



Targeting epigenetics as atherosclerosis treatment: an updated view

Annette E. Neele^a, Lisa Willemsen^a, Hung-Jen Chen^a, Kim E. Dzobo^b, and Menno P.J. de Winther^a

Purpose of review

This review discusses the current developments on epigenetic inhibition as treatment for atherosclerosis.

Recent findings

The first phase III clinical trial targeting epigenetics in cardiovascular disease (CVD), BETonMACE, using the bromodomain inhibitor apabetalone (RVX-208) showed no significant effect on major adverse cardiovascular events (MACE) in patients with type II diabetes, low HDL-c and a recent acute coronary artery event compared with its placebo arm.

Summary

Preclinical and clinical studies suggest that targeting epigenetics in atherosclerosis is a promising novel therapeutic strategy against CVD. Interfering with histone acetylation by targeting histone deacetylases (HDACs) and bromodomain and extraterminal domain (BET) proteins demonstrated encouraging results in modulating disease progression in model systems. Although the first phase III clinical trial targeting BET in CVD showed no effect on MACE, we suggest that there is sufficient potential for future clinical usage based on the outcomes in specific subgroups and the fact that the study was slightly underpowered. Lastly, we propose that there is future window for targeting repressive histone modifications in atherosclerosis.

Keywords

apabetalone, bromodomain, cardiovascular disease, histone deacetylase, histone modification

INTRODUCTION

Cardiovascular disease (CVD) is still the major cause of death worldwide, with atherosclerosis as the main underlying disease [1]. It is known that traditional risk factors, such as elevated levels of LDL contribute to disease development. Recently, more clinical evidence showed that also nontraditional risk factors such as inflammation play a crucial role in the development of human atherosclerosis and contribute to the risk of CVD. The CANTOS trial demonstrates that interleukin-1 β (IL-1 β) blockade by monoclonal antibodies targeting IL-1 β (Canakinumab) lowers recurrent cardiovascular events in patients who previously had a myocardial infarction (MI) and high levels of C-reactive protein (CRP) independent of lipid-lowering strategies [2]. In addition, the COLCOT trial shows that in patients with a recent MI, daily treatment with the anti-inflammatory drug colchicine also significantly lowered the risk of cardiovascular events compared with its placebo arm [3]. Although the observed effects in these recent clinical trials are limited and patients suffer from side effects, they open a window for novel

treatment strategies that modulate the inflammatory response in disease.

Epigenetic pathways control DNA accessibility and usage and thereby regulate gene expression. DNA methylation and posttranslational histone modifications are the most common mechanisms controlling DNA accessibility. Histone modifications are regulated by a large number of histone-modifying

^aDepartment of Medical Biochemistry, Amsterdam Cardiovascular Sciences, Amsterdam Infection and Immunity and ^bDepartment of Experimental Vascular Medicine, Amsterdam Cardiovascular Sciences, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands

Correspondence to Menno P.J. de Winther, PhD, Department of Medical Biochemistry, Amsterdam UMC, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. Tel: +31 20 5666762; e-mail: m.dewinther@amsterdamumc.nl

Curr Opin Lipidol 2020, 31:324–330

DOI:10.1097/MOL.0000000000000711

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

KEY POINTS

- HDAC3 and HDAC9 are promising targets for atherosclerosis treatment.
- Apabetalone has the potential to reduce inflammation *in vitro* and *in vivo*.
- Although the first phase III clinical trial using apabetalone to target BET proteins in CVD showed no effect on MACE, it suggests sufficient potential to warrant further future studies.

enzymes that affect either activating or repressive histone marks. Histone acetylation is in general associated with gene transcription, whereas histone methylation can either be activating or repressive depending on the type and position of the methylation. Several preclinical studies show a role for histone modifications and its enzymes in CVD, and recently, the outcomes of the first phase III clinical trial (BETonMACE), which used an inhibitor of the bromodomain and extraterminal (BET) proteins of histone acetylation in CVD, were published. Here, we will discuss the recent development on targeting epigenetics as treatment for atherosclerosis with a focus on histone deacetylases (HDACs), BETs and future opportunity for targeting repressive histone modifications.

