

Bromodomain and Extra-Terminal Protein Inhibition in Atherosclerosis: Advantages and Disappointments

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ABSTRACT

Major cardiovascular events (MACEs) are leading cause of premature death in general population, as well as in the patients with known atherosclerosis and coronary artery disease (CAD). Manifestation and progression of atherosclerosis is tightly related to systemic and local microvascular inflammation that accelerate vascular remodeling, triggers endothelial dysfunction and promote negative impact of the dyslipidemia on the vasculature. The bromodomain and extra terminal (BET) proteins were first proposed as anticancer drugs and

then cardio and vascular protective effects were found. The aim of the mini review is summarize knowledge for BET protein inhibitors in prevention of poor clinical outcomes in patients with atherosclerosis and CAD. The results of pool analysis of some clinical trials, such as ASSERT, ASSURE-I, SUSTAIN, are disputed. It has been shown positive effects of apabetalone on the levels of Apo A-I and high-density lipoprotein cholesterol that was associated with significant declining of the systemic and local inflammatory reaction. There is evidence regarding that the apabetalone induced reverse of the atherosclerotic plaque volume.

Key words: Atherosclerosis; Coronary artery disease; Dyslipidemia; inflammation; Bromodomain and extra terminal proteins

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INTRODUCTION

Atherosclerosis is a leading cause of vascular remodeling that relates to cardiovascular (CV) complications, such as acute coronary syndrome (ACS), myocardial infarction (MI), stroke, and other major cardiovascular events (MACEs), which are associated with a high rate of morbidity and mortality^[1]. It has been established that atherosclerosis is maladaptive process encompassing systemic, microvascular and in-plaque inflammation, altered immune response, dyslipidemia and lipid infiltration of sub-intima, endothelial dysfunction and impaired vascular repairment^[2,3]. Activated macrophages play a pivotal role in atherosclerosis progression through initiating local inflammatory reaction, maintaining foam cells formation, triggering plaque cap instability and rupture, as well as supporting subsequent endothelial dysfunction and coagulative potency^[4,5]. The numerous molecular mechanisms including TRIM59, SMAD, Janus Kinase (JNK) / Signal Transducer and Activator of Transcription (STAT) signaling pathway and PI3K/Akt, mitogen activated protein kinase and AMPK transduction mechanisms, which ensure a transformation of normal tissue-

Table 1 Clinical studies dedicated an efficacy, safety and tolerability of apabetalone

Trial acronym	Full name	Study population	Treatment	Concomitant medication	The primary efficacy parameter
ASSERT	Clinical Trial for Dose Finding and Safety of RVX000222 in Subjects With Stable Coronary Artery Disease	299 patients with CAD	RVX-208 100-300 mg bid or placebo for 12 weeks	Statins	percent change in ApoA1 from baseline to 12 weeks post-randomization, and comparison of Compare the dose and time response relationships for major lipids (ApoA1, total cholesterol, HDL-C, LDL-C, non-HDL-C, TG, ApoB, LDL, and HDL-subclasses) over 4, 8 and 12 weeks' time course
ASSURE-I	The ApoA-I Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation	310 patients with angiographic coronary artery disease and low HDL-C levels	RVX-208 100 mg bid or placebo for 26 weeks	Statins	the nominal change in percent atheroma volume
SUSTAIN	Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation	172 with low HDL levels	RVX-208 100 mg bid or placebo for 24 weeks	Statins	percentage change in HDL-C levels
BETonMACE	Effect of RVX000222 on Time to Major Adverse Cardiovascular Events in High-Risk T2DM Subjects With CAD	2425 patients with CAD and diabetes mellitus	RVX-208 100 mg bid or placebo for 120 weeks	Intensive statin therapy	MACEs

Abbreviations: ApoA1, apo-lipoprotein A1, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; ApoB, apo-lipoprotein B; CAD, coronary artery disease.

resident mononuclear-derived macrophages into atherosclerotic macrophages with various phenotypes (M1 and M2), are under close epigenetic control^[6-8]. The histones' acetylation is one of core element in the epigenetic regulation of post-processing transcription, which is realized by gene expression of the bromodomain and extra terminal (BET) proteins^[9]. At physiological condition BET proteins occupy promoter sites on chromatin supporting an expression of housekeeping genes that ensure cell growth, differentiation, and metabolism^[10]. At pathophysiological conditions pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha, mediate translocation of transcription factor nuclear factor kappa-B (NF-κB) from cytoplasm to the nucleus with further binding to cis-regulatory regions of DNA^[11]. The next step of DNA activation is p65 subunit-dependent acetylation of NF-κB RelA complex resulting in a recruitment of the BRD4 from housekeeping genes, chromatin remodeling, and modification of transcription activity through cell cycle regulation of positive transcription elongation factor (P-TEFb) component stimulates RNA polymