2DM is ongoing[186]).

ALP targeted to mineralization in hypophosphatasia

Hypophosphatasia is a rare hereditary metabolic disease caused by inactivating mutations in *ALPL*[130]. This disorder manifests as a wide spectrum of mineralization defects, ranging from mild disease with only slight dental abnormality to severe infantile and prenatal forms with high mortality[130]. Although patients with hypophosphatasia often have hyperphosphataemia, this disease is not associated with accelerated vascular calcification. This finding suggests that TNALP is required for vascular calcification, as P_i is a strong inducer of vascular calcification[58]. Enzyme-replacement therapy with asfotase α, a recombinant mineral-targeted human TNALP, was recently approved to treat children with severe infantile hypophosphatasia. This therapy has resulted in dramatic improvements in bone mineralization[187] and survival[188]. Vascular calcification is presumably not yet present in these young individuals; however, long-term administration of asfotase α, especially in the presence of hyperphosphataemia, needs to be carefully monitored, as it could theoretically promote accelerated vascular calcification and resulting cardiovascular complications.

Targeting IALP

IALP supplementation prevented the development of features of the metabolic syndrome in mice fed a high fat-diet[109]. These findings suggest that oral IALP supplementation could represent a novel prevention and treatment strategy for metabolic syndrome in humans. Clinical studies have shown renoprotective and anti-inflammatory effects of bovine IALP in sepsis[189] and in patients undergoing cardiac surgery[190].

A phase I study showed that intravenous administration of a novel recombinant form of human IALP in healthy volunteers was safe and well tolerated[191]. Further clinical studies are needed to elucidate whether IALP could prevent and combat systemic and intestinal inflammation, metabolic syndrome and gut dysbiosis. The traditional herbal remedy curcumin increases the expression of IALP, corrects gut permeability in CKD[192], and inhibits manifestations of metabolic syndrome[193]. These findings raise the possibility of using nutritional approaches to treat metabolic syndrome, uraemic inflammation and gut dysbiosis via IALP upregulation.

Conclusions

Cardiovascular complications remain the leading cause of increased morbidity and mortality in CKD, T2DM, and ageing. The complexity of the underlying pathophysiological processes advocates for a multidimensional approach to treatment and prevention. Despite the introduction of novel treatment strategies targeting the cardiorenal and bone–vascular axes, the burden of CVD is still high. In view of the robust evidence presented in this Review, we propose that ALP is an evolving treatment target for CVD and metabolic syndrome. Thus, the development of compounds that inhibit TNALP either directly or by epigenetic modulation, is a promising novel approach to the treatment and prevention of cardiovascular complications in a large proportion of the general population.

Vascular calcification and inflammation might both drive the association between high ALP levels and increased mortality. BALP, which exerts its main effect on local calcification inhibitors, increases in the circulation in the setting of extensive vascular calcification. The levels of IALP and liver ALP on the other hand increase as part of an inflammatory defence mechanism. This association of high levels of serum ALP with inflammation might explain why in some cases statistical correction for CRP weakens the association of ALP or BALP with mortality. The development of novel TNALP inhibitors and modulators for the prevention of cardiovascular complications, and the development of systemically administered IALP for the treatment of acute inflammatory disorders are promising approaches to reduce the increased mortality associated with CKD and with features of the metabolic syndrome.