TARGETING HISTONE DEACETYLASES AS ATHEROSCLEROSIS TREATMENT

In general, histone acetylation is associated with open chromatin and gene transcription. This modification is mediated by histone acetyltransferases (HATs) and counteracted by HDACs. HDACs remove activating acetyl marks resulting in closed chromatin and repressed gene expression. HDACs can be divided into four families: class I HDACs (HDAC1–3, 8), class II HDACs (HDAC4–7, 9, 10), which are Zn²⁺-dependent enzymes, class III HDACs, which are the sirtuins (SIRT1–7) and class IV HDAC, which only comprises HDAC11 [4]. Currently, six HDAC inhibitors are FDA approved and various HDAC inhibitors are used in clinical trials for cancer and neurological disease (Table 1). Up to date, there are no phase III clinical trials with HDAC inhibitors for CVD treatment, but preclinical studies do suggest a future opportunity for targeting specific HDACs in CVD. Although *in vitro* HDAC inhibition has anti-inflammatory properties in immune cells such as macrophages [5] and T cells [6], the first *in vivo* mice study using HDAC inhibitors for atherosclerosis surprisingly showed that treatment with the pan-

HDAC inhibitor trichostatin A (TSA) enhanced atherosclerotic lesion formation in a *Ldlr*^{-/-} mice model for atherosclerosis [7]. On the contrary, treatment with the more specific class I HDAC inhibitor valproate attenuated atherosclerosis development in a hyperglycaemic *ApoE*-deficient mouse model [8], and recently, it was shown that also treatment with the pan-HDAC inhibitor vorinostat (SAHA) decreased atherosclerotic lesion size in an ApoE-deficient mice [9]. Differences in outcomes might not only be explained by the specificity and selectivity of the compounds, but also by the type of cells that are affected by the HDAC inhibitors. Cell-type specific targeting of HDACs, such as targeting macrophages as key immune regulators of atherosclerosis, might be a better approach. Needham *et al.* [10] developed a method to specifically target HDACs in monocytes and macrophages by use of an esterase-sensitive chemical motif (ESM). Carboxylesterase-1 is predominantly expressed in human monocytes and macrophages and inhibitors with an ESM-motif will be hydrolyzed specifically in these cells mediating accumulation of the drugs [10]. We were the first to show that use of a HDACi coupled to an ESM motif specifically targets monocytes and macrophages in a mouse model for atherosclerosis [11]. Although ESM-HDACi reduced the maturation and pro-inflammatory responses of macrophages, lesion size was not affected by ESM-HDACi treatment. Yet, lesions treated with the ESM-HDACi showed a less advanced phenotype compared with its controls suggesting clinically relevant benefits. In addition to cell-specific targeting, preclinical studies suggest a role for targeting specific HDACs in atherosclerosis. HDAC9 has been linked to abdominal aortic calcification [12] and risk for stroke [13]. In addition, both systemic and haematopoietic deletion of *Hdac9* reduced atherosclerosis in *Ldlr*^{-/-} mice [14]. Improved outcome on atherosclerosis was recently supported by Asare *et al.* [15[□]] who showed that haematopoietic *Hdac9* deficiency in mice results in a more stable plaque phenotype by reducing macrophage content and increasing cap thickness. Furthermore, they showed that treatment with the class IIa HDAC inhibitor TMP196, with a high affinity for HDAC9, also reduced atherosclerosis in mice [15[□]]. The authors propose a mechanism by which HDAC9 directly deacetylates IKK α and β resulting in its activation leading to a pro-inflammatory response in macrophages and endothelial cells. Apart from HDAC9, we reason that also HDAC3 might be of interest for future targeting, as we showed that myeloid *Hdac3* deficiency stabilized atherosclerotic lesions in *Ldlr*^{-/-} mice via direct regulation of the pro-fibrotic *Tgfb1* [16]. We propose that both isoform-specific HDAC

