Atherosclerosis 290 (2019) 59–65

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Apabetalone lowers serum alkaline phosphatase and improves cardiovascular risk in patients with cardiovascular disease

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HIGHLIGHTS

- Alkaline phosphatase (ALP) predicts residual cardiovascular risk in patients with cardiovascular disease on statin treatment.
- Apabetalone treatment reduces circulating ALP in a dose-dependent fashion.
- The reduction of circulating ALP by apabetalone is associated with a reduction in major adverse cardiovascular events (MACE).
- The association of ALP reduction with MACE is independent of concurrent levels of high-sensitivity C-reactive protein.

ARTICLE INFO

Keywords:
Epigenetic
Alkaline phosphatase
Major adverse cardiovascular event
Residual cardiovascular risk
apabetalone

ABSTRACT

Background and aims: In patients with cardiovascular disease, considerable residual risk remains despite evidence-based secondary prevention measures. Alkaline phosphatase (ALP) has been suggested as a modifiable cardiovascular risk factor. We sought to determine whether cardiovascular risk reduction by the bromodomain and extra-terminal (BET) protein inhibitor apabetalone is associated with the concomitant lowering of serum ALP.

Methods: In a *post-hoc* analysis of 795 patients with established coronary heart disease and statin treatment, who participated in phase 2 placebo-controlled trials of apabetalone, we determined the effect of assigned treatment for up to 24 weeks on the incidence of major adverse cardiovascular events (MACE) and serum ALP.

Results: Baseline ALP (median 72 U/L) predicted MACE (death, non-fatal myocardial infarction, coronary revascularization, or hospitalization for cardiovascular causes), independent of high-sensitivity C-reactive protein (hsCRP), sex, age, race, study, cardiovascular risk factors, chronic kidney disease (CKD), liver function markers and treatment allocation (hazard ratio [HR] per standard deviation [SD] 1.6, 95% CI 1.19–2.16, $p = 0.002$). Mean placebo-corrected decreases in ALP from baseline were 9.2% ($p < 0.001$) after 12–14 weeks and 7.7%

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https://doi.org/10.1016/j.atherosclerosis.2019.09.002
Received 6 April 2019; Received in revised form 19 August 2019; Accepted 12 September 2019
Available online 17 September 2019

($p < 0.001$) after 24–26 weeks of apabetalone treatment. In the apabetalone group, a 1-SD reduction in ALP was associated with a HR for MACE of 0.64 (95% CI 0.46–0.90, $p = 0.009$).
Conclusions: Serum ALP predicts residual cardiovascular risk, independent of hsCRP, established cardiovascular risk factors and CKD, in patients with cardiovascular disease on statin treatment. Apabetalone lowers serum ALP, which was associated with a lower risk of cardiovascular events. Whether the beneficial cardiovascular effects of apabetalone are causally related to ALP reduction remains undetermined.

1. Introduction

Despite evidence-based secondary prevention strategies including statins, patients with cardiovascular disease, type 2 diabetes, and/or chronic kidney disease remain at considerable risk for ischemic cardiovascular events. In the search for additional modifiable targets, alkaline phosphatase (ALP) has emerged as a cardiovascular risk marker, and may also be a pathogenic cardiovascular risk factor [1]. Experimental and clinical studies link ALP to increased vascular calcification and inflammation. Epidemiological studies have demonstrated correlations between serum ALP activity and the incidence of death [2,3] or major adverse cardiovascular events (MACE) [4,5], myocardial infarction [6], stroke [7], cerebral small vessel disease [8], re-stenosis after coronary revascularization [9], coronary slow flow phenomenon [10], and peripheral arterial disease [11]. Circulating ALP is associated with coronary calcification [12,13] and ALP activity is implicated in calcification of vascular smooth muscle cells [14]. Furthermore, ALP is expressed by inflammatory cells [15,16], and the association of circulating ALP with cardiovascular events is modulated, but not eliminated by adjustment for other markers of inflammation and oxidative stress [17–19].