1. Levey, A. S. *et al.* Chronic kidney disease as a global public health problem: approaches and initiatives — a position statement from kidney disease improving global outcomes. *Kidney Int.* **72**, 247–259 (2007).
2. Stenvinkel, P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *J. Intern. Med.* **268**, 456–467 (2010).
3. Go, A., Chertow, G., Fan, D., McCulloch, C. & Hsu, C. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N. Engl. J. Med.* **351**, 1296–1305 (2004).
4. Xie, X. *et al.* Renin-angiotensin system inhibitors and kidney and cardiovascular outcomes in patients with CKD: a Bayesian network meta-analysis of randomized clinical trials. *Am. J. Kidney Dis.* **67**, 728–741 (2016).
5. Palmer, S. C. *et al.* HMG CoA reductase inhibitors (statins) for people with chronic kidney disease not requiring dialysis. *Cochrane Database Syst. Rev.* **5**, CD007784 (2014).
6. Stenvinkel, P. & Larsson, T. E. Chronic kidney disease: a clinical model of premature aging. *Am. J. Kidney Dis.* **62**, 339–351 (2013).
7. Kooman, J. P., Kotanko, P., Schols, A. M., Shiels, P. G. & Stenvinkel, P. Chronic kidney disease and premature ageing. *Nat. Rev. Nephrol.* **10**, 732–742 (2014).
8. Di Lullo, L. *et al.* Chronic kidney disease and cardiovascular complications. *Heart Fail. Rev.* **20**, 259–272 (2015).
9. Li, J. W., Xu, C., Fan, Y., Wang, Y. & Xiao, Y. B. Can serum levels of alkaline phosphatase and phosphate predict cardiovascular diseases and total mortality in individuals with preserved renal function? A systemic review and meta-analysis. *PLoS ONE* **9**, e102276 (2014).
10. Reid, I. R., Gamble, G. D. & Bolland, M. J. Circulating calcium concentrations, vascular disease and mortality: a systematic review. *J. Intern. Med.* **279**, 524–540 (2016).
11. Drechsler, C. *et al.* Bone alkaline phosphatase and mortality in dialysis patients. *Clin. J. Am. Soc. Nephrol.* **6**, 1752–1759 (2011).
12. Millan, J. L. *Mammalian Alkaline Phosphatase: From Biology to Applications in Medicine and Biotechnology* (Wiley, 2006).
13. Magnusson, P., Degerblad, M., Sääf, M., Larsson, L. & Thorén, M. Different responses of bone alkaline phosphatase isoforms during recombinant insulin-like growth factor-i (IGF-I) and during growth hormone therapy in adults with growth hormone deficiency. *J. Bone Miner. Res.* **12**, 210–220 (1997).
14. Magnusson, P., Löfman, O. & Larsson, L. Determination of alkaline phosphatase isoenzymes in serum by high-performance liquid chromatography with post-column reaction detection. *J. Chromatogr.* **576**, 79–86 (1992).
15. Magnusson, P. & Farley, J. Differences in sialic acid residues among bone alkaline phosphatase isoforms: a physical, biochemical, and immunological characterization. *Calcif. Tissue Int.* **71**, 508–518 (2002).
16. Halling Linder, C., Narisawa, S., Millán, J. & Magnusson, P. Glycosylation differences contribute to distinct catalytic properties among bone alkaline phosphatase isoforms. *Bone* **45**, 987–993 (2009).
17. Weiss, M. J. *et al.* Structure of the human liver/bone/kidney alkaline phosphatase gene. *J. Biol. Chem.* **263**, 12002–12010 (1988).
18. Millán, J. L. & Fishman, W. H. Biology of human alkaline phosphatases with special reference to cancer. *Crit. Rev. Clin. Lab. Sci.* **32**, 1–39 (1995).
19. Magnusson, P. *et al.* Monoclonal antibodies against tissue-nonspecific alkaline phosphatase. Report of the ISOBM TD9 workshop. *Tumour Biol.* **23**, 228–248 (2002).
20. Mornet, E. *et al.* Structural evidence for a functional role of human tissue nonspecific alkaline phosphatase in bone mineralization. *J. Biol. Chem.* **276**, 31171–31178 (2001).
21. Alcantara, E. H. *et al.* Zinc deprivation inhibits extracellular matrix calcification through decreased synthesis of matrix proteins in osteoblasts. *Mol. Nutr. Food Res.* **55**, 1552–1560 (2011).
22. Heaton, F. W. Effect of magnesium deficiency on plasma alkaline phosphatase activity. *Nature* **207**, 1292–1293 (1965).
23. Hoylaerts, M. F. *et al.* Functional significance of calcium binding to tissue-nonspecific alkaline phosphatase. *PLoS ONE* **10**, e0119874 (2015).
24. Fedde, K. N., Lane, C. C. & Whyte, M. P. Alkaline phosphatase is an ectoenzyme that acts on micromolar concentrations of natural substrates at physiologic pH in human osteosarcoma (SAOS-2) cells. *Arch. Biochem. Biophys.* **264**, 400–409 (1988).
25. Udenfriend, S. & Kodukula, K. How glycosylphosphatidylinositol-anchored membrane proteins are made. *Annu. Rev. Biochem.* **64**, 563–591 (1995).
26. Low, M. G. The glycosyl-phosphatidylinositol anchor of membrane proteins. *Biochim. Biophys. Acta* **988**, 427–454 (1989).
27. Hooper, N. M. Glycosyl-phosphatidylinositol anchored membrane enzymes. *Clin. Chim. Acta* **266**, 3–12 (1997).
28. Magnusson, P., Sharp, C. A. & Farley, J. R. Different distributions of human bone alkaline phosphatase isoforms in serum and bone tissue extracts. *Clin. Chim. Acta* **325**, 59–70 (2002).
29. Anh, D. J., Eden, A. & Farley, J. R. Quantitation of soluble and skeletal alkaline phosphatase, and insoluble alkaline phosphatase anchor-hydrolase activities in human serum. *Clin. Chim. Acta* **311**, 137–148 (2001).
30. Magnusson, P., Löfman, O. & Larsson, L. Methodological aspects on separation and reaction conditions of bone and liver alkaline phosphatase isoform analysis by high-performance liquid chromatography. *Anal. Biochem.* **211**, 156–163 (1993).
31. Magnusson, P. *et al.* Effect of chronic renal failure on bone turnover and bone alkaline phosphatase isoforms. *Kidney Int.* **60**, 257–265 (2001).
32. Magnusson, P., Häger, A. & Larsson, L. Serum osteocalcin and bone and liver alkaline phosphatase isoforms in healthy children and adolescents. *Pediatr. Res.* **38**, 955–961 (1995).
33. Magnusson, P. *et al.* Differences of bone alkaline phosphatase isoforms in metastatic bone disease and discrepant effects of clodronate on different skeletal sites indicated by the location of pain. *Clin. Chem.* **44**, 1621–1628 (1998).
34. Magnusson, P., Davie, M. & Sharp, C. Circulating and tissue-derived isoforms of bone alkaline phosphatase in Paget's disease of bone. *Scand. J. Clin. Lab. Invest.* **70**, 128–135 (2010).