Table 1. Histone deacetylase inhibitors

Class	Drug	Targets	Stage
HDACi	Belinostat (PXD101)	Class I, II, IV	FDA
	Panobinostat (LBH589)	Class I, II, IV	FDA
	Vorinostat (SAHA)	Class I, II, IV	FDA
	Romidepsin (FK228)	Class I	FDA
	Sodium Butyrate	Class I, II	FDA
	Valproic acid/Valproate	Class I, II	FDA
	Tucidinostat/Chidamide (CS055, HBI-8000)	Class I, II	Phase IV (China)
	Givinostat (ITF2357)	Class I, II, IV	Phase III
	Pracinostat (SB939)	Class I, II, IV (except HDAC6)	Phase III
	Entinostat (MS-275)	Class I	Phase III
	Tacedinaline (CI994)	Class I	Phase III
	Nicotinamide (Vitamin B3)	Class III	Phase III
	Abexinostat (PCI-24781)	Class I, II, IV	Phase II
	AR-42 (HDAC-42)	Class I, II, IV	Phase II
	Dacinostat (LAQ824)	Class I, II, IV	Phase II
	Remetinostat (SHP-141)	Class I, II, IV	Phase II
	Quisinostat (JNJ-26481585)	Class I, II, IV	Phase II
	Mocetinostat (MGCD0103)	Class I, IV	Phase II
	Resminostat (RAS2410)	HDAC1/3/6	Phase II
	Ricolinostat (ACY-1215)	HDAC6	Phase II
	Trichostatin A (TSA)	Class I, II, IV	Phase I
	Tinostamustine (EDO-S101)	Class I, II	Phase I
	CXD-101	Class I	Phase I
	Domatinostat (4SC-202)	Class I	Phase I
	R306465 (JNJ-16241199)	Class I	Phase I
	RG2833 (RGFP109)	HDAC1/3	Phase I
	Pyroxamide	HDAC1	Phase I
	Scriptaid	Class I, II, IV	Preclinical
	TMP195	Class II	Preclinical
	TMP269	Class II	Preclinical
	UF010	Class I	Preclinical
	RGFP966	HDAC3	Preclinical
	LMK-235	HDAC4/5	Preclinical
	Droxinostat	HDAC6/8 (3)	Preclinical
	Nexturastat A	HDAC6	Preclinical
	Tubastatin A	HDAC6	Preclinical
	Citarinostat (ACY-241)	HDAC6	Preclinical
	PCI-34051	HDAC8	Preclinical

List of available HDAC inhibitors with their selectivity and clinical stage they are in. Clinical stages are for the treatment of various types of cancer or neurological disease, not for CVD.

and cell-type specific targeting is of great interest for future therapy and an important next step in targeting HDACs in atherosclerosis, as both influence its outcomes. Targeting HDACs is not only of interest for atherosclerosis but also other CVDs, as it was recently shown that HDAC inhibition can improve cardiopulmonary function in a large animal model (feline) of diastolic dysfunction [17]. An extensive overview of

HATs and HDACs in the broader arena of CVD was recently given by Li *et al.* [18].

CLINICAL INSIGHTS IN TARGETING BET PROTEINS IN CARDIOVASCULAR DISEASE

BET proteins can recognize and read acetylated histones and mediate recruitment of RNA polymerase II

Table 2. BRD2/3/4/T inhibitors

Class	Drug	Targets	Stage
BETi	Apabetalone (RVX-208)	BD2; BRD2/3	phase III for CVD
	Molibresib (I-BET762, GSK525762)	BRD2/3/4/T	Phase II
	CPI-0610	BD1; BRD2/4	Phase II
	Birabresib (OTX015, MK-8628)	BRD2/3/4	Phase II ^a
	INCB054329	BRD2/3/4/T	Phase I/II ^a
	INCB057643	BRD2/3/4/T	Phase I ^a
	BMS-986158	BRD2/3/4/T	Phase I
	FT-1101	BRD2/3/4/T	Phase I
	TEN-010 (JQ2)	BRD2/3/4/T	Phase I
	ABBV-744	BD2; BRD2/3/4	Phase I
	PLX51107	BRD2/3/4/T	Phase I
	AZD5153	BRD4	Phase I
	JQ1	BRD2/3/4/T	Preclinical
	Bromosporine	BRD2/4/9, CECR2	Preclinical
	Mivebresib (ABBV-075)	BRD2/4/T	Preclinical
	I-BET151 (GSK1210151A)	BRD2/3/4	Preclinical
	I-BET726 (GSK1324726A)	BRD2/3/4	Preclinical
	PFI-1 (PF-6405761)	BRD2/4	Preclinical
	ARV-771	BRD2/3/4	Preclinical
	ARV-825	BRD4	Preclinical
CPI-203	BRD4	Preclinical	

List of preclinical and clinical BET inhibitors with their selectivity. Clinical stages are for the treatment of various types of cancer or neurological disease; only apabetalone is used in clinical trials for CVD.