Bromodomain and extra-terminal (BET) proteins are a family of bromodomain (BD) containing proteins that bind acetylated lysines on the histones that compact DNA to chromatin, thereby regulating gene transcription [20]. Through this epigenetic mechanism, BET proteins have been shown to regulate the expression of proteins implicated in the development of many disease states [21–23]. Apabetalone (RVX-208) is an oral small molecule that targets the second bromodomain of BET proteins BRD2, BRD3, and BRD4, inhibiting the transcriptional interactions of BET proteins with acetylated lysines on histones and transcription factors. In experimental models and human studies, apabetalone has been shown to target multiple processes that may underlie cardiovascular disease, including vascular calcification [24], inflammation, atherogenesis, thrombosis and reverse cholesterol transport [25,26]. In phase 2 studies, treatment with apabetalone was associated with a reduction of MACE [27], favorable modulation of coronary atherosclerotic plaque [28], and improvement of kidney function [29], and the drug is currently under study in a phase 3 trial in patients with type 2 diabetes and a recent acute coronary syndrome [30]. No safety concerns have arisen in experimental, toxicological, or clinical studies except for a transient elevation of liver transaminases, which resolves without sequelae, either with continued dosing or following therapy discontinuation.

Here, we report the effect of apabetalone on ALP activity in a *post-hoc* aggregate analysis of phase 2 clinical trials, and the association of its effects on ALP and MACE.

2. Patients and methods

2.1. ASSERT, SUSTAIN and ASSURE study design

The rationale and design of the ASSERT (clinicaltrials.gov NCT01058018), SUSTAIN (NCT01423188), and ASSURE (NCT01067820) studies have been published previously [31–33]. The ASSERT study evaluated the safety and tolerability of three doses of apabetalone in 299 statin-treated patients with coronary artery disease. Patients were treated with placebo or apabetalone at a dose of 50 mg, 100 mg, or 150 mg twice daily for 12 weeks. In SUSTAIN, 176 statin-treated patients with low levels of HDL-C were randomized to receive placebo or apabetalone 100 mg twice daily for 24 weeks and evaluated for changes in lipid parameters including HDL-C. In ASSURE, 323 patients with angiographic coronary artery disease and low HDL-C levels were randomized to receive placebo or apabetalone 100 mg twice daily for 26 weeks and evaluated for progression of coronary atherosclerosis using serial intravascular ultrasound measurements. Adherence to study medication, determined by counting tablets of study medication returned by participants, did not differ between active and placebo groups or among groups receiving different doses. In each study, patients were evaluated for cardiovascular events until 30 days following end of treatment phase. MACE was a pre-specified endpoint for ASSURE but not for ASSERT or SUSTAIN. The effect of apabetalone on ALP was evaluated in all three studies. Three patients from the ASSURE trial were excluded from the current study due to missing information on baseline ALP. Written informed consent was obtained from each patient included in the original studies. The study protocols conform to the ethical guidelines of the 1975 Declaration of Helsinki and have been priorly approved by the respective Institution's ethics committee on research on humans.

2.2. Study visits and laboratory tests

In ASSERT, ALP activity was measured at baseline, every 2 weeks during the randomized treatment phase, and at follow-up 4 weeks following the termination of the study drug. In SUSTAIN and ASSURE, ALP was measured at baseline, at Week 12 or 14, and at Week 24 or 26 of study treatment. A central laboratory performed all biochemical determinations (Icon, Farmingdale, New York, for ASSERT or ACM, Rochester, NY, USA and York, UK, for SUSTAIN and ASSURE). Normal ranges for serum ALP were 53–129 U/L for males, 42–98 U/L for females 18–59 years, and 53–141 U/L for females ≥60 years. MACE (death, myocardial infarction, defined by 2 out of 3 criteria: 1. clinical signs/symptoms, 2. elevated circulating cardiac troponin or creatinine kinase isoenzyme MB, 3. EKG changes, coronary revascularization, and hospitalization for cardiovascular causes) were investigator-reported and were not centrally adjudicated [27].

2.3. Statistical tests

Demographic and laboratory characteristics are summarized for all randomized participants in the ASSERT, SUSTAIN, or ASSURE trials who had a baseline ALP measurement and took at least 1 dose of study drug. The categorical variables are summarized using frequencies (%), while continuous variables are reported as median (interquartile range). To improve comparability, continuous variables were standardized in regression analyses and expressed as standard deviation (1-SD) when appropriate. Throughout the study the χ-square test was employed for paired comparisons of categorical variables and the non-parametric Mann Whitney test was used for continuous variables, if not specified otherwise.

In ASSERT, analysis of ALP was performed in participants with a baseline and at least 1 post-baseline value through the 12-week treatment period. Last observation carried forward (LOCF) was used when the Week 12 value was not available. A nonparametric rank analysis of covariance (ANCOVA) was employed to compare the percentage

change from baseline at Weeks 4, 6, 8, 10 and 12 in each active treatment group with placebo, after controlling for the ranked baseline value. Pairwise *p*-values were adjusted for multiple comparisons with Dunnett's method. The placebo-corrected changes of ALP in the ASSURE and SUSTAIN trials were calculated using Bayes Factor Independent Sample Test.