35. Magnusson, P., Larsson, L., Magnusson, M., Davie, M. & Sharp, C. Isoforms of bone alkaline phosphatase: characterization and origin in human trabecular and cortical bone. *J. Bone Miner. Res.* **14**, 1926–1933 (1999).
36. Swolin-Eide, D., Hansson, S., Larsson, L. & Magnusson, P. The novel bone alkaline phosphatase b1x isoform in children with kidney disease. *Pediatr. Nephrol.* **21**, 1723–1729 (2006).
37. Haarhaus, M., Fernström, A., Magnusson, M. & Magnusson, P. Clinical significance of bone alkaline phosphatase isoforms, including the novel b1x isoform, in mild to moderate chronic kidney disease. *Nephrol. Dial. Transplant.* **24**, 3382–3389 (2009).
38. Haarhaus, M., Monier-Faugere, M. C., Magnusson, P. & Malluche, H. H. Bone alkaline phosphatase isoforms in hemodialysis patients with low versus non-low bone turnover: a diagnostic test study. *Am. J. Kidney Dis.* **66**, 99–105 (2015).
39. Shioi, A. *et al.* Induction of bone-type alkaline phosphatase in human vascular smooth muscle cells: roles of tumor necrosis factor-alpha and oncostatin M derived from macrophages. *Circ. Res.* **91**, 9–16 (2002).
40. Haarhaus, M., Arnqvist, H. J. & Magnusson, P. Calcifying human aortic smooth muscle cells express different bone alkaline phosphatase isoforms, including the novel b1x isoform. *J. Vasc. Res.* **50**, 167–174 (2013).
41. Grabowski, P. Physiology of bone. *Endocr. Dev.* **28**, 33–55 (2015).
42. Bleicher, F. Odontoblast physiology. *Exp. Cell Res.* **325**, 65–71 (2014).
43. Golub, E. E. Biomineralization and matrix vesicles in biology and pathology. *Semin. Immunopathol.* **33**, 409–417 (2011).
44. New, S. E. *et al.* Macrophage-derived matrix vesicles: an alternative novel mechanism for microcalcification in atherosclerotic plaques. *Circ. Res.* **113**, 72–77 (2013).
45. Allahverdian, S., Chehroudi, A. C., McManus, B. M., Abraham, T. & Francis, G. A. Contribution of intimal smooth muscle cells to cholesterol accumulation and macrophage-like cells in human atherosclerosis. *Circulation* **129**, 1551–1559 (2014).
46. Kramann, R. *et al.* Adventitial msc-like cells are progenitors of vascular smooth muscle cells and drive vascular calcification in chronic kidney disease. *Cell Stem Cell* **19**, 628–642 (2016).
47. Chen, N., O'Neill, K., Chen, X. & Moe, S. Annexin-mediated matrix vesicle calcification in vascular smooth muscle cells. *J. Bone Miner. Res.* **23**, 1798–1805 (2008).
48. Millan, J. L. The role of phosphatases in the initiation of skeletal mineralization. *Calcif. Tissue Int.* **93**, 299–306 (2013).
49. Ali, S. Y., Sajdera, S. W. & Anderson, H. C. Isolation and characterization of calcifying matrix vesicles from epiphyseal cartilage. *Proc. Natl Acad. Sci. USA* **67**, 1513–1520 (1970).
50. Dai, X. Y. *et al.* Phosphate-induced autophagy counteracts vascular calcification by reducing matrix vesicle release. *Kidney Int.* **83**, 1042–1051 (2013).
51. Goettsch, C. *et al.* Sortilin mediates vascular calcification via its recruitment into extracellular vesicles. *J. Clin. Invest.* **126**, 1323–1336 (2016).
52. Fleish, H. & Neuman, W. F. Mechanisms of calcification: role of collagen, polyphosphates, and phosphatase. *Am. J. Physiol.* **200**, 1296–1300 (1961).
53. Fleisch, H. & Bisaz, S. Mechanism of calcification: inhibitory role of pyrophosphate. *Nature* **195**, 911 (1962).
54. Hessle, L. *et al.* Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proc. Natl Acad. Sci. USA* **99**, 9445–9449 (2002).
55. Jono, S., Peinado, C. & Giachelli, C. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. *J. Biol. Chem.* **275**, 20197–20203 (2000).
56. Halling Linder, C. *et al.* Bone alkaline phosphatase and tartrate-resistant acid phosphatase: potential co-regulators of bone mineralization. *Calcif. Tissue Int.* http://dx.doi.org/10.1007/s00223-017-0259-2 (2017).
57. Robison, R. The possible significance of hexosephosphoric esters in ossification. *Biochem. J.* **17**, 286–293 (1923).
58. Villa-Bellosta, R. & Egido, J. Phosphate, pyrophosphate, and vascular calcification: a question of balance. *Eur. Heart J.* http://dx.doi.org/10.1093/eurheartj/ehv605 (2015).
59. Lomashvili, K. A., Narisawa, S., Millan, J. L. & O'Neill, W. C. Vascular calcification is dependent on plasma levels of pyrophosphate. *Kidney Int.* **85**, 1351–1356 (2014).
60. de Oliveira, R. A. *et al.* Peritoneal dialysis per se is a risk factor for sclerostin-associated adynamic bone disease. *Kidney Int.* **87**, 1039–1045 (2015).
61. Murali, S. K. *et al.* Fgf23 regulates bone mineralization in a $1,25(OH)_2D_3$ and Klotho-independent manner. *J. Bone Miner. Res.* **31**, 129–142 (2016).
62. Lieben, L. *et al.* Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. *J. Clin. Invest.* **122**, 1803–1815 (2012).
63. Villa-Bellosta, R., Wang, X., Millan, J. L., Dubyak, G. R. & O'Neill, W. C. Extracellular pyrophosphate metabolism and calcification in vascular smooth muscle. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H61–H68 (2011).
64. Moe, S. *et al.* Medial artery calcification in ESRD patients is associated with deposition of bone matrix proteins. *Kidney Int.* **61**, 638–647 (2002).
65. Shroff, R. *et al.* Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. *Circulation* **118**, 1748–1757 (2008).
66. Shanahan, C. *et al.* Medial localization of mineralization-regulating proteins in association with mönckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. *Circulation* **100**, 2168–2176 (1999).
67. Chen, N. & Moe, S. Arterial calcification in diabetes. *Curr. Diab. Rep.* **3**, 28–32 (2003).
68. St Hilaire, C. *et al.* Nt5e mutations and arterial calcifications. *N. Engl. J. Med.* **364**, 432–442 (2011).
69. Villa-Bellosta, R. *et al.* Defective extracellular pyrophosphate metabolism promotes vascular calcification in a mouse model of Hutchinson–Gilford progeria syndrome that is ameliorated on pyrophosphate treatment. *Circulation* **127**, 2442–2451 (2013).
70. Sheen, C. R. *et al.* Pathophysiological role of vascular smooth muscle alkaline phosphatase in medial artery calcification. *J. Bone Miner. Res.* **30**, 824–836 (2015).

