Pharmacologic epigenetic modulators of alkaline phosphatase in chronic kidney disease

Mathias Haarhaus*a,b,c, Dean Gilhamd, Ewelina Kulikowski,d Per Magnussonb, and Kamyar Kalantar-Zadehe,f,g

Purpose of review
In chronic kidney disease (CKD), disturbance of several metabolic regulatory mechanisms cause premature ageing, accelerated cardiovascular disease (CVD), and mortality. Single-target interventions have repeatedly failed to improve the prognosis for CKD patients. Epigenetic interventions have the potential to modulate several pathogenetic processes simultaneously. Alkaline phosphatase (ALP) is a robust predictor of CVD and all-cause mortality and implicated in pathogenic processes associated with CVD in CKD.

Recent findings
In experimental studies, epigenetic modulation of ALP by microRNAs or bromodomain and extraterminal (BET) protein inhibition has shown promising results for the treatment of CVD and other chronic metabolic diseases. The BET inhibitor apabetalone is currently being evaluated for cardiovascular risk reduction in a phase III clinical study in high-risk CVD patients, including patients with CKD [ClinicalTrials.gov Identifier: NCT02586155]. Phase II studies demonstrate an ALP-lowering potential of apabetalone, which was associated with improved cardiovascular and renal outcomes.

Summary
ALP is a predictor of CVD and mortality in CKD. Epigenetic modulation of ALP has the potential to affect several pathogenetic processes in CKD and thereby improve cardiovascular outcome.

Keywords
alkaline phosphatase, apabetalone, bromodomain and extraterminal inhibition, chronic kidney disease, epigenetic, microRNA, vascular calcification

INTRODUCTION
Chronic kidney disease (CKD) is a state of imbalance of several important physiologic regulatory mechanisms, among them mineral balance, acid–base balance, nutritional balance, and energy balance, resulting in accelerated cardiovascular disease (CVD) and mortality. In addition, CKD is also associated with chronic inflammation and resembles a model for premature ageing [1,2]. In CKD, numerous pathways are upregulated that are associated with immunity and inflammation, oxidative stress, endothelial dysfunction, vascular calcification, and coagulation [3]. Pharmacologic epigenetic modulation has the advantage of targeting several disease-related processes simultaneously. Due to its expression in multiple tissues and organs, which is upregulated in response to different pathogenic stimuli, alkaline phosphatase (ALP, EC 3.1.3.1) may be a suitable target for epigenetic modulation (Fig. 1).
ALKALINE PHOSPHATASE IN HEALTH AND DISEASE

ALP is a ubiquitously expressed enzyme that catalyzes the hydrolytic removal of phosphate groups from biochemical compounds [4]. Four different isozymes are known in humans. The tissue-nonspecific isozyme (TNALP) is expressed in different organs, for example, bone, liver, kidneys, brain, cardiovascular system, and leukocytes, whereas tissue-specific isozymes are expressed in the intestine (IALP), the placenta, and the testis (germ cell ALP) [5]. In most healthy individuals, circulating total ALP activity is comprised of approximately 50% of bone-specific isoforms of TNALP (BALP) and an equal percentage of liver-specific TNALP isoforms. However, in patients with blood groups B and 0, IALP can contribute up to 10% of the circulating ALP activity. In individuals with blood group A, IALP contributes less than 3% of total ALP activity, as

KEY POINTS

- Circulating ALP is a robust risk marker for CVD and all-cause mortality in the general population and in CKD.
- ALP is ubiquitously expressed and is involved in several pathophysiological processes associated with cardiovascular complications in CKD, for example vascular calcification, chronic inflammation, oxidative stress, and fibrosis.
- BET inhibitors and microRNAs are epigenetic modulators with the potential to simultaneously target several different pathogenic mechanisms upregulated in chronic diseases.
- The novel epigenetic modulator apabetalone targets pathogenetic processes associated with the induction of ALP and improves cardiovascular prognosis in high-risk patients, including patients with CKD, while lowering circulating ALP activity.

FIGURE 1. Alkaline phosphatase is ubiquitously expressed; however, the contribution of alkaline phosphatase from different tissues to the circulating alkaline phosphatase activity may vary. Under healthy conditions, liver and bone isoforms of tissue-nonspecific isozyme alkaline phosphatase comprise approximately 50% each of the total circulating alkaline phosphatase activity. Intestine alkaline phosphatase can comprise up to 10% of the circulating alkaline phosphatase activity in individuals with blood group B or 0, but less than 3% in individuals with blood group A. Circulating alkaline phosphatase predicts disease-related outcomes, for example cardiovascular disease or mortality, but to which extend alkaline phosphatase derived from specific tissues contributes to the total circulating alkaline phosphatase activity in pathologic conditions remains largely undetermined. Designed by Macrovector and Brgfx - Freepik.com.
blood group A red cells bind IALP in the circulation. ALP is an ectoenzyme attached to the outer layer of cell membranes. It is released into circulation as a soluble homodimer and cleared from the circulation via hepatic asialoglycoprotein receptors after desialylation by circulating neuraminidase [6–8].

TNALP is involved in the regulation of biomineralization, inflammation, oxidative stress and endothelial dysfunction, fibrosis, and cellular hypertrophy [9,10–12]. TNALP dephosphorylates compounds of the extracellular matrix quite unspecifically. Known biological functions of ALP include the inactivation of calcification inhibitors, the dephosphorylation of nucleotides in purinergic signaling, the activation of matrix metalloproteinases (MMPs), and the local regulation of vitamin B6 metabolism (Fig. 2). IALP contributes to the regulation of the gut microbiome, nutrient uptake, and the systemic immune response [5].