After combining baseline data from the three trials, characteristics of patients who did or did not experience MACE were compared. For comparison between patients with high and low ALP, all participants were stratified according to the median baseline ALP. The prediction of the first occurrence of MACE by baseline ALP was calculated using log rank analysis in a Kaplan-Meier procedure, stratified by median ALP and adjusted for treatment allocation, and Cox proportional hazards models, adjusted for baseline hsCRP, age, sex, established cardiovascular risk factors (diabetes status, smoking status, hyperlipidemia, defined as elevated LDL-C or HDL-C < 45 mg/dL for women, < 40 mg/dL for men, hypertension, and history of cardiovascular disease as conveyed by the patient), chronic kidney disease (CKD, defined as estimated glomerular filtration rate < 60 mL/min/1.73 m^2, calculated from serum creatinine, using the Chronic Kidney Disease Epidemiology Collaboration formula), and study.

For the determination of the association between the change from baseline in ALP and the incidence of MACE, pooled data from patients participating in the ASSERT, SUSTAIN, or ASSURE trials with ALP at baseline and follow-up at 12–14 weeks from randomization were analyzed. In addition, data from patients with a baseline and follow-up ALP at 24–26 weeks from randomization in the SUSTAIN or ASSURE trial were analyzed. As the ALP-lowering effect of apabetalone occurred as soon as 4 weeks after treatment start and was sustained throughout the trials, all events after randomization were included in the primary analyses, with the exception that patients with MACE within 2 weeks before blood sampling were excluded to avoid a potential acute effect of MACE on ALP, which has a serum half-life of 1 week. Thus, 794 and 492 patients were included in the Cox proportional hazard models at 12–14 and 24–26 weeks, respectively. Sensitivity analyses were performed including all patients. Additional sensitivity analyses were performed after excluding patients with events before 4 and 8 weeks, however, after 12 weeks, the remaining incidence of events was insufficient for further statistical analyses (data not shown). The associations of changes in ALP with time to first MACE were assessed in each treatment group with Cox proportional hazards models adjusted for baseline ALP, baseline hsCRP, baseline liver function markers, change of hsCRP, sex, age, race, established cardiovascular risk factors, CKD, and study. For further determination of the association of ALP lowering by apabetalone with the incidence of MACE, apabetalone-treated patients were categorized according to median on-treatment ALP after 12–14 weeks. Relationships of MACE to 1-SD decrease of on-treatment ALP or hsCRP at Week 12–14 and to the change of ALP or hsCRP from baseline to Week 12–14 were examined in Cox proportional hazard models adjusted for sex, age, race, established cardiovascular risk factors, CKD, study, treatment modality, and baseline ALP, hsCRP, and liver function tests.

3. Results

Baseline ALP was available in a total of 795 patients from the ASSERT, ASSURE and SUSTAIN trials (Supplementary Fig. 1). Demographic data and laboratory parameters are summarized in Table 1. Median baseline ALP was 72 U/L. Patients with baseline ALP below the median also had lower baseline hsCRP and were more often of male sex. No difference was found in age or prevalence of diabetes at baseline (Table 1).

3.1. Apabetalone treatment reduces serum ALP

Aggregate follow-up for the 795 patients included in this analysis was 4343 months, with a median (interquartile range) follow-up time of 197 (114–211) days. In ASSERT, ALP levels were decreased under apabetalone treatment in a dose-dependent manner. Table 2 summarizes the percentage change in ALP from baseline at each time point in each treatment group; detailed comparisons between groups are listed in Supplementary Table 1. Each dose of apabetalone produced a significant reduction in ALP. The decrease in ALP was observed by Week 4 and was sustained throughout the course of the study. In SUSTAIN, during the 24 weeks of treatment with apabetalone, the mean placebo-corrected reduction in ALP at Week 12 was 13.1% ($p < 0.001$). The reduction of ALP observed at Week 12 was maintained at Week 24 (Supplementary Fig. 2). In ASSURE, during the 26 weeks of treatment with apabetalone, the mean placebo-corrected reduction in ALP at Week 14 was 9.9% ($p = 0.003$). The reduction observed at Week 14 was maintained at Week 26 (Supplemental Fig. 3). The pooled analysis of all trials resulted in mean placebo-corrected decreases in ALP from baseline of 9.2% ($p < 0.001$) after 12–14 weeks and 7.7% ($p < 0.001$) after 24–26 weeks of apabetalone treatment. The decrease of ALP in all studies was not accompanied by a change in other liver enzymes.