71. Savinov, A. Y. *et al.* Transgenic overexpression of tissue-nonspecific alkaline phosphatase (TNAP) in vascular endothelium results in generalized arterial calcification. *J. Am. Heart Assoc.* **4**, e002499 (2015).
72. Chen, N. X. & Moe, S. M. Pathophysiology of vascular calcification. *Curr. Osteoporos. Rep.* **13**, 372–380 (2015).
73. Graciolli, F. *et al.* Phosphorus overload and PTH induce aortic expression of Runx2 in experimental uraemia. *Nephrol. Dial. Transplant.* **24**, 1416–1421 (2009).
74. Tangri, N. *et al.* Effect of bone mineral guideline target achievement on mortality in incident dialysis patients: an analysis of the United Kingdom Renal Registry. *Am. J. Kidney Dis.* **57**, 415–421 (2011).
75. Sardiwal, S., Magnusson, P., Goldsmith, D. J. & Lamb, E. J. Bone alkaline phosphatase in CKD-mineral bone disorder. *Am. J. Kidney Dis.* **62**, 810–822 (2013).
76. Palmer, S. C. *et al.* Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *JAMA* **305**, 1119–1127 (2011).
77. Fahrleitner-Pammer, A. *et al.* Bone markers predict cardiovascular events in chronic kidney disease. *J. Bone Miner. Res.* **23**, 1850–1858 (2008).
78. Damera, S. *et al.* Serum alkaline phosphatase levels associate with elevated serum c-reactive protein in chronic kidney disease. *Kidney Int.* **79**, 228–233 (2011).
79. Nayeem, F., Anderson, K. E., Nagamani, M., Grady, J. J. & Lu, L. J. Alkaline phosphatase and percentage body fat predict circulating c-reactive protein in premenopausal women. *Biomarkers* **15**, 663–670 (2010).
80. Lee, C. T. *et al.* Association between c-reactive protein and biomarkers of bone and mineral metabolism in chronic hemodialysis patients: a cross-sectional study. *J. Ren. Nutr.* **19**, 220–227 (2009).
81. Cheung, B. M. *et al.* Association between plasma alkaline phosphatase and c-reactive protein in Hong Kong Chinese. *Clin. Chem. Lab. Med.* **46**, 523–527 (2008).
82. Kunutsor, S. K. *et al.* Serum alkaline phosphatase and risk of incident cardiovascular disease: interrelationship with high sensitivity c-reactive protein. *PLoS ONE* **10**, e0132822 (2015).
83. Filipowicz, R. *et al.* Associations of serum skeletal alkaline phosphatase with elevated c-reactive protein and mortality. *Clin. J. Am. Soc. Nephrol.* **8**, 26–32 (2013).
84. Wannamethee, S. G., Sattar, N., Papcosta, O., Lennon, L. & Whincup, P. H. Alkaline phosphatase, serum phosphate, and incident cardiovascular disease and total mortality in older men. *Arterioscler. Thromb. Vasc. Biol.* **33**, 1070–1076 (2013).
85. Cho, I. J. *et al.* Effects of c-reactive protein on bone cells. *Life Sci.* **145**, 1–8 (2016).
86. Lencel, P., Delplace, S., Hardouin, P. & Magne, D. TNF-α stimulates alkaline phosphatase and mineralization through PPARγ inhibition in human osteoblasts. *Bone* **48**, 242–249 (2011).
87. Aghagolzadeh, P. *et al.* Calcification of vascular smooth muscle cells is induced by secondary calciprotein particles and enhanced by tumor necrosis factor-alpha. *Atherosclerosis* **251**, 404–414 (2016).
88. Shanmugham, L. N. *et al.* Il-1beta induces alkaline phosphatase in human phagocytes. *Arch. Med. Res.* **38**, 39–44 (2007).
89. Kukulski, F., Levesque, S. A. & Sevigny, J. Impact of ectoenzymes on p2 and p1 receptor signaling. *Adv. Pharmacol.* **61**, 263–299 (2011).
90. Antonioli, L., Pacher, P., Vizi, E. S. & Hasko, G. Cd39 and cd73 in immunity and inflammation. *Trends Mol. Med.* **19**, 355–367 (2013).
91. Fawley, J. & Gourlay, D. M. Intestinal alkaline phosphatase: a summary of its role in clinical disease. *J. Surg. Res.* **202**, 225–234 (2016).
92. Okada, T., Zinchuk, V. S. & Seguchi, H. Lipopolysaccharide administration increases acid and alkaline phosphatase reactivity in the cardiac muscle. *Microsc. Res. Tech.* **58**, 421–426 (2002).
93. Pike, A. F., Kramer, N. I., Blaauboer, B. J., Seinen, W. & Brands, R. A novel hypothesis for an alkaline phosphatase 'rescue' mechanism in the hepatic acute phase immune response. *Biochim. Biophys. Acta* **1832**, 2044–2056 (2013).
94. Jalkanen, J. *et al.* Aberrant circulating levels of purinergic signaling markers are associated with several key aspects of peripheral atherosclerosis and thrombosis. *Circ. Res.* **116**, 1206–1215 (2015).