ALP is present in many species including humans, and is routinely applied as a marker for liver disease or bone turnover; however, until recently, its biologic relevance was poorly understood. Similar to the evolutionary science behind the emergence of the C-reactive protein (CRP) from an inflammatory modulator to now a novel CVD marker, over the past 2 decades, ALP, too, has been emerging with newly discovered roles in biological homeostasis [9]. Emerging evidence suggests that circulating ALP is a strong predictor of adverse cardiovascular outcome and all-cause mortality [9]. In spite of being a novel cardiovascular risk marker and potential therapeutic target for cardiovascular risk, no clinical stage therapeutics aimed at lowering serum ALP are available to date.

**Alkaline phosphatase and biomineralization**

Biomineralization is regulated by a complex interplay of calcification promoters and inhibitors. In CKD, disturbance of this interplay is common and can cause extensive soft-tissue calcification such as medial artery calcification or calcification of atherosclerotic plaques. ALP is essential for bone mineralization, as demonstrated by hypophosphatasia, a hereditary disease with loss-of-function mutations of the *ALPL* gene that encodes TNALP [13]. In addition, ALP plays a central role in pathological processes. LPS, lipopolysaccharides; MMP, metalloproteinase; OPN, osteopontin; Pi, phosphate; PL, pyridoxal; PLP, pyridoxal phosphate; Ppi, pyrophosphate.

**FIGURE 2.** Summary of mechanisms linking dephosphorylation by alkaline phosphatase to normal and pathophysiological processes. LPS, lipopolysaccharides; MMP, metalloproteinase; OPN, osteopontin; Pi, phosphate; PL, pyridoxal; PLP, pyridoxal phosphate; Ppi, pyrophosphate.
soft-tissue calcification [14,15]. ALP is actively enhanced in matrix vesicles derived from mineralization-competent cells. These vesicles function as nidi for matrix mineralization. The process is similar in physiologically mineralizing tissues, such as bone and dentin, and in pathological soft-tissue calcification. ALP promotes the propagation of matrix mineralization by dephosphorylation of mineralization inhibitors such as pyrophosphate and the phosphoprotein osteopontin, and by generation of inorganic phosphate, rendering a more procalcific extracellular milieu [16–18]. A role in the regulation of additional phosphoproteins in the extracellular matrix can be speculated. Matrix Gla protein (MGP) is one of the most important physiological mineralization inhibitors [19]. Its activity is determined by post-translational phosphorylation in addition to vitamin K-dependent carboxylation [20,21]. The effect of MGP inhibition by pharmacological vitamin K antagonists on the propagation of medial artery calcification and calcific uremic arteriolopathy in CKD is well known [22,23]. Lower circulating levels of the nonphosphorylated form of MGP are associated with vascular calcification and mortality in dialysis patients, independent of its carboxylation status [24]. However, the mechanisms of MGP dephosphorylation are yet unknown and a role for ALP in this process can only be hypothesized.

**Alkaline phosphatase and fibrosis**

A novel mechanism has been suggested for ALP in fibrosis and cardiovascular fibrocalcification, which is a feature of congestive heart failure [25]. The upregulation of ALP in cardiac myocytes leads to increased fibrosis via dephosphorylation of metalloproteinases 2 and 9 [26]. Indeed, increased circulating ALP activities have been observed in CKD patients with myocardial hypertrophy and congestive heart failure [27–29]. Further, ALP in bronchoalveolar lavage has been identified as a marker of pulmonary fibrosis, connecting ALP to fibrotic processes in the lung [30].

**Alkaline phosphatase and inflammation**

Several mechanisms link ALP to inflammation. Circulating ALP correlates well with circulating CRP, and ALP has been suggested as a component of the hepatic acute phase reaction [31]. Also, circulating IALP is enhanced in inflammatory conditions [32]. However, CRP and inflammatory cytokines have an inhibitory effect on ALP activity in osteoblasts [33,34] as circulating CRP was only associated with total ALP, not BALP, in a large cohort of dialysis patients [35], suggesting an extra-skeletal source for the increased circulating ALP activity during inflammation. In contrast to the effect of inflammation on ALP in bone, inflammatory mediators can increase ALP activity in vascular smooth muscle cells (VSMCs) and mesenchymal stem cells [36,37], which is concordant with the clinical finding of opposing effects of inflammation on bone versus vascular mineralization in CKD [38]. ALP modulates the cellular inflammatory response via purinergic signaling by contributing to the enzymatic conversion of proinflammatory extracellular adenosine triphosphate to anti-inflammatory adenosine [39]. ALP is also expressed by inflammatory cells in the vascular wall, and may mediate a link between inflammation and vascular calcification, commonly seen in the atherosclerotic plaque and in diseases of the metabolic syndrome, such as type 2 diabetes mellitus and CKD [40–43].

Sepsis-induced inflammation can cause acute kidney injury and loss of renal function that leads to morbidity and mortality [44]. Serum ALP predicts infection-related mortality [45] and has been proposed as a component of a clinical prediction model for bacteremia in CKD stage 5D patients [46]. Circulating ALP has the potential to inactivate endotoxins and other highly phosphorylated proinflammatory compounds [31,32]. Intestinal ALP detoxifies lipopolysaccharide (LPS) to reduce its inflammatory properties and interaction with Toll-like receptors and prevents inflammation in zebrafish in response to the gut microbiota [47]. Indeed Resolvin E1-induced intestinal ALP promotes resolution of inflammation through LPS detoxification [48]. This concept is being challenged in clinical trials. For example, in patients with acute kidney injury and sepsis, injection of recombinant ALP promoted a decrease in all-cause mortality, supporting a physiological role for ALP in mitigating the deleterious and morbid actions arising from sepsis [49]. Hence, similar to CRP, there is a biologically plausible role for increased levels of ALP under such pathologic circumstance, which may elicit maladaptive consequences. IALP may also exert a protective effect against inflammation-induced complications of diabetes mellitus type 1, such as CVD or diabetic nephropathy [50].