3.2. Baseline ALP associates with MACE

Forty-three of 795 (5.4%) patients experienced MACE (Table 3). Types of events were coronary revascularization (N = 23), hospitalization for acute coronary syndrome or heart failure (N = 12), myocardial infarction (N = 4), and death (N = 4). Patients who experienced MACE had higher baseline levels of ALP than those who did not (Table 3). Similarly, when baseline ALP was dichotomized at its median, patients with levels above the median had an higher risk of developing MACE during follow-up ($p = 0.04$, Fig. 1). The association of baseline ALP with the occurrence of MACE remained significant after adjustment for treatment allocation, age, sex, race, established cardiovascular risk factors, CKD, hsCRP, calcium, markers of liver function, and study (hazard ratio per 1-SD 1.6, 95% CI 1.19–2.16, $p = 0.002$; 1-SD = 21 U/L).

3.3. ALP reduction by apabetalone associates with prevention of MACE

Serum ALP after 24–26 weeks of treatment was available in 467 of

Table 1
Baseline demographic and laboratory data.

	All patients	ALP groups		
	N = 795	< 72U/L (N = 400)	≥72 U/L (N = 395)	*p*
Female sex [N (%)]	211 (26.5)	88 (22.3)	123 (30.8)	0.007
Caucasian [N (%)]	679 (85.5)	356 (90.1)	323 (80.8)	< 0.001
Diabetes [N (%)]	278 (35)	133 (33.7)	145 (36.3)	0.5
Smoking [N (%)]	191 (24)	88 (22.3)	103 (25.8)	0.3
Hyperlipidemia [N (%)]	468 (58.9)	235 (59.5)	233 (58.3)	0.7
Hypertension [N (%)]	655 (82.4)	332 (84.1)	323 (80.8)	0.2
Previous CVD [N (%)]	743 (92.5)	375 (94.5)	368 (92)	0.1
CKD [N (%)]	94 (11.8)	49 (12.4)	45 (11.3)	0.6
Age (years)	61 (14)	62 (14)	61 (15)	0.4
BMI	29.4 (6.8)	29.4 (6.7)	29.6 (7.1)	0.9
ALP (U/L)	72 (26)	59 (14)	85 (19)	< 0.001
ALT (U/L)	24 (12)	24 (11)	24 (12)	0.9
AST (U/L)	20 (8)	20 (8)	20 (8)	0.8
GGT (U/L)	26 (18)	25 (19)	27 (17)	0.1
Bilirubin (mg/dL)	0.6 (0.5)	0.5 (0.4)	0.5 (3.5)	0.2
Calcium (mg/dL)	9.2 (1.5)	9.2 (1.1)	9.2 (7.1)	0.8
hsCRP (mg/L)	2.2 (4.1)	1.5 (3.1)	3.1 (4.9)	< 0.001

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVD, cardiovascular disease; CKD, chronic kidney disease; BMI, body mass index; GGT, gamma-glutamyl transferase; hsCRP, high sensitivity C-reactive protein.
Data for continuous variables are expressed as median (interquartile range).

Table 2
Percentage change of ALP from baseline in ASSERT.

Time Point	Placebo (n = 74)	Apabetalone 100 mg (n = 76)	*p vs.* placebo	Apabetalone 200 mg (n = 75)	*p vs.* placebo	Apabetalone 300 mg (n = 74)	*p vs.* placebo
Week 4	0.0	−6.6	< 0.0001	−9.6	< 0.0001	−13.5	< 0.0001
Week 6	+1.2	−6.2	< 0.0001	−11.5	< 0.0001	−13.7	< 0.0001
Week 8	+1.1	−7.1	< 0.0001	−11.4	< 0.0001	−14.5	< 0.0001
Week 10	−0.48	−6.3	0.001	−11.4	< 0.0001	−14.0	< 0.0001
Week 12/End of Treatment	+1.8	−4.2	< 0.0001	−12.5	< 0.0001	−12.7	< 0.0001

Table 3
Baseline demographic and biochemical data in patients with or without MACE.