95. Qian, S. *et al.* The P2Y2 nucleotide receptor is an inhibitor of vascular calcification. *Atherosclerosis* **257**, 38–46 (2017).
96. Lalles, J. P. Intestinal alkaline phosphatase: novel functions and protective effects. *Nutr. Rev.* **72**, 82–94 (2014).
97. Alpers, D. H. *et al.* Intestinal alkaline phosphatase in patients with chronic renal failure. *Gastroenterology* **94**, 62–67 (1988).
98. De Broe, M. E., Bosteels, V. & Wieme, R. J. Letter: increased intestinal alkaline phosphatase in serum of patients on maintenance haemodialysis. *Lancet* **303**, 753–754 (1974).
99. Stepan, J., Pilarova, T. & Melicharova, D. The source and clinical significance of serum alkaline phosphatases in patients treated by chronic dialysis. *Acta Univ. Carol. Med. Monogr.* **78**, 75–79 (1977).
100. Tibi, L., Chhabra, S. C., Sweeting, V. M., Winney, R. J. & Smith, A. F. Multiple forms of alkaline phosphatase in plasma of hemodialysis patients. *Clin. Chem.* **37**, 815–820 (1991).
101. Zetterberg, H. Increased serum concentrations of intestinal alkaline phosphatase in peritoneal dialysis. *Clin. Chem.* **51**, 675–676 (2005).
102. Raj, D. S. *et al.* Soluble CD14 levels, interleukin 6, and mortality among prevalent hemodialysis patients. *Am. J. Kidney Dis.* **54**, 1072–1080 (2009).
103. Andersen, K. *et al.* Intestinal dysbiosis, barrier dysfunction, and bacterial translocation account for CKD-related systemic inflammation. *J. Am. Soc. Nephrol.* **28**, 76–83 (2016).
104. Kieffer, D. A. *et al.* Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. *Am. J. Physiol. Renal Physiol.* **310**, F857–F871 (2016).
105. Malo, M. S. *et al.* Intestinal alkaline phosphatase preserves the normal homeostasis of gut microbiota. *Gut* **59**, 1476–1484 (2010).
106. Aronsson, B., Barany, P., Nord, C. E., Nystrom, B. & Stenvinkel, P. *Clostridium difficile*-associated diarrhoea in uremic patients. *Eur. J. Clin. Microbiol.* **6**, 352–356 (1987).
107. Alam, S. N. *et al.* Intestinal alkaline phosphatase prevents antibiotic-induced susceptibility to enteric pathogens. *Ann. Surg.* **259**, 715–722 (2014).
108. Singh, A. K. & Kari, J. A. Metabolic syndrome and chronic kidney disease. *Curr. Opin. Nephrol. Hypertens.* **22**, 198–203 (2013).
109. Kaliannan, K. *et al.* Intestinal alkaline phosphatase prevents metabolic syndrome in mice. *Proc. Natl Acad. Sci. USA* **110**, 7003–7008 (2013).
110. Malo, M. S. A high level of intestinal alkaline phosphatase is protective against type 2 diabetes mellitus irrespective of obesity. *EBioMedicine* **2**, 2016–2023 (2015).
111. Schultz-Hector, S., Balz, K., Bohm, M., Ikehara, Y. & Rieke, L. Cellular localization of endothelial alkaline phosphatase reaction product and enzyme protein in the myocardium. *J. Histochem. Cytochem.* **41**, 1813–1821 (1993).
112. Deracinois, B., Lenfant, A. M., Dehouck, M. P. & Flahaut, C. Tissue non-specific alkaline phosphatase (TNAP) in vessels of the brain. *Subcell. Biochem.* **76**, 125–151 (2015).
113. Liu, Y., Drozdov, I., Shroff, R., Beltran, L. E. & Shanahan, C. M. Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. *Circ. Res.* **112**, e99–e109 (2013).
114. Yamazaki, Y. *et al.* Vascular cell senescence contributes to blood-brain barrier breakdown. *Stroke* **47**, 1068–1077 (2016).
115. Pike, A. F., Kramer, N. I., Blaauboer, B. J., Seinen, W. & Brands, R. An alkaline phosphatase transport mechanism in the pathogenesis of Alzheimer's disease and neurodegeneration. *Chem. Biol. Interact.* **226**, 30–39 (2015).
116. Perticone, F. *et al.* Serum alkaline phosphatase negatively affects endothelium-dependent vasodilation in naive hypertensive patients. *Hypertension* **66**, 874–880 (2015).
117. Mody, N., Parhami, F., Sarafian, T. & Demer, L. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. *Free Radic. Biol. Med.* **31**, 509–519 (2001).
118. Muteliefu, G., Enomoto, A., Jiang, P., Takahashi, M. & Niwa, T. Indoxyl sulphate induces oxidative stress and the expression of osteoblast-specific proteins in vascular smooth muscle cells. *Nephrol. Dial. Transplant.* **24**, 2051–2058 (2009).