**Alkaline phosphatase and oxidative stress**

Increased oxidative stress is associated with adverse cardiovascular outcomes [51]. Oxidative stress induces ALP and calcification in calcifying vascular cells [52]. Increased oxidative stress is also associated with osteoporosis [53] because mineralization is inhibited in osteoblasts [52]. The reduction of cardiovascular oxidative stress in CKD patients by
exercise treatment is associated with a reduction of circulating ALP [54]. However, the origin of the increased serum ALP activity in patients with oxidative stress has yet to be determined.

**Alkaline phosphatase and hypertension**

ALP contributes to regulation of hypertension and vascular tone. Inhibition of ALP in isolated perfused kidneys and in experimental animals in vivo decreased the hypertensive blood pressure (BP) response to norepinephrine [55]. The effect is partially explained by the role of ALP in purinergic signaling and increased adenosine production. Circulating ALP activity is inversely correlated to maximal vasodilatory response to acetylcholine, indicative of endothelial dysfunction [56]. An additional mechanism linking ALP to BP control is the association with arterial stiffness [57], possibly explained by vascular calcification [58]. A contribution of ALP to increased fibrotic transformation of capacity arteries can also be speculated [59].

**Alkaline phosphatase and cognitive impairment**

Circulating ALP is associated with impaired cognition [60–62]. Cognitive impairment is a serious complication in ageing and CKD. Underlying abnormalities include neurodegenerative processes and impaired microcirculation. In Alzheimer's disease, ALP in the brain and circulation is inversely correlated with cognitive function, and dephosphorylation of tau has been suggested as a putative pathomechanism [63]. Increased circulating ALP is also associated with cerebral small vessel disease, a hallmark of vascular cognitive impairment [64]. ALP contributes to the regulation of gammaaminobutyrate and other neurotransmitters [65]. The association of reduced circulating ALP after parathyroidectomy in CKD patients with improved cognition suggests a possible therapeutic implication for ALP lowering in cognitive impairment [66].

**Alkaline phosphatase in chronic kidney disease**

In CKD, circulating ALP is commonly used in conjunction with parathyroid hormone for the approximation of bone turnover due to its association with bone formation [10,67]. In the absence of liver disease, variations in total ALP typically arise from BALP, and can identify extremes of high and low bone turnover [68]. Furthermore, circulating ALP is a better predictor of incident fractures in dialysis patients than bone mineral density [69]. Circulating ALP is also a strong and independent predictor of mortality and cardiovascular complications in CKD [9]. In non-CKD populations, the association between ALP and inflammation is predictive of mortality [35]. In contrast, circulating BALP levels in patients with advanced CKD are an even stronger predictor of mortality than total ALP [70]. This could be due to its association with the extensive vascular calcification arising in patients with CKD on dialysis [71]. As all of the pathomechanisms discussed above are upregulated in CKD [3], the contribution of ALP to the increased CKD-related mortality, cardiovascular complications, and impaired cognition is presumably multifactorial.

**REGULATION OF ALPL GENE EXPRESSION**

Human TNALP is encoded by the ALPL gene (accession number, NM_000478), which is located on the short arm of chromosome 1, 1p36.12 [72–74]. The ALPL gene exceeds 50 kb and comprises 12 exons. The first exon is part of the 5'-untranslated region of the TNALP mRNA, which consists of either exon 1A or 1B that respond to different promoters and results in two mRNAs, each encoding an identical polypeptide, but with different 5'-untranslated regions [75]. The expression of TNALP is ubiquitous; however, transcription of the two variants of exon 1 results in cell-specific and tissue-specific expression. One of these transcripts is termed ‘bone ALPL transcript’ in active osteoblasts comprising exon 1A, whereas exon 1B is driven by a separate promoter active in liver and kidney tissues [75,76].

The regulation of ALPL expression is best studied in osteoblast-like cells. Bone formation by cells from the osteoblast lineage and functional actions, for example, biomineralization, involve multiple developmental signals such as hormones, growth factors, cytokines, Wingless-related integration site (WNT) ligands, and bone morphogenetic proteins. In addition, there are also several transcription factors that regulate the expression of a variety of osteoblast-specific genes expressing proteins pivotal for biomineralization, for example, collagen type I, bone-specific alkaline phosphatase, and osteocalcin [77]. The bone essential transcription factor runt-related transcription factor 2 (Runx2) has been identified as the master regulator for osteoblast differentiation [78]. Osterix (Osx; Sp7 gene), a zinc finger-containing transcription factor with a Runx2-binding sequence, is also essential for osteoblast differentiation and bone mineralization. Osx is not expressed in Runx2-deficient mice, whereas the expression of Runx2 is not affected in Osx-deficient mice [79], which implies that Osx regulates osteoblast differentiation downstream of Runx2 [80].

---

**Human TNALP is encoded by the ALPL gene (accession number, NM_000478), which is located on the short arm of chromosome 1, 1p36.12 [72–74].**

**The ALPL gene exceeds 50 kb and comprises 12 exons. The first exon is part of the 5′-untranslated region of the TNALP mRNA, which consists of either exon 1A or 1B that respond to different promoters and results in two mRNAs, each encoding an identical polypeptide, but with different 5′-untranslated regions [75].**

**The expression of TNALP is ubiquitous; however, transcription of the two variants of exon 1 results in cell-specific and tissue-specific expression. One of these transcripts is termed ‘bone ALPL transcript’ in active osteoblasts comprising exon 1A, whereas exon 1B is driven by a separate promoter active in liver and kidney tissues [75,76].**