^aTerminated.

to promote transcription. The BET reader proteins include BRD2–4 and BRDT of which BRD4 is most dominant in transcriptional regulation. BET proteins contain two bromodomains BD1 and BD2, which are suggested to regulate distinct transcriptional outcomes. A more detailed description on the transcriptional mechanisms of BET regulation in CVD is greatly reviewed by Borck *et al.* [19[¶]]. Multiple small molecule BET inhibitors have been developed and used in clinical trials for cancer and recently also in CVD (Table 2). JQ-1 was one of the first developed compounds that can bind acetylated histones and thereby prevents binding of BETs to histone tails resulting in transcriptional blockade. JQ-1 is a broad-spectrum BET inhibitor, as it targets both BD1 and -2 of all BRDs. Daily treatment with JQ-1 in hypercholesterolemic *Ldlr*^{-/-} mice reduced aortic plaque area by 40%, an effect that was independent of LDL and HDL levels. JQ-1 attenuated endothelial activation and reduced transmigration of leukocytes *in vitro* and *ex vivo* [20]. The BET inhibitor RVX-208, also called apabetalone, is a selective BET inhibitor for the BD-2 domain of mainly BRD2/3, with low affinity for BRD4 [21]. Apabetalone was originally discovered as ApoA1-inducing compound [22], which also holds true for I-BET, a GSK developed compound [23].

Treatment of atherosclerotic *ApoE*^{-/-} mice for 12 weeks with apabetalone increased circulating levels of HDL-c and decreased circulating LDL-c [24]. Treatment also resulted in a significant reduction of aortic lesion formation. Follow-up experiments with lower dosage and lower diet also reduced atherosclerotic lesion formation without changes in plasma lipid levels. Moreover, in both set-ups, apabetalone significantly reduced pro-inflammatory cytokines in the plasma suggesting that apabetalone can have both lipid-lowering and anti-inflammatory properties [24]. The phase I clinical trial showed that apabetalone significantly increased ApoA-1 and cholesterol efflux towards ABCA1 after 1 week of treatment in healthy volunteers [22]. In the phase IIa ASSERT trial, patients with stable coronary artery disease (CAD) were treated with apabetalone or placebo for 12 weeks on top of standard statin therapy [25]. Apabetalone treatment significantly increased HDL-c and ApoA-1 with a rapid increase from week 8 to 12, suggesting that longer treatment could even have more profound effects, which was assessed in the phase IIb trials SUSTAIN and ASSURE [26]. In the phase II SUSTAIN trial, 176 patients with established atherosclerotic CVD and low levels of HDL-c were treated with apabetalone 100 mg twice daily (b.i.d.) or

placebo for 24 weeks. Apart from lipid efficacy, also safety and tolerability of RVX-208 was measured. Although the effects were limited, the percentage changes in HDL-c and ApoA-I were significantly lower in apabetalone-treated patients. The phase IIb ASSURE trial measured percentage change of atheroma volume as primary end point after 26 weeks of treatment with 100 mg b.i.d. apabetalone or placebo in 323 patients with angiographic CAD and low levels of HDL-c. No significant difference in primary end point was observed compared with its placebo control group, but the primary end point was decreased compared with baseline levels [27]. The relative risk of major adverse cardiovascular events (MACE) in patients with CVD was decreased by 44% and even 57% in diabetic CVD patients. Additional analysis on attenuated coronary atherosclerotic plaque as measurement of vulnerable plaques was based on the intravascular ultrasound measures. Attenuated coronary Atherosclerotic plaque was observed in 27 patients treated with apabetalone from the ASSURE trial, which was significantly lower after treatment than baseline [28]. Furthermore, apabetalone not only reduced vascular inflammation *in vitro* but also lowered pro-atherogenic proteins in plasma of CVD patients [29]. Apabetalone also lowers serum alkaline phosphate (ALP), which itself predicts residual cardiovascular risk independent of high sensitivity CRP (hsCRP), that associated with a lower risk of cardiovascular events [30]. Although hsCRP was not significantly different between the apabetalone and placebo groups in the phase II ASSURE trial, pooled analysis of the phase II clinical trials (ASSERT, SUSTAIN and ASSURE) showed a significant increase in ApoA-1 and HDL-C and a decrease in hsCRP. MACE was significantly lower in apabetalone-treated patients and this effect was even more profound in diabetic patients, patients with low levels of HDL-C or patients with elevated hsCRP [31]. The effects of apabetalone to reduce atherogenesis in CVD patients was assessed in the phase III clinical trial BETonMACE. In the BETonMACE trial, 2425 patients with type II diabetes, low HDL-C and a recent acute coronary artery event (within 3 months) were treated with apabetalone 100 mg b.i.d. or placebo control on top of high-intensity statin therapy and followed for 26.5 months [32[■], 33[■]]. The primary outcome of this study was the first time to MACE, which included cardiovascular death, nonfatal MI or stroke. No significant change in MACE was observed upon apabetalone treatment (apabetalone-treated 10.3 vs. placebo 12.4%; hazard ratio 0.82; $P=0.11$) and a nonsignificant trend was observed for the MACE sensitivity analysis (excluding deaths of undetermined cause) (hazard ratio 0.79; $P=0.06$). The negative outcomes on MACE in this phase III clinical trial might have