119. Watanabe, H. *et al.* P-cresyl sulfate, a uremic toxin, causes vascular endothelial and smooth muscle cell damages by inducing oxidative stress. *Pharmacol. Res. Perspect.* **3**, e00092 (2015).
120. Raghuraman, G. *et al.* Eotaxin augments calcification in vascular smooth muscle cells. *J. Cell. Biochem.* **118**, 647–654 (2017).
121. Torino, C. *et al.* Oxidative stress as estimated by gamma-glutamyl transferase levels amplifies the alkaline phosphatase-dependent risk for mortality in ESKD patients on dialysis. *Oxid. Med. Cell. Longev.* **2016**, 8490643 (2016).
122. Oh, S. W., Han, K. H. & Han, S. Y. Associations between renal hyperfiltration and serum alkaline phosphatase. *PLoS ONE* **10**, e0122921 (2015).
123. Bulum, T., Kolaric, B., Duvnjak, M. & Duvnjak, L. Alkaline phosphatase is independently associated with renal function in normoalbuminuric type 1 diabetic patients. *Ren. Fail.* **36**, 372–377 (2014).
124. Pfleiderer, G. *et al.* Change in alkaline phosphatase isoenzyme pattern in urine as possible marker for renal disease. *Kidney Int.* **17**, 242–249 (1980).
125. Tobar, A. *et al.* Proximal tubular hypertrophy and enlarged glomerular and proximal tubular urinary space in obese subjects with proteinuria. *PLoS ONE* **8**, e75547 (2013).
126. Canales, B. K. *et al.* Renal glomerular and tubular injury after gastric bypass in obese rats. *Nutrition* **28**, 76–80 (2012).
127. Kapojos, J. J. *et al.* Induction of glomerular alkaline phosphatase after challenge with lipopolysaccharide. *Int. J. Exp. Pathol.* **84**, 135–144 (2003).
128. Briere, N., Petitclerc, C. & Plante, G. Presence of alkaline phosphatase and gamma-glutamyl transpeptidase on the parietal layer of Bowman's capsule. *Acta Histochem.* **73**, 237–241 (1983).
129. Briere, N., Plante, G. E. & Petitclerc, C. Electron-microscopic demonstration of alkaline-phosphatase activity in the juxtaglomerular apparatus. *Cell. Tissue Res.* **234**, 147–151 (1983).
130. Whyte, M. P. Hypophosphatasia — aetiology, nosology, pathogenesis, diagnosis and treatment. *Nat. Rev. Endocrinol.* **12**, 233–246 (2016).
131. Kunutsor, S. K., Apekey, T. A. & Khan, H. Liver enzymes and risk of cardiovascular disease in the general population: a meta-analysis of prospective cohort studies. *Atherosclerosis* **236**, 7–17 (2014).
132. Iqbal, M. P., Mehboobali, N., Azam, I. & Tareen, A. K. Association of alkaline phosphatase with acute myocardial infarction in a population with high prevalence of hypovitaminosis D. *Clin. Chim. Acta* **425**, 192–195 (2013).
133. Park, J. B. *et al.* Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. *Eur. Heart J.* **34**, 920–931 (2013).
134. Shantouf, R. *et al.* Association of serum alkaline phosphatase with coronary artery calcification in maintenance hemodialysis patients. *Clin. J. Am. Soc. Nephrol.* **4**, 1106–1114 (2009).
135. London, G. *et al.* Arterial calcifications and bone histomorphometry in end-stage renal disease. *J. Am. Soc. Nephrol.* **15**, 1943–1951 (2004).
136. Barreto, D. V. *et al.* Association of changes in bone remodeling and coronary calcification in hemodialysis patients: a prospective study. *Am. J. Kidney Dis.* **52**, 1139–1150 (2008).
137. Kurz, P. *et al.* Evidence for abnormal calcium homeostasis in patients with adynamic bone disease. *Kidney Int.* **46**, 855–861 (1994).
138. Bover, J. *et al.* Adynamic bone disease: from bone to vessels in chronic kidney disease. *Semin. Nephrol.* **34**, 626–640 (2014).
139. Briet, M. *et al.* Age-independent association between arterial and bone remodeling in mild-to-moderate chronic kidney disease. *Nephrol. Dial. Transplant.* **25**, 191–197 (2010).
140. Lomashvili, K., Garg, P., Narisawa, S., Millan, J. & O'Neill, W. Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. *Kidney Int.* **73**, 1024–1030 (2008).
141. Lomashvili, K., Khawandi, W. & O'Neill, W. Reduced plasma pyrophosphate levels in hemodialysis patients. *J. Am. Soc. Nephrol.* **16**, 2495–2500 (2005).
142. O'Neill, W. C., Lomashvili, K. A., Malluche, H. H., Faugere, M. C. & Riser, B. L. Treatment with pyrophosphate inhibits uremic vascular calcification. *Kidney Int.* **79**, 512–517 (2011).