	MACE (N = 43)	No MACE (N = 752)	*p*
Female sex [N (%)]	13 (30.2)	198 (26.3)	0.6
Caucasian [N (%)]	38 (88.4)	641 (85.2)	0.6
Diabetes [N (%)]	18 (41.9)	260 (34.6)	0.3
Smoking [N (%)]	13 (30.2)	178 (23.7)	0.3
Hyperlipidaemia [N (%)]	32 (74.4)	436 (58.0)	0.03
Hypertension [N (%)]	37 (86.0)	618 (82.2)	0.5
Previous CVD [N (%)]	42 (97.7)	701 (93.2)	0.2
CKD [N (%)]	4 (9.3)	90 (12.0)	0.6
Age (years)	62 (12)	61 (14)	0.7
BMI	29.9 (8)	29.4 (6.7)	1.0
ALP (U/L)	80 (34)	71 (26)	0.004
ALT (U/L)	24 (12)	24 (12)	0.6
AST (U/L)	20 (8)	20 (8)	0.7
GGT (U/L)	26 (18)	28 (23)	0.3
Total bilirubin (mg/dL)	0.6 (0.6)	0.5 (0.4)	0.9
Calcium (mg/dL)	9.2 (1.5)	9.2 (1.3)	0.7
hsCRP (mg/L)	3.6 (4.8)	2.1 (3.9)	0.02

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVD, cardiovascular disease; CKD, chronic kidney disease; BMI, body mass index; GGT, gamma-glutamyl transferase; hsCRP, high sensitivity C-reactive protein.
Data for continuous variables are expressed as median (interquartile range).

the 496 participants in the SUSTAIN or ASSURE trial; one patient with an event 2 weeks prior to blood sampling at 12–14 weeks and 4 patients with events within 2 weeks prior to blood sampling at 24–26 weeks were excluded from the following analyses. Apabetalone significantly reduced ALP at 12–14 weeks of treatment and this effect was sustained at 24–26 weeks of treatment, whereas there were no significant changes in ALP among the placebo group (Fig. 2). Among the apabetalone treated group, the decrease in ALP from baseline at Week 12–14 was associated with lower risk of MACE (HR 0.64 per 1-SD (95% CI 0.46–0.90), $p = 0.009$; 1-SD = 13 U/L), independent of age, sex, race, established cardiovascular risk factors, CKD; baseline ALP, hsCRP, calcium, and liver function, changes of hsCRP from baseline, and study. Similar associations were observed when assessing the change in ALP at Week 24–26 with subsequent outcome (HR 0.66 per 1-SD (95% CI 0.43–0.99), $p = 0.045$; 1-SD = 14 U/L).

Among all patients, each 1-SD lower ALP on assigned study treatment at Week 12–14 was associated with a lower risk of MACE (HR 0.54 (95% CI 0.32–0.89), $p = 0.02$; 1-SD = 21 U/L). A similar association with lower risk of MACE was found for the absolute change in ALP from baseline to Week 12–14 (HR 0.68 (95% CI 0.49–0.93), $p = 0.02$; 1-SD = 13 U/L). In contrast, no significant associations were observed between on-treatment hsCRP or absolute change of hsCRP level from baseline to Week 12–14 and risk of MACE (Fig. 3).

4. Discussion

Apabetalone is the first epigenetic oral therapeutic in the bromodomain and extra-terminal (BET) protein inhibitor class to be evaluated as a treatment for patients with cardiovascular disease [23]. Apabetalone acts by inhibiting BET proteins from binding to acetylated lysines on the histone tails of chromatin, thus representing a mechanism by which gene expression can be modulated [23]. This approach has the