several explanations. First, the study was underpowered to detect smaller differences between groups due to lower than expected event rates and the fact that the study population was based on an 80% power to detect a 30% event reduction, which was not met. Outcomes might thus be different in a larger cohort. In line with this, the Kaplan–Meier estimate of time to first occurrence of the primary efficacy end point graph shows effects at early stage of the study, with a peak difference at 24 months. The explanation for this remains uncertain. Also, BETonMACE was performed in patients with a recent acute coronary artery event (within 3 months) on high-intensity statins, while phase II clinical trials were mainly performed in patients with stable CAD and standard statin therapy. Lastly, hsCRP levels were not reduced in the present study and surprisingly only measured in a small subset of patients ($\pm 20\%$, baseline levels 2.9 mg/l). Positive outcomes on MACE by targeting inflammation in both the CANTOS and COLCOT clinical trials were performed in patients with elevated levels of hsCRP, which was lowered upon treatment (CANTOS pre: 4.25 mg/l, post: 2.10 mg/l; COLCOT pre: 4.27 mg/l, post 1.37 mg/l). Additional subgroup analysis suggests larger effects of apabetalone on primary outcomes in patients with decreased kidney function (Estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m²) or low levels of LDL-c (LDL $<$ median). Furthermore, secondary end point analysis and other prespecified analysis show beneficial effects of apabetalone on patients with congestive heart failure (hazard ratio 0.59 for first hospitalization for congestive heart failure and HR 0.49 for first and recurrent hospitalization). Therefore, we reason that there is still potential for future clinical trials targeting BET proteins in CVD in specific subsets of patients.

FUTURE OPPORTUNITY: A POTENTIAL ROLE FOR TARGETING REPRESSIVE HISTONE MODIFICATIONS IN ATHEROSCLEROSIS?

Although most studies focus on targeting histone acetylation processes in CVD, preclinical evidence suggests that also targeting repressive histone modifications might be of future interest. Repressive histone modifications include H3K9me2/3 and H3K27me3, which results in closed chromatin and thus gene repression. H3K27me3 is catalyzed by the polycomb repressive complex 2 (PRC2) that consist of multiple proteins of which enhancer of the zeste homolog 1 or 2 EZH1/2 contains the catalytic SET domain of the complex. On the contrary, H3K27me3 is removed by the demethylases KDM6A and KDM6B (JMJD3). We showed that pro-fibrotic pathways were suppressed in *Kdm6b*-deleted foam cells, which is an

important hallmark for atherosclerosis [34]. Surprisingly, we also reported that myeloid *Kdm6b* deficiency in mice results in advanced atherosclerosis [35]. This might have been caused by the fact that KDM6B in macrophages is regulated in response to various triggers and that deletion or inhibition of KDM6B is reported to reduce both pro and anti-inflammatory responses [36,37]. In contrast to KDM6B, recent studies suggest that targeting the opposing enzyme, EZH2 of the PRC2 complex, might be a better approach. It was shown that IFN- γ activation of human macrophages suppresses a set of anti-inflammatory genes, which was mediated by EZH2 [38]. Moreover, treatment with GSK126, an EZH2-selective inhibitor, reduced macrophage pro-inflammatory responses [39]. In addition, myeloid *Ezh2*-deficiency in mice showed improvement in the chronic inflammatory disorders experimental autoimmune encephalomyelitis (EAE) and colitis, suggesting a potential role for targeting repressive histone modification in chronic inflammatory disorders such as CVD [39,40]. Indeed, overexpression of *Ezh2* in mice was shown to increase atherosclerosis plaque size by downregulation of *Abga1/Abcg1* making macrophages foamier [41]. Various inhibitors against PRC2 (of which most target EZH2) are used in clinical trials for cancers, but their impact on CVD remains unknown (Table 3). The selective EZH2 inhibitor Tazemetostat (EPZ6438) is the first PRC2i that got FDA approval for the use in patients with epithelioid sarcoma [42] or follicular lymphoma.