143. Tonelli, M. *et al.* Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. *Circulation* **120**, 1784–1792 (2009).
144. Kalantar-Zadeh, K. *et al.* Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. *Kidney Int.* **70**, 771–780 (2006).
145. Regidor, D. *et al.* Serum alkaline phosphatase predicts mortality among maintenance hemodialysis patients. *J. Am. Soc. Nephrol.* **19**, 2193–2203 (2008).
146. Blayney, M. J. *et al.* High alkaline phosphatase levels in hemodialysis patients are associated with higher risk of hospitalization and death. *Kidney Int.* **74**, 655–663 (2008).
147. Beddhu, S., Ma, X., Baird, B., Cheung, A. & Greene, T. Serum alkaline phosphatase and mortality in African Americans with chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* **4**, 1805–1810 (2009).
148. Kovesdy, C. P., Ureche, V., Lu, J. L. & Kalantar-Zadeh, K. Outcome predictability of serum alkaline phosphatase in men with pre-dialysis CKD. *Nephrol. Dial. Transplant.* **25**, 3003–3011 (2010).
149. Beddhu, S., Baird, B., Ma, X., Cheung, A. K. & Greene, T. Serum alkaline phosphatase and mortality in hemodialysis patients. *Clin. Nephrol.* **74**, 91–96 (2010).
150. Lukowsky, L. R. *et al.* Mineral and bone disorders and survival in hemodialysis patients with and without polycystic kidney disease. *Nephrol. Dial. Transplant.* **27**, 2899–2907 (2012).
151. Rhee, C. M. *et al.* Comparative mortality-predictability using alkaline phosphatase and parathyroid hormone in patients on peritoneal dialysis and hemodialysis. *Perit. Dial. Int.* **34**, 732–748 (2014).
152. Liu, X. *et al.* Alkaline phosphatase and mortality in patients on peritoneal dialysis. *Clin. J. Am. Soc. Nephrol.* **9**, 771–778 (2014).
153. Maruyama, Y. *et al.* A higher serum alkaline phosphatase is associated with the incidence of hip fracture and mortality among patients receiving hemodialysis in Japan. *Nephrol. Dial. Transplant.* **29**, 1532–1538 (2014).
154. Fein, P. A. *et al.* Relationship between alkaline phosphatase and all-cause mortality in peritoneal dialysis patients. *Adv. Perit. Dial.* **29**, 61–63 (2013).
155. Lertdumrongluk, P. *et al.* Impact of age on survival predictability of bone turnover markers in hemodialysis patients. *Nephrol. Dial. Transplant.* **28**, 2535–2545 (2013).
156. Taliercio, J. J. *et al.* Prognostic importance of serum alkaline phosphatase in CKD stages 3–4 in a clinical population. *Am. J. Kidney Dis.* **62**, 703–710 (2013).
157. Beige, J. *et al.* Association of serum alkaline phosphatase with mortality in non-selected European patients with CKD5D: an observational, three-centre survival analysis. *BMJ Open* **4**, e004275 (2014).
158. Soohoo, M. *et al.* Changes in markers of mineral and bone disorders and mortality in incident hemodialysis patients. *Am. J. Nephrol.* **43**, 85–96 (2016).
159. Molnar, M. Z., Kovesdy, C. P., Mucsi, I., Salusky, I. B. & Kalantar-Zadeh, K. Association of pre-kidney transplant markers of mineral and bone disorder with post-transplant outcomes. *Clin. J. Am. Soc. Nephrol.* **7**, 1859–1871 (2012).
160. Zelle, D. M. *et al.* Markers of the hepatic component of the metabolic syndrome as predictors of mortality in renal transplant recipients. *Am. J. Transplant.* **10**, 106–114 (2010).
161. Foley, R. N. *et al.* Hypocalcemia, morbidity, and mortality in end-stage renal disease. *Am. J. Nephrol.* **16**, 386–393 (1996).
162. Chang, J. F. *et al.* Combined alkaline phosphatase and phosphorus levels as a predictor of mortality in maintenance hemodialysis patients. *Medicine (Baltimore)* **93**, e106 (2014).
163. Chua, H. R. *et al.* Predicting first-year mortality in incident dialysis patients with end-stage renal disease — the UREA5 study. *Blood Purif.* **37**, 85–92 (2014).
164. Lin, Y. C. *et al.* Effect modifying role of serum calcium on mortality-predictability of PTH and alkaline phosphatase in hemodialysis patients: an investigation using data from the Taiwan Renal Registry Data System from 2005 to 2012. *PLoS ONE* **10**, e0129737 (2015).
165. Zhu, J. G. *et al.* Serum alkaline phosphatase levels are not associated with increased death risk in prevalent hemodialysis patients: 5-year experience in a single hemodialysis center. *Kidney Blood Press. Res.* **41**, 498–506 (2016).
166. Scialla, J. J. *et al.* Race, mineral homeostasis and mortality in patients with end-stage renal disease on dialysis. *Am. J. Nephrol.* **42**, 25–34 (2015).
167. Sumida, K. *et al.* Prognostic significance of pre-end-stage renal disease serum alkaline phosphatase for post-end-stage renal disease mortality in late-stage chronic kidney disease patients transitioning to dialysis. *Nephrol. Dial. Transplant.* http://dx.doi.org/10.1093/ndt/gfw412 (2017).
168. Bretaudiere, J. P. *et al.* Criteria for establishing a standardized method for determining alkaline phosphatase activity in human serum. *Clin. Chem.* **23**, 2263–2274 (1977).
169. Tietz, N. W. *et al.* Transferability studies for the AACC reference method and the IFCC method for measurement of alkaline phosphatase activity. *Clin. Chem.* **30**, 704–706 (1984).