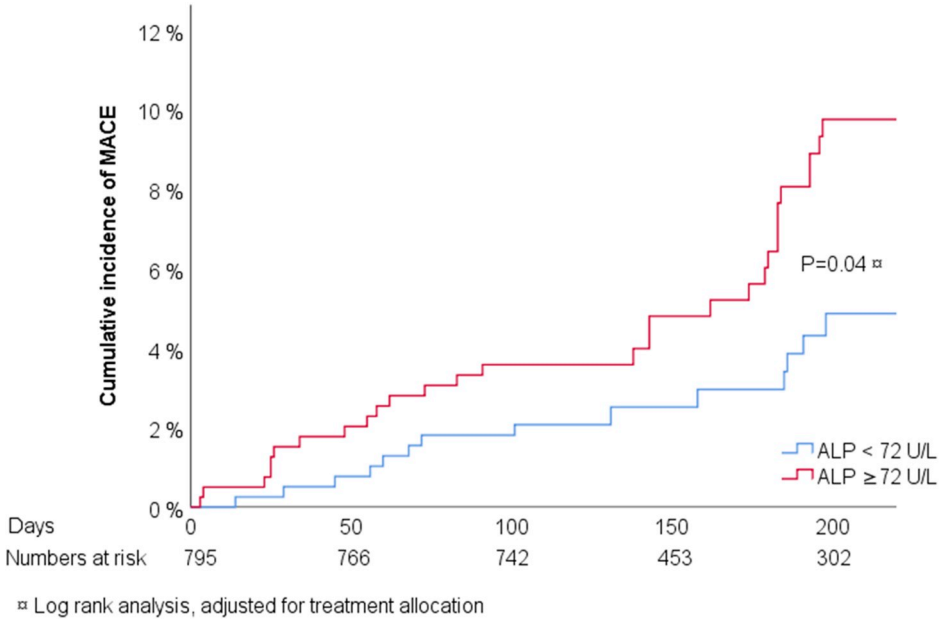

Fig. 1. Incidence of first MACE according to median baseline level of ALP.
Numbers at risk reflect the fact that contributing trials had different lengths of follow-up.

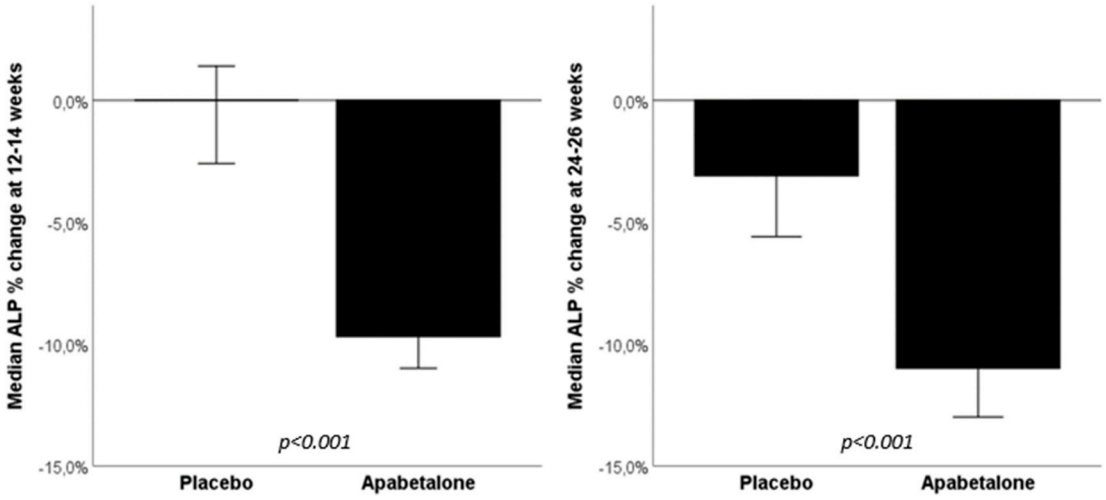

Fig. 2. Change of ALP from baseline.
Error bars represent 95% CI.

potential to impact multiple dysregulated genes and biological pathways that may be involved in the pathogenesis or progression of cardiovascular disease.

Here, we report the reduction of ALP in CVD patients following treatment with the epigenetic modulator apabetalone. Baseline ALP was strongly and independently associated with the occurrence of MACE, and ALP was significantly reduced with apabetalone, compared with placebo. ALP reduction with apabetalone was paralleled by a reduction in the incidence of MACE compared to the placebo group, as previously reported [27]. The trend for MACE event reduction was consistent across three studies (Supplementary Fig. 4).

Our finding of higher baseline ALP in patients who experienced a MACE compared to patients who did not is in accordance with earlier reports of an association of increased ALP with the incidence of MACE in STEMI patients after PCI [4,5] and is further supported by a large body of evidence for associations of increased serum ALP with all-cause and cardiovascular mortality and with the incidence of cardiovascular events [1,34]. The association of an increased cardiovascular risk with higher ALP activities within the range of normal values in the current study is in accordance with most previous studies. The current study is the first indication that a therapeutic intervention in humans may simultaneously lower ALP and improve cardiovascular outcome.