**The regulation of ALPL expression is best studied in osteoblast-like cells. Bone formation by cells from the osteoblast lineage and functional actions, for example, biomineralization, involve multiple developmental signals such as hormones, growth factors, cytokines, Wingless-related integration site (WNT) ligands, and bone morphogenetic proteins. In addition, there are also several transcription factors that regulate the expression of a variety of osteoblast-specific genes expressing proteins pivotal for biomineralization, for example, collagen type I, bone-specific alkaline phosphatase, and osteocalcin [77]. The bone essential transcription factor runt-related transcription factor 2 (Runx2) has been identified as the master regulator for osteoblast differentiation [78].**

**Osterix (Osx; Sp7 gene), a zinc finger-containing transcription factor with a Runx2-binding sequence, is also essential for osteoblast differentiation and bone mineralization. Osx is not expressed in Runx2-deficient mice, whereas the expression of Runx2 is not affected in Osx-deficient mice [79], which implies that Osx regulates osteoblast differentiation downstream of Runx2 [80].**
Other key transcription factors involved in osteoblast differentiation are the homeobox gene Msx2 and members of the distal-less homeobox (Dlx) family. Msx2 represses the expression of ALPL by directly binding to its promoter, whereas Dlx5 activates ALPL expression by interfering with the action of Msx2 [81]. Dlx3 is another potent regulator of Runx2 activation during osteogenic differentiation [82]. It has also been demonstrated that overexpression of Dlx2 has no effect on RUNX2, DLXS, and MSX2 expression upon osteogenic induction, but stimulated ALPL and osteocalcin expression [83]. Thus, Dlx2 may directly upregulate ALPL to promote osteoblastogenesis.

**EPIGENETIC REGULATION OF ALKALINE PHOSPHATASE**

The term *Epigenotype* was coined in 1942 by Waddington who concluded that ‘between genotype and phenotype lies a whole complex of development processes’ [84]. The modern definition of epigenetics includes modifications of DNA and associated proteins, not involving changes to the underlying DNA sequence, that are influenced by the environment and maintained during cell division that cause stable changes in gene expression [85*]. The main epigenetic factors are DNA methylation, posttranslational changes of histones, and higher order chromatin structure. Post translational modifications of histones impact chromatin structure, accessibility, and recruitment of transcription machinery to dictate whether genes are switched on or off. These dynamic modifiCations orchestrate cellular responses to environmental, developmental, or metabolic stimuli through modification of the transcriptome. However, epigenetics can underlie dysregulated gene expression in disease states including cancer [86] and pathological inflammatory processes [87]. Enzymes or proteins that generate or interact with epigenetic alterations can be classified as writers, erasers, or readers, depending on whether they add, remove, or recognize a posttranslational modification (Fig. 3).

**Histone acetylation**

Histone acetylation is associated with open chromatin structure, accessibility for transcription factor binding, and active transcription [88*]. Histone acetylation impacts TNALP expression. Histone deacetylase inhibitors (HDACi) increase chromatin acetylation. *In vitro*, HDACi increase expression of ALPL and promoted osteogenic differentiation of human mesenchymal stem cells [89]. Mechanistically, histone acetylation has been associated with the regulation of bone morphogenic proteins, WNT signaling, and RUNX2 induction [90]. Whether acetylation directly impacts promoters of ALPL expression is an area of ongoing research.

**DNA methylation**

Studies have demonstrated that the ALPL promoter A1E is highly methylated [91]. Delgado-Calle et al. [92], demonstrated that DNA methylation has an important role in the modulation of ALPL expression in human osteoblast-like cells. They showed an inverse relationship between the methylation status of a CpG island extending from −579 to +836 bp of the ALPL gene including the promoter region, which implies that epigenetic regulation by DNA demethylation strongly enhances TNALP expression and activity [92]. In VSMC, both phosphate and hydroxyapatite nanocrystals modulate DNA methylation, which results in an increased ALP activity and the induction of an osteoblast-like phenotype [93,94].

**MicroRNAs**

Long noncoding RNAs and microRNAs (miRNAs) are also key epigenetic factors that are involved in posttranscriptional gene regulation [77,95*]. The miRNAs are small noncoding single-stranded RNA molecules, approximately 18–25 nucleotides, that inhibit protein synthesis by binding to the 3’-untranslated region of mRNA to block protein translation and/or modulate mRNA stability. It has been estimated, through computational predictions, that more than 50% of all human protein-coding genes are potentially regulated by miRNAs [96]. Bone-regulating miRNAs play a key role in osteogenic differentiation and signaling pathways involved in osteogenesis [77,95*,97,98]. The key transcription factors Runx2 and Osx are downregulated by numerous miRNAs in pluripotent mesenchymal cells to suppress the bone phenotype in nonosseous cells and tissues [77,99].

Some miRNAs have been found to suppress and promote distinct signaling pathways related with osteogenic differentiation [95*,100*]. Reduced mRNA expression for collagen I, TNALP, and osteocalcin has been found while overexpressing miR-375, thus suggesting that miR-375 is able to suppress osteogenic differentiation by targeting Runx2 [101]. Overexpression of miR-133a-5p has also been reported to inhibit ALPL expression and mineralization through targeting Runx2 [102]. Li et al. [103], demonstrated that miR-216a promotes osteoblast differentiation and enhanced bone formation.
PHARMACOLOGIC EPINEGENIC INTERVENTIONS TARGETING ALKALINE PHOSPHATASE