170. Kidney Disease: Improving Global Outcomes (KDIGO) CKD–MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int. Suppl.* **76**, S1–S130 (2009).
171. Kobayashi, I. *et al.* Higher serum bone alkaline phosphatase as a predictor of mortality in male hemodialysis patients. *Life Sci.* **90**, 212–218 (2012).
172. Sardiwal, S. *et al.* Bone-specific alkaline phosphatase concentrations are less variable than those of parathyroid hormone in stable hemodialysis patients. *Kidney Int.* **82**, 100–105 (2012).
173. Gardham, C. *et al.* Variability of parathyroid hormone and other markers of bone mineral metabolism in patients receiving hemodialysis. *Clin. J. Am. Soc. Nephrol.* **5**, 1261–1267 (2010).
174. Garrett, G., Sardiwal, S., Lamb, E. J. & Goldsmith, D. J. PTH — a particularly tricky hormone: why measure it at all in kidney patients? *Clin. J. Am. Soc. Nephrol.* **8**, 299–312 (2013).
175. Lamb, E. J. & Delaney, M. P. Does PTH offer additive value to ALP measurement in assessing CKD-MBD? *Perit. Dial. Int.* **34**, 687–691 (2014).
176. Kozlenkov, A. *et al.* Residues determining the binding specificity of uncompetitive inhibitors to tissue-nonspecific alkaline phosphatase. *J. Bone Miner. Res.* **19**, 1862–1872 (2004).
177. Dahl, R. *et al.* Discovery and validation of a series of aryl sulfonamides as selective inhibitors of tissue-nonspecific alkaline phosphatase (TNAP). *J. Med. Chem.* **52**, 6919–6925 (2009).
178. Sergienko, E. *et al.* Identification and characterization of novel tissue-nonspecific alkaline phosphatase inhibitors with diverse modes of action. *J. Biomol. Screen.* **14**, 824–837 (2009).
179. Sidique, S. *et al.* Design and synthesis of pyrazole derivatives as potent and selective inhibitors of tissue-nonspecific alkaline phosphatase (TNAP). *Bioorg. Med. Chem. Lett.* **19**, 222–225 (2009).
180. Debray, J. *et al.* Inhibitors of tissue-nonspecific alkaline phosphatase: design, synthesis, kinetics, biomineralization and cellular tests. *Bioorg. Med. Chem.* **21**, 7981–7987 (2013).
181. Marques, S., Buchet, R., Popowycz, F., Lemaire, M. & Mebarek, S. Synthesis of benzofuran derivatives as selective inhibitors of tissue-nonspecific alkaline phosphatase: effects on cell toxicity and osteoblast-induced mineralization. *Bioorg. Med. Chem. Lett.* **26**, 1457–1459 (2016).
182. Picaud, S. *et al.* RVX-208, an inhibitor of bet transcriptional regulators with selectivity for the second bromodomain. *Proc. Natl Acad. Sci. USA* **110**, 19754–19759 (2013).
183. Gilham, D. *et al.* RVX-208, a bet-inhibitor for treating atherosclerotic cardiovascular disease, raises Apoa-I/HDL and represses pathways that contribute to cardiovascular disease. *Atherosclerosis* **247**, 48–57 (2016).
184. Kalantar-Zadeh, K. *et al.* Alkaline phosphatase lowering by selective bet inhibition, a novel mechanism for mace reduction in high risk cvd, diabetes and CKD patients — a post-hoc analysis of phase 2b studies with RVX-208. *J. Am. Soc. Nephrol.* **26**, 227A (2015).
185. Wong, N. C. W. *et al.* Apabetalone (RVX-208), a selective bromodomain and extra-terminal (bet) protein inhibitor, decreases abundance and activity of complement proteins *in vitro*, in mice and in clinical studies. *Nephrol. Dial. Transplant.* **31**, i109 (2016).
186. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT02586155 (2016)
187. Whyte, M. P. *et al.* Enzyme-replacement therapy in life-threatening hypophosphatasia. *N. Engl. J. Med.* **366**, 904–913 (2012).
188. Whyte, M. P. *et al.* Asfotase alfa treatment improves survival for perinatal and infantile hypophosphatasia. *J. Clin. Endocrinol. Metab.* **101**, 334–342 (2016).
189. Heemskerk, S. *et al.* Alkaline phosphatase treatment improves renal function in severe sepsis or septic shock patients. *Crit. Care Med.* **37**, 417–423 (2009).
190. Kats, S. *et al.* Anti-inflammatory effects of alkaline phosphatase in coronary artery bypass surgery with cardiopulmonary bypass. *Recent Pat. Inflamm. Allergy Drug Discov.* **3**, 214–220 (2009).
191. Peters, E. *et al.* Pharmacokinetic modeling and dose selection in a randomized, double-blind, placebo-controlled trial of a human recombinant alkaline phosphatase in healthy volunteers. *Clin. Pharmacokinet.* **55**, 1227–1237 (2016).
192. Ghosh, S. S., Gehr, T. W. & Ghosh, S. Curcumin and chronic kidney disease (CKD): major mode of action through stimulating endogenous intestinal alkaline phosphatase. *Molecules* **19**, 20139–20156 (2014).
193. Ghosh, S. S., Bie, J., Wang, J. & Ghosh, S. Oral supplementation with non-absorbable antibiotics or curcumin attenuates Western diet-induced atherosclerosis and glucose intolerance in LDLR$^{-/-}$ mice — role of intestinal permeability and macrophage activation. *PLoS ONE* **9**, e108577 (2014).

Author contributions

All authors researched data for the article. M.H. wrote the article. M.H., K.K.-Z. and P.M. provided the figures. M.H. and P.M. made substantial contributions to discussions of the content. All authors contributed to discussion of the content and reviewed and/or edited the manuscript before submission.

Competing interests statement

The authors declare no competing interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.