Table 3. Polycomb repressive complex 2 inhibitors

Class	Drug	Targets	Stage
PRC2i	Tazemetostat (EPZ6438)	EZH2	FDA
	CPI-1205	EZH2	Phase II
	GSK126 (GSK2816126)	EZH2	Phase I ^a
	JQ-EZ-05	EZH1/2	Preclinical
	UNC1999	EZH1/2	Preclinical
	CPI-360	EZH1	Preclinical
	CPI-169	EZH2	Preclinical
	EBI-2511	EZH2	Preclinical
	EI1	EZH2	Preclinical
	EPZ005687	EZH2	Preclinical
	EPZ011989	EZH2	Preclinical
	GSK343	EZH2	Preclinical
	GSK503	EZH2	Preclinical
	PF-06726304	EZH2	Preclinical
	EED226	EED	Preclinical
	A-395	EED	Preclinical

List of preclinical and clinical PRC2 inhibitors with their selectivity. Clinical stages are for the treatment of various types of cancer not for CVD.
^aTerminated.

CONCLUSION

Interfering with histone acetylation by targeting HDACs and BET proteins demonstrated encouraging results in modulating inflammatory responses and suggest a promising novel therapeutic strategy against CVD. Although the first phase III clinical trial targeting BET in CVD (BETonMACE) showed no effect on MACE (HR 0.82), we propose that there is sufficient potential for future clinical practice in targeting BET proteins based on the limitations in the design of the current clinical trial. Future studies with apabetalone or other BET inhibitors should be performed in larger cohorts, and possibly target more specific patient groups such as in patients with established CVD and an inflammatory risk profile (high hsCRP/high ALP). Moreover, apabetalone is a BD2-selective compound with higher affinity for BRD2/3 over BRD4 and outcomes of future clinical trials with other BET inhibitors targeting both BD1 and BD2, for example might have different outcomes. Bioavailability and dosing of such drugs remain points of attention. As a next step in targeting HDACs as treatment for atherosclerosis, we propose that both isoform-specific cell-type specific targeting of HDACs is of great interest for future therapy. Lastly, we think that there is an upcoming opportunity for targeting repressive histone modifications in atherosclerotic disease, which should be exploited in the future.

Acknowledgements

None.

Financial support and sponsorship

Our work is supported by grants from Amsterdam Cardiovascular Sciences, The Netherlands Heart Foundation (CVON 2017–2 and Dekker-fellowship 2020T029), The Netherlands Heart Foundation and Spark-Holding BV (2015B002, 2019B016), Fondation Leducq (LEAN-Transatlantic Network Grant), the European Union (EPI-MAC-ITN and REPROGRAM; EU Horizon 2020) and ZonMW (Open competition 09120011910025).

Conflicts of interest

MdW receives funding from GSK.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. World Health Organization. Cardiovascular diseases. 2020. <https://www.who.int/health-topics/cardiovascular-diseases>.
2. Ridker PM, Everett BM, Thuren T, *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017; 377:1119–1131.