MicroRNAs
Given the ubiquitous expression of ALP, its central role in biomineralization and the high incidence of vascular calcification in patients with CKD, it is reasonable to explore pharmacologic epigenetic modulation of ALP as a potential therapeutic measure aimed at the prevention of cardiovascular complications in CKD [9*]. Recent evidence indicates that miRNAs are deregulated in CKD – mineral and bone disorder [104]. Experimental studies support the concept that miRNAs are potential targets to ameliorate vascular calcification [100*]. According to the miRBase version 22, sequences of 2656 mature human miRNAs have been catalogued so far [105]. Hence, it is a challenging task to include most of the miRNAs that have been investigated over the years in this review. However, recent data demonstrate that phosphate-induced aortic calcification trigger miRNA modulation by upregulating miR-200c, miR-155 and miR-322, whereas miR-708 and miR-331 were downregulated [106*]. Other miRNAs that are involved in vascular calcification, thus potential treatment targets, are miR-29a/b, miR-30d/e, miR-125b, miR-135a, miR-143, miR-145, miR-204, miR223 and miR-762 [107]. Most of these miRNAs target the two main transcription factors Runx2 and Osx that influence TNALP activity and biomineralization. Undoubtedly, miRNAs have a key role in regulating the progression of vascular calcification; however, the high abundance of miRNAs requires extended large-scale epigenome-wide studies to fully exploit the potential of epigenetic regulation by miRNAs for novel therapeutic approaches to ameliorate vascular calcification.
Bromodomain and extraterminal inhibition

Bromodomain and extraterminal (BET) proteins BRD2, BRD3, BRD4, and BRDT are chromatin readers that not only bind acetylated lysine on histone tails and transcription factors via bromodomains 1 and 2, but also recruit transcriptional machinery to drive expression of bromodomain and extraterminal sensitive genes. Apabetalone is an orally available small molecule inhibitor of bromodomain and extraterminal bromodomains that causes bromodomain and extraterminal protein release from chromatin and, as a consequence, downregulation of bromodomain and extraterminal sensitive gene transcription. Apabetalone preferentially targets bromodomain 2 (represented by yellow halo), a characteristic that differentiates it from pan-bromodomain and extraterminal inhibitors that bind bromodomains 1 and 2 with equal affinity [109]. In clinical trials, apabetalone treatment reduced major adverse cardiac events (MACE) in patients with CVD, and was associated with 44% relative risk reduction on top of standard of care [110–112]. The reduction of MACE by apabetalone was associated with a reduction of serum ALP, independent of traditional cardiovascular risk factors and inflammation [111–112]. Studies showed this drug concurrently modulated factors that promote atherosclerotic plaque stabilization and MACE reduction. HDL cholesterol increased [110,112], while the complement cascade, acute phase reaction, and mediators of vascular inflammation were suppressed [113,114].
Novel therapeutic approaches in nephrology and hypertension

In CKD patients with a history of CVD, apabetalone treatment improved kidney function and reduced circulating levels of ALP [115]. Mechanistically, apabetalone downregulated ALPL expression in primary human hepatocytes and VSMC [116], and as a consequence, reduced TNALP protein levels and enzymatic activity. Small molecule inhibitors of TNALP have been evaluated as a therapeutic for vascular calcification [14], however, apabetalone may be the first clinical stage molecule to modify TNALP production. In vitro, apabetalone opposed calcification of VSMCs cultured in osteogenic conditions through an epigenetic mechanism involving BRD4 that suppressed induction of procalcific genes, including RUNX2 and ALPL [116].

A single dose of apabetalone in CKD stage 4–5 patients rapidly resulted in reduction of numerous inflammatory cytokines, including IL-6 [2]. In the same study, proteomic profiling of more than 1300 plasma proteins predicted several immune and inflammatory pathways were activated in patients with impaired kidney function, including nuclear factor κB (NF-κB), IL-6, or bone morphogenetic protein signaling. These canonical pathways were downregulated with one dose of apabetalone, which would favourably impact progression of renal impairment and associated vascular calcification.

Bromodomain and extraterminal inhibition in metabolic bone disorders: implication for renal osteodystrophy

Distinct preclinical models of metabolic bone diseases have demonstrated that BETi do not diminish bone structure or mechanical properties, and may instead increase bone volume and restore mechanical strength [117–120]. These studies show that beneficial effects of BETi on bone disorders stems from anti-inflammatory effects, as well as epigenetic modulation of key factors in bone remodelling, including TNALP. N-methylpyrrolidone (NMP) is a U.S. Food and Drug Administration-approved drug excipient identified as a bioactive BETi [121]. Studies with NMP in preclinical models of bone degeneration have positioned BETi as a pharmacologic strategy for the prevention or treatment of bone diseases characterized by excessive bone resorption. Numerous studies have demonstrated BETi suppresses inflammatory responses mediated by TNFα and NF-κB [3,122–124]. NMP promoted growth of mineralized bone that was blocked by TNFα and recovered TNFα-inhibited expression of essential osteoblastic genes, including ALPL, RUNX2 and SP7/Osterix [125]. In addition, NMP promoted bone regeneration by enhancing BMP2 signaling in osteoblasts [126] and inhibited osteoclast differentiation to attenuate bone resorption induced by receptor activator of NF-κB ligand [127]. NMP was shown to increase osteoblast viability during hypoxia, and countered hypoxia-mediated downregulation of key genes involved in mineralization, including ALPL [128]. Mechanistically, the NMP treatment was protective in maintaining osteoblast differentiation during hypoxia in part by inhibiting NF-κB signaling, NMP preserved bone mineral density and quality of bones in ovariectomized rats [121], essentially ameliorating estrogen depletion-induced osteoporosis. Results were verified in similar studies using N,N-dimethylacetamide [127], or the more potent BETi JQ1, where treatment actually reversed bone loss induced by estrogen deficiency [117]. These data imply that BETi therapy can increase bone mass and improve bone turnover in inflammatory bone disorders and potentially in CKD.

CONCLUSION

Circulating ALP is a robust and independent risk marker for CVD and mortality in the general population and in CKD. The ubiquitous expression of ALP and its involvement in several pathophysiologic processes associated with CVD, bone disease, CKD progression, and cognitive dysfunction renders it suitable for multifactorial epigenetic interventions. Positive results from clinical studies with the novel BETi apabetalone implicate a role for ALP as a possible novel cardiovascular treatment target. Experimental studies with additional BETis and miRNAs suggest a wider therapeutic potential for epigenetic modulation of ALP. Further research is required to definitively establish ALP as a clinical treatment target levels and to elucidate the effect of lowering of serum ALP towards specific targets levels on clinical outcomes.