A major finding of the current study is the association between reduction of ALP and reduction of MACE by apabetalone. ALP may promote vascular calcification and arterial stiffness [12,13,35], vascular inflammation [18], destabilization of atherosclerotic plaques [15,36], and oxidative stress [19], and thereby contribute to cardiovascular disease. A pathogenic role for ALP in promoting vascular calcification and atherosclerosis progression is supported by animal studies of targeted overexpression of ALP in endothelial cells [37] or vascular smooth muscle cells [38], where vascular calcification and the incidence of cardiovascular events could be reduced by treatment with

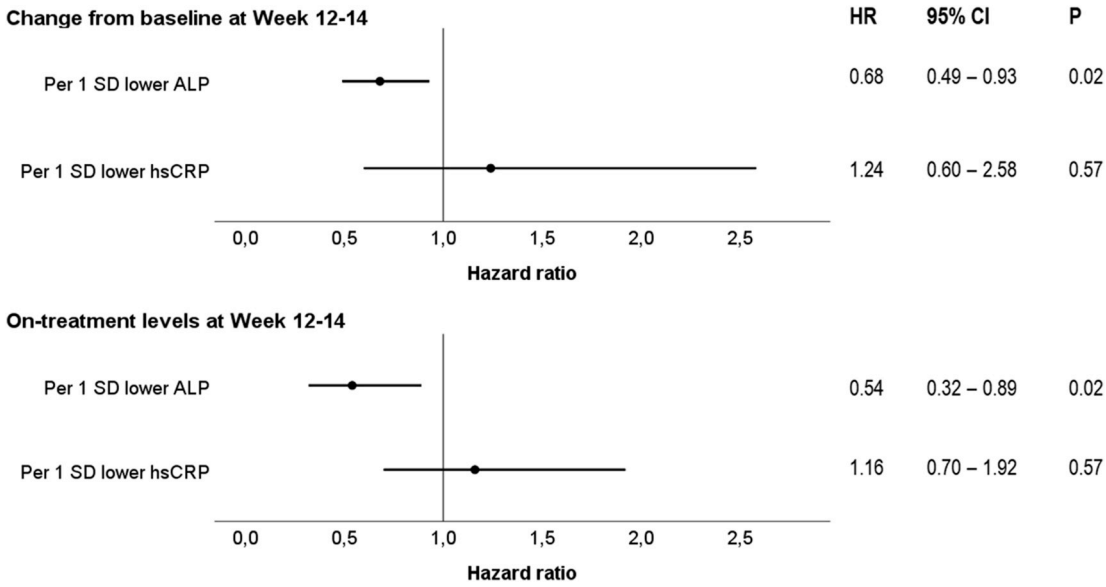

Fig. 3. Associations of ALP and hsCRP level reduction or on-treatment levels with risk of MACE.
On-treatment levels are defined as levels at Week 12–14 of therapy. Markers represent hazard ratios (HR) and lines represent 95% confidence intervals (CI) per standard deviation (SD) decrease, calculated by Cox proportional hazard models adjusted for sex, age, race, established cardiovascular risk factors, chronic kidney disease, study, treatment modality, and baseline alkaline phosphatase (ALP), high-sensitivity C-reactive protein (hsCRP), and liver function tests. A 1 SD of on-treatment ALP at Week 12–14 corresponds to 21 U/L and a 1-SD of absolute change in ALP from baseline to Week 12–14 corresponds to 13 U/L. The corresponding 1-SD values for on-treatment hsCRP and hsCRP change from baseline to Week 12–14 are 5.4 mg/L and 7.9 mg/L, respectively.

pharmacological doses of a direct ALP inhibitor [38]. Vascular calcification is driven by cells of mesenchymal origin that adapt a calciotropic phenotype in response to pro-calcific stimuli. A common feature of these mineralization-competent cells is the secretion of ALP-rich matrix vesicles that propagate the mineralization of extracellular matrix [39]. Apabetalone attenuates calcification and gene expression of ALP and other key factors that promote calcification, paralleled by a reduction of ALP protein levels and enzyme activity, in vascular smooth muscle cells (VSMCs) during osteogenic transdifferentiation [24].

Inflammatory processes are pivotal to the progression of atherosclerosis, and inflammatory biomarkers predict risk in atherosclerotic cardiovascular disease [40]. Circulating ALP correlates with hsCRP in the current study, but the association of baseline ALP with MACE incidence was independent of hsCRP, which is consistent with previous studies [9,18,41]. Likewise, the association of the change of ALP with MACE remained significant after adjustment for baseline hsCRP and change of hsCRP. This implies that ALP provides additional prognostic information not conveyed by the "standard" inflammatory marker hsCRP. In atherosclerotic plaque, ALP expressing inflammatory cells may contribute to increased plaque vulnerability [15,36]. ALP interacts with TNF alpha and other inflammatory cytokines in the stimulation of VSMC calcification [16] and may thus be involved in processes linking vascular inflammation to calcification. Oxidative stress also induces vascular calcification and the up-regulation of ALP in vascular smooth muscle cells [42] and the mortality risk associated with ALP is amplified in the presence of increased oxidative stress [19]. Oxidative stress was not determined in the current study, but apabetalone was previously shown to inhibit mediators of inflammation and oxidative stress at the gene expression and protein level [25,43].