3. Tardif JC, Kouz S, Waters DD, *et al.* Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med* 2019; 381:2497–2505.
 4. Park SY, Kim JS. A short guide to histone deacetylases including recent progress on class II enzymes. *Exp Mol Med* 2020; 52:204–212.
 5. Van den Bossche J, Neele AE, Hoeksema MA, *et al.* Inhibiting epigenetic enzymes to improve atherogenic macrophage functions. *Biochem Biophys Res Commun* 2014; 455(3–4):396–402.
 6. Glauben R, Sonnenberg E, Wetzal M, *et al.* Histone deacetylase inhibitors modulate interleukin 6-dependent CD4+ T cell polarization in vitro and in vivo. *J Biol Chem* 2014; 289:6142–6151.
 7. Choi JH, Nam KH, Kim J, *et al.* Trichostatin A exacerbates atherosclerosis in low density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2005; 25:2404–2409.
 8. Bowes AJ, Khan MI, Shi Y, *et al.* Valproate attenuates accelerated atherosclerosis in hyperglycemic apoE-deficient mice: evidence in support of a role for endoplasmic reticulum stress and glycogen synthase kinase-3 in lesion development and hepatic steatosis. *Am J Pathol* 2009; 174:330–342.
 9. Manea SA, Vlad ML, Fenyó IM, *et al.* Pharmacological inhibition of histone deacetylase reduces NADPH oxidase expression, oxidative stress and the progression of atherosclerotic lesions in hypercholesterolemic apolipoprotein E-deficient mice; potential implications for human atherosclerosis. *Redox Biol* 2020; 28:101338.
 10. Needham LA, Davidson AH, Bawden LJ, *et al.* Drug targeting to monocytes and macrophages using esterase-sensitive chemical motifs. *J Pharmacol Exp Ther* 2011; 339:132–142.
 11. Luque-Martin R, Van den Bossche J, Furze RC, *et al.* Targeting histone deacetylases in myeloid cells inhibits their maturation and inflammatory function with limited effects on atherosclerosis. *Front Pharmacol* 2019; 10:1242.
 12. Malhotra R, Mauer AC, Lino Cardenas CL, *et al.* HDAC9 is implicated in atherosclerotic aortic calcification and affects vascular smooth muscle cell phenotype. *Nat Genet* 2019; 51:1580–1587.
 13. International Stroke Genetics C, Wellcome Trust Case Control C, Bellenguez C, *et al.* Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. *Nat Genet* 2012; 44:328–333.
 14. Cao Q, Rong S, Repa JJ, *et al.* Histone deacetylase 9 represses cholesterol efflux and alternatively activated macrophages in atherosclerosis development. *Arterioscler Thromb Vasc Biol* 2014; 34:1871–1879.
 15. Asare Y, Campbell-James TA, Bokov Y, *et al.* Histone deacetylase 9 activates IKK to regulate atherosclerotic plaque vulnerability. *Circ Res* 2020; 127:811–823.
- This study provides mechanistic insights into how Hdac9 regulates atherosclerosis.
16. Hoeksema MA, Gijbels MJ, Van den Bossche J, *et al.* Targeting macrophage histone deacetylase 3 stabilizes atherosclerotic lesions. *EMBO Mol Med* 2014; 6:1124–1132.
 17. Wallner M, Eaton DM, Berretta RM, *et al.* HDAC inhibition improves cardiopulmonary function in a feline model of diastolic dysfunction. *Sci Transl Med* 2020; 12:eaay7205.
 18. Li P, Ge J, Li H. Lysine acetyltransferases and lysine deacetylases as targets for cardiovascular disease. *Nat Rev Cardiol* 2020; 17:96–115.
 19. Borck PC, Guo LW, Plutzky J. BET epigenetic reader proteins in cardiovascular transcriptional programs. *Circ Res* 2020; 126:1190–1208.
- An excellent review on the transcriptional mechanisms of BET regulation in CVD.
20. Brown JD, Lin CY, Duan Q, *et al.* NF-kappaB directs dynamic super enhancer formation in inflammation and atherogenesis. *Mol Cell* 2014; 56:219–231.
 21. Picaud S, Wells C, Felletar I, *et al.* RVX-208, an inhibitor of BET transcriptional regulators with selectivity for the second bromodomain. *Proc Natl Acad Sci U S A* 2013; 110:19754–19759.
 22. Bailey D, Jahagirdar R, Gordon A, *et al.* RVX-208: a small molecule that increases apolipoprotein A-I and high-density lipoprotein cholesterol in vitro and in vivo. *J Am Coll Cardiol* 2010; 55:2580–2589.