Acknowledgements

P.M. is supported by ALF grants Region Östergötland, Sweden. K.K.-Z. is supported by the NIDDK grants R01-DK095668 and K24-DK091419 as well as philanthropic grants from Mr Harold Simmons, Mr Louis Chang, Dr Joseph Lee and AVEO.

Financial support and sponsorship

None.

Conflicts of interest

M.H. is a member of the renal clinical advisory board of Resverlogix Inc. and an employee of Diaverum Sweden, AB. He has received consultancy and speaker honoraria from Resverlogix and Amgen. D.G. and E.K. are employees of Resverlogix. K.K.-Z. is a member of the renal clinical advisory board of Resverlogix. P.M. has no conflict of interest related to this article.
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Stenvinkel P, Larsson TE. Chronic kidney disease: a clinical model of premature aging. Am J Kidney Dis 2013; 62:399–351.

2. Kooman JP, Kotanko P, Schols AM, et al. Chronic kidney disease and premature ageing. Nat Rev Nephrol 2014; 10:732–742.

3. Wakisaka S, Tsujikawa LM, Halliday C, et al. Benefit of apatobalmin on plasma proteins in renal disease. Kidney Int Rep 2018; 3:711–721.

4. Millan J. Mammalian alkaline phosphatase: from biology to applications in medicine and biotechnology. Weinheim: Wiley; 2006.

5. Buchel R, Millan JL, Magne D. Multisystemic functions of alkaline phosphatases. Methods Mol Biol 2013; 1053:27–51.

6. Anh DJ, Eden A, Farley JR. Quantitation of soluble and skeletal alkaline phosphatase, and insoluble alkaline phosphatase anchor-hydrolase activities in human serum. Clin Chim Acta 2001; 311:137–148.

7. Anh DJ, Dmai HP, Hall SL, Farley JR. Skeletal alkaline phosphatase activity is primarily released from human osteoblasts in an insoluble form, and the net release is inhibited by calcium and skeletal growth factors. Calcif Tissue Int 1998; 62:332–340.

8. Magnusson P, Sharp CA, Farley JR. Different distributions of human alkaline phosphatase tissue isoforms in serum and bone tissue extracts. Clin Chim Acta 2002; 325:59–70.

9. Haahr M, Brandenburg V, Kalantar-Zadeh K, et al. Alkaline phosphatase: a novel serum-targeted marker for cardiovascular disease in CKD. Nat Rev Nephrol 2017; 13:429–442.

A comprehensive discussion of the link between serum alkaline phosphatase (ALP), mortality and cardiovascular disease (CVD) in chronic kidney disease and the general population.

10. Sardwal S, Magnusson P, Goldsmith DJ, Lamb EJ. Bone alkaline phosphatase in CKD-mineral bone disorder. Am J Kidney Dis 2013; 62:810–822.

11. Schuetze KB, McKinsey TA. TNAP: a new player in cardiac fibrosis? Focus 2011; 26:48.

12. Whyte MP, Simmons JH, Moseley S, et al. Opposing TNF-alpha/IL-1beta- and BMP-2-activated MAPK signaling pathways converge on Runx2 to regulate BMP-2-induced osteoblastic differentiation. Cell Death Dis 2014; 5:e1187.

13. Lee HL, Woo KM, Ryoo HM, Baek JH. Tumor necrosis factor-alpha increases alkaline phosphatase expression in vascular smooth muscle cells via MSX2 induction. Biochem Biophys Res Commun 2010; 391:1087–1092.

14. Ding J, Ghali O, Lencel P, et al. TNF-alpha and IL-1beta inhibit RUNX2 and collagen expression but increase alkaline phosphatase activity and mineralization in human mesenchymal stem cells. Life Sci 2009; 84:499–504.

15. Vlaene L, Behets GJ, Heye S, et al. Inflammation and the bone-vascular axis in end-stage renal disease. J Intern Med 2016; 284:499–507.

16. Rader DA. Alkaline phosphatase, an unconventional immune protein. Front Immunol 2017; 8:897.

17. Tompkins G. Is inflammation the link between atherosclerosis and vascular calcification in chronic kidney disease? Blood Purif 2007; 25:179–182.

18. Shannughm LN, Petracca C, Castellani ML, et al. IL-1beta induces alkaline phosphatase in human phagocytes. Arch Med Res 2007; 38:39–44.

19. Shioi A, Katagi M, Okuno Y, et al. Induction of osteoblast-like alkaline phosphatase in human vascular smooth muscle cells: roles of tumor necrosis factor-alpha and oncostatin M derived from macrophages. Circ Res 2002; 91:9–16.

20. Collin J, Gossel M, Matsuo Y, et al. Osteogenic monocyes within the coronary circulation and their association with plaque vulnerability in patients with early atherosclerosis. Int J Cardiol 2015; 181:57–64.

21. Poston JT, Koyner JL. Sepsis associated acute kidney injury. BMJ 2019; 364:k4891.

22. Hwang SD, Kim SH, Kim YO, et al. Serum alkaline phosphatase levels predict infection-related mortality and hospitalization in peritoneal dialysis patients. PLoS One 2016; 11:e0157361.

23. Sasaki S, Hasegawa T, Kawarazaki H, et al. Development and validation of a clinical prediction rule for bacteremias among maintenance hemodialysis patients in outpatient settings. PLoS One 2017; 12:e0169975.

24. Bates JM, Akerlund J, Mitteg E, Guillemin K. Intestinal alkaline phosphatase deoxynucleotides: lipo-pyrophosphatase. Calcif Prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe 2007; 2:371–382.