Although clinical and experimental evidence supports an active role for ALP in cardiovascular disease, the current study does not allow us to determine whether ALP is a marker or a mediator of cardiovascular risk. Another limitation of the current analysis is that it is a *post-hoc* analysis of the association of a post-randomization biomarker with cardiovascular outcomes in pooled data from three different phase 2 clinical studies, none of which was individually powered to identify a significant difference in cardiovascular outcomes. ASSERT was intended to examine apolipoprotein A1 percent changes; SUSTAIN for HDL-C percent changes; and ASSURE for coronary atheroma percent change assessed with intravascular ultrasound. Although there are similarities among the trials in study design, differences in study populations may have affected outcomes and effects of treatment. The analysis is also limited by a low event rate, short follow-up, and a small number of MACE events, some of which were associated with cardiovascular procedures rather than spontaneous events. Investigator-reported outcomes may be subject to misclassification. Nonetheless, each of the trials was double blind, making it unlikely that a difference in MACE between treatment groups resulted from biased reporting by investigators. The phase 3 BETonMACE trial will accumulate a much larger number of MACE events and has a primary endpoint of cardiovascular death, non-fatal myocardial infarction, or stroke [30]. Forthcoming data from BETonMACE will determine whether reduction of ALP by apabetalone is associated with reduction of hard cardiovascular outcomes.

In summary, the present *post-hoc* analysis of pooled data from the ASSERT, ASSURE and SUSTAIN studies shows that serum ALP is associated with residual cardiovascular risk in patients with pre-existing cardiovascular disease on statin treatment, providing further evidence that ALP is a cardiovascular risk factor. The novel BET inhibitor apabetalone lowers serum ALP activity in a dose dependent fashion and reduces the incidence of MACE, and the magnitude of benefit appeared to be correlated with the extent of ALP reduction. However, the current analysis is hypothesis generating. Further experimental and clinical data are needed to determine whether ALP is a direct participant in atherothrombotic events and therefore a therapeutic target in its own right.

Conflicts of interest

EK, JOJ, MS, CH, KL, and NW are salaried employees of Resverlogix. and shareholders of Resverlogix, which supported the study financially. MH, VB, SB, MT, CZ and KKZ are members of the renal clinical advisory board of Resverlogix. KKR, SJN and GGS are members of the clinical steering committee of BET on MACE of Resverlogix. In addition, MH has acted as consultant and as speaker for Resverlogix Corp and Amgen. KKR has acted as a consultant for Resverlogix, Amgen, Sanofi, Regeneron, MedCo, Cerenis, Lilly, Ionis Pharma, Esperion, and Abbvie; and as a speaker for Kowa, AstraZeneca, Pfizer, Takeda, Boehringer Ingelheim, Algorithm, Cipla. He has received research grants from Sanofi-Regeneron, Amgen, Merck Sharpe & Dohme, and Pfizer through his institution and he acknowledges support from the NIHR Imperial Biomedical Research Centre. SJN reports grants from AstraZeneca, Amgen, Anthera, Eli Lilly, Esperion, Novartis, Cerenis, The Medicines Company, Resverlogix, InfraReDx, Roche, Sanofi, Regeneron, and LipoScience and personal fees from AstraZeneca, Eli Lilly, Anthera, Omthera, Merck, Takeda, Resverlogix, Sanofi-Regeneron, CSL Behring, Esperion, and Boehringer Ingelheim. GGS receives research support to his institution from Resverlogix, Sanofi, and The Medicines Company. SB is supported by research grants RO1-DK115814, R21-DK106574 and research funding from Bayer. MT has received a research grant from Merk Canada (funds to institution). KKZ 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

The study was supported by Resverlogix Corp.

Author contributions

Research idea and study design: MH, KKZ; clinical trials design and management: SJN, JOJ, MS; data analysis/interpretation: MH, KKR, SJN, GGS, JOJ, MS, KKZ; statistical analysis: MH, CH; writing of the manuscript: MH; revision of the manuscript: MH, KKR, SJN, GGS, EK, JOJ, CH, KL, NW, VB, SB, MT, CZ, KKZ. Each author contributed important intellectual content during drafting or revision of the manuscript and accepts accountability for the overall work. MH and CH had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.09.002.

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