 23. Gosmini R, Nguyen VL, Toum J, *et al.* The discovery of I-BET726 (GSK1324726A), a potent tetrahydroquinoline ApoA1 up-regulator and selective BET bromodomain inhibitor. *J Med Chem* 2014; 57:8111–8131.
 24. Jahagirdar R, Zhang H, Azhar S, *et al.* A novel BET bromodomain inhibitor, RVX-208, shows reduction of atherosclerosis in hyperlipidemic ApoE deficient mice. *Atherosclerosis* 2014; 236:91–100.
 25. Nicholls SJ, Gordon A, Johansson J, *et al.* Efficacy and safety of a novel oral inducer of apolipoprotein a-I synthesis in statin-treated patients with stable coronary artery disease a randomized controlled trial. *J Am Coll Cardiol* 2011; 57:1111–1119.
 26. Nicholls SJ, Gordon A, Johansson J, *et al.* ApoA-I induction as a potential cardioprotective strategy: rationale for the SUSTAIN and ASSURE studies. *Cardiovasc Drugs Ther* 2012; 26:181–187.
 27. Nicholls SJ, Puri R, Wolski K, *et al.* Effect of the BET protein inhibitor, RVX-208, on progression of coronary atherosclerosis: results of the phase 2b, randomized, double-blind, multicenter, ASSURE trial. *Am J Cardiovasc Drugs* 2016; 16:55–65.
 28. Shishikura D, Kataoka Y, Honda S, *et al.* The effect of bromodomain and extra-terminal inhibitor apabetalone on attenuated coronary atherosclerotic plaque: insights from the ASSURE trial. *Am J Cardiovasc Drugs* 2019; 19:49–57.
 29. 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.
 30. 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.
 31. Nicholls SJ, Ray KK, Johansson JO, *et al.* Selective BET protein inhibition with apabetalone and cardiovascular events: a pooled analysis of trials in patients with coronary artery disease. *Am J Cardiovasc Drugs* 2018; 18:109–115.
 32. Ray KK, Nicholls SJ, Buhr KA, *et al.* Effect of apabetalone added to standard therapy on major adverse cardiovascular events in patients with recent acute coronary syndrome and type 2 diabetes: a randomized clinical trial. *JAMA* 2020; 323:1565–1573.
- Outcomes of the first phase III clinical trial targeting epigenetics (BETs) in CVD.
33. Ray KK, Nicholls SJ, Ginsberg HD, *et al.* Effect of selective BET protein inhibitor apabetalone on cardiovascular outcomes in patients with acute coronary syndrome and diabetes: rationale, design, and baseline characteristics of the BETonMACE trial. *Am Heart J* 2019; 217:72–83.
- Design of the first phase III clinical trial targeting BET proteins in CVD.
34. Neele AE, Prange KH, Hoeksema MA, *et al.* Macrophage Kdm6b controls the pro-fibrotic transcriptome signature of foam cells. *Epigenomics* 2017; 9:383–391.
 35. Neele AE, Gijbels MJ, van der Velden S, *et al.* Myeloid Kdm6b deficiency results in advanced atherosclerosis. *Atherosclerosis* 2018; 275:156–165.
 36. Van den Bossche J, Neele AE, Hoeksema MA, *et al.* Macrophage polarization: the epigenetic point of view. *Curr Opin Lipidol* 2014; 25:367–373.
 37. Kuznetsova T, Prange KHM, Glass CK, *et al.* Transcriptional and epigenetic regulation of macrophages in atherosclerosis. *Nat Rev Cardiol* 2019.
 38. Qiao Y, Kang K, Giannopoulou E, *et al.* IFN-gamma induces histone 3 lysine 27 trimethylation in a small subset of promoters to stably silence gene expression in human macrophages. *Cell Rep* 2016; 16:3121–3129.
 39. Zhang X, Wang Y, Yuan J, *et al.* Macrophage/microglial Ezh2 facilitates autoimmune inflammation through inhibition of Socs3. *J Exp Med* 2018; 215:1365–1382.
 40. Neele AE, de Winther MPJ. Repressing the repressor: Ezh2 mediates macrophage activation. *J Exp Med* 2018; 215:1269–1271.
 41. Lv YC, Tang YY, Zhang P, *et al.* Histone methyltransferase enhancer of Zeste homolog 2-mediated ABCA1 promoter DNA methylation contributes to the progression of atherosclerosis. *PLoS One* 2016; 11:e0157265.
 42. Rothbart SB, Baylin SB. Epigenetic therapy for epithelioid sarcoma. *Cell* 2020; 181:211.