25. Campbell EL, Marcusus CF, Kominsky DJ, et al. Resolvin E1-induced intestinal alkaline phosphatase promotes resolution of inflammation through LPS detoxification. Proc Natl Acad Sci U S A 2010; 107:14298–14303.

26. Pickkers P, Mehta RL, Murray PT, et al. Effect of human recombinant alkaline phosphatase on 7-day creatinine clearance in patients with sepsis-associated acute kidney injury: a randomized clinical trial. JAMA 2018; 320:1998–2009.

27. Lassenius MI, Fogarty CL, Blaut M, et al. Intestinal alkaline phosphatase at the crossroad of intestinal health and disease – a putative role in type 1 diabetes. J Intern Med 2017; 281:586–600.

28. Yang X, Li Y, Li Y, et al. Oxidative stress-mediated atherosclerosis: mechanisms and therapies. Front Physiol 2017; 8:600.

29. Nody M, Parham F, Sarafian T, Demer L. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. Free Radic Biol Med 2001; 31:509–519.

30. Cervellati C, Bonacorsi G, Cremonini E, et al. Oxidative stress and bone resorption interplay as a possible trigger for postmenopausal osteoporosis. Biomed Res Int 2014; 2014:596593.

31. Wilund KR, Tomayko EJ, Wu PT, et al. Intralabyrinthic exercise training reduces oxidative stress and epicardial fat: a pilot study. Nephrol Dial Transplant 2010; 25:2695–2701.

32. Jackson KE, Zhang Y, Cheng D. Alkaline phosphatase inhibitors attenuate renal function impairment in mice. Front Physiol 2017; 8:897.

33. Pantoni F, Perticone M, Maio R, et al. Serum alkaline phosphatase negatively affects endothelium-dependent vasodilation in naive hypertensive patients. Hypertension 2015; 66:874–880.
Novel therapeutic approaches in nephropathy and hypertension

57. Manghat P, Souleimanova I, Cheung J, et al. Association of bone turnover markers and arterial stiffness in predialysis chronic kidney disease (CKD). Bone 2011; 48:1127–1132.

58. Sigirits M, Taal M, Bangar P, McIntyre C. Progressive vascular calcification over 2 years is associated with arterial stiffening and increased mortality in patients with stages 4 and 5 chronic kidney disease. Clin J Am Soc Nephrol 2007; 2:1041–1048.

59. Jiang L, Zhang J, Monticone RE, et al. Calpain-1 regulation of matrix metalloproteinase 2 activity in vascular smooth muscle cells facilitates age-associated aortic wall calcification and fibrosis. Hypertension 2012; 59:1109–1120.

60. Brown WR, Thore CR. Review: cerebral microvascular pathology in aging and neurodegeneration. Neuropathol Appl Neurobiol 2011; 37:56–74.

61. Vasanthan R, Priyanka HP, Swamilanad T, et al. Interrelationship between Mini-Mental State Examination scores and biochemical parameters in patients with mild cognitive impairment and Alzheimer’s disease. Genatr Gerontol Int 2017; 17:1737–1745.

62. Kollet KA, Williams J, Vardy ER, et al. Plasma alkaline phosphatase is elevated in Alzheimer’s disease and inversely correlates with cognitive function. Int J Epidemiol Genet 2011; 2:114–121.

63. Kollet KA, Hooper NM. The role of tissue nonspeciﬁc alkaline phosphatase (TNAP) in neurodegenerative diseases: Alzheimer’s disease in the focus. Subcell Biochem 2015; 76:363–374.

64. Ryu WS, Lee SH, Kim CK, et al. High serum alkaline phosphatase in relation to cerebral small vessel disease. Atherosclerosis 2014; 232:313–318.

65. Coburn SP. Vitamin B6 metabolism and interactions with TNAP. Subcell Biochem 2015; 76:207–233.

66. Chou FF, Chen JB, Hsieh KC, Liou CW. Cognitive changes after parathyroidectomy in patients with secondary hyperparathyroidism. Surgery 2008; 143:590–592.

67. Haarhaus M, Fernstrom 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 2009; 24:3382–3389.

68. Haarhaus M, Monier-Faguer MC, Magnusson P, Malluche HH. Bone alkaline phosphatase isoforms in hemodialysis patients with low versus nonlow bone turnover: a diagnostic test study. Am J Kidney Dis 2015; 66:99–105.

69. Imoni S, Mori Y, Akita W, et al. Diagnostic usefulness of bone mineral density and biochemical markers of bone turnover in predicting fracture in CKD stage 5D patients: a single-center cohort study. Nephrol Dial Transplant 2012; 27:345–351.

70. Drexhler C, Verduijn M, Plz S, et al. Bone alkaline phosphatase and mortality in dialysis patients. Clin J Am Soc Nephrol 2011; 6:1752–1759.

71. Yan J, Li L, Zhang M, et al. Circulating bone-speciﬁc alkaline phosphatase and abdominal aortic calcification in maintenance hemodialysis patients. Biomark Med 2018; 12:1231–1239.

72. Swallow DM, Posey S, Parker M, et al. Mapping of the gene coding for the human liver/bone/kidney isozyme of alkaline phosphatase to chromosome 1. Ann Hum Genet 1986; 50:229–235.

73. Smith M, Weiss MJ, Griffin CA, et al. Regional assignment of the gene for the human liver/bone/kidney alkaline phosphatase to chromosome 1p36.1-p34. Genomics 1986; 2:139–143.

74. Weiss MJ, Ray K, Henstrom PS, et al. Structure of the human liver/bone/kidney alkaline phosphatase gene. J Biol Chem 1988; 263:12002–12010.

75. Matsuura S, Kishi F, Kajii T. Characterization of a 5′-flanking region of the human liver/bone/kidney alkaline phosphatase gene: two kbp of mRNA from a single gene. Biochem Biophys Res Commun 1990; 168:993–1000.

76. Studer M, Terso M, Gianni M, Garattini E. Characterization of a second promoter in the mouse liver/bone/kidney-type alkaline phosphatase gene; cell and tissue speciﬁc expression. Biochem Biophys Res Commun 1991; 179:1352–1360.

77. Lian JB, Stein GS, van Wijnen AJ, et al. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol 2012; 8:212–227.

78. Otto F, Thomell AP, Crompton T, et al. Cbfα1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 1999; 99:785–791.

79. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc ﬁnger-containing transcription factor osteos is required for osteoblast differentiation and bone formation. Cell 2002; 108:17–29.

80. Komori T. Regulation of proliferation, differentiation and functions of osteoblasts by Runx2. Int J Mol Med 2019; 20:109–103.

81. Shirdashekar S, Terasawai K, Miyama K, et al. Regulation of the activity of the transcription factor Runx2 by two homeobox proteins, Msx2 and Dlx5. Genes Cells 2001; 6:851–856.

82. Hassan MQ, Tare RS, Lee SH, et al. BMP2 commitment to the osteogenic lineage involves activation of Runx2 by DLX5 and a homeodomain transcriptional network. J Biol Chem 2006; 281:40515–40526.

83. Zhang J, Zhang W, Dai J, et al. Overexpression of Dlx9 enhances osteogenic differentiation of BMSCs and MC3T3-E1 cells via direct upregulation of Osteocalcin and Alp. Int J Oral Sci 2019; 11:12.

84. Waddington CH. The epigenotype. 1942. Int J Epidemiol 2012; 41:10–13.

85. Feinberg AP. The key role of epigenetics in human disease prevention and mitigation. N Engl J Med 2018; 378:1333–1334.

Important discussion of the therapeutic potential of epigenetic modulation.
111. Haarhaus M, Ray KK, Nicholls SJ, et al. Apabetalone lowers serum alkaline phosphatase and improves cardiovascular risk in patients with cardiovascular disease. Atherosclerosis 2019; 290:59–65.

This is the first clinical report of an association of pharmacologic lowering of serum ALP activity with improved cardiovascular outcome.

112. Gilham D, Wasiak S, Tsujikawa LM, 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 2016; 247:48–57.

113. Tsujikawa LM, Fu L, Das S, et al. Apabetalone (RVX-208) reduces vascular inflammation in vitro and in CVD patients by a BET-dependent epigenetic mechanism. Clin Epigenetics 2019; 11:102.

114. Wasiak S, Gilham D, Daze E, et al. Downregulation of the complement cascade in vitro, in mice and in patients with cardiovascular disease by the BET protein inhibitor apabetalone (RVX-208). J Cardiovasc Transl Res 2017; 10:337–347.

115. Kulikowski E, Halliday C, Johansson J, et al. Apabetalone mediated epigenetic modulation is associated with favorable kidney function and alkaline phosphatase profile in patients with chronic kidney disease. Kidney Blood Press Res 2018; 43:449–457.

116. Gilham D, Tsujikawa LM, Sarsons CD, et al. Apabetalone downregulates factors and pathways associated with vascular calcification. Atherosclerosis 2019; 280:75–84.

The article is the first demonstration that BET proteins are involved in epigenetic changes leading to calcification of vascular smooth muscle cells, a process ameliorated with BET inhibitors.

117. Baud’huin M, Lamoureux F, Jacques C, et al. Inhibition of BET proteins and epigenetic signaling as a potential treatment for osteoporosis. Bone 2017; 94:10–21.

118. Lamoureux F, Baud’huin M, Rodriguez Calleja L, et al. Selective inhibition of BET bromodomain epigenetic signalling interferes with the bone-associated tumour vicious cycle. Nat Commun 2014; 5:3511.

119. Meng S, Zhang L, Tang Y, et al. BET inhibitor JQ1 blocks inflammation and bone destruction. J Dent Res 2014; 93:657–662.

120. Park-Min KH, Lim E, Lee MJ, et al. Inhibition of osteoclastogenesis and inflammatory bone resorption by targeting BET proteins and epigenetic regulation. Nat Commun 2014; 5:5418.

121. Gjoksi B, Ghayor C, Siegenthaler B, et al. The epigenetically active small chemical N-methyl pyrrolidone (NMP) prevents estrogen depletion induced osteoporosis. Bone 2015; 78:114–121.

122. Brown JD, Lin CY, Duan Q, et al. NF-kappaB directs dynamic super enhancer formation in inflammation and atherosclerosis. Mol Cell 2014; 56:219–231.

123. Jahagirdar R, Atwell S, Marusic S, et al. RVX-297, a BET bromodomain inhibitor, has therapeutic effects in preclinical models of acute inflammation and autoimmune disease. Mol Pharmacol 2017; 92:694–706.

124. Nicodeme E, Jeffrey KL, Schaefer U, et al. Suppression of inflammation by a synthetic histone mimic. Nature 2010; 468:1119–1123.

125. Chen TH, Weber FE, Malina-Altzinger J, Ghayor C. Epigenetic drugs as new therapy for tumor necrosis factor-alpha-compromised bone healing. Bone 2019; 127:49–68.

126. Miguel BS, Ghayor C, Ehrbar M, et al. N-Methyl pyrrolidone as a potent bone morphogenetic protein enhancer for bone tissue regeneration. Tissue Eng Part A 2009; 15:2955–2963.

127. Ghayor C, Gjoksi B, Dong J, et al. N,N-Dimethylacetamide a drug excipient that acts as bromodomain ligand for osteoporosis treatment. Sci Rep 2017; 7:42108.

128. Li Q, Liu R, Zhao J, Lu Q. N-methyl pyrrolidone (NMP) ameliorates the hypoxia-reduced osteoblast differentiation via inhibiting the NF-kappaB signaling. J Toxicol Sci 2016; 41:701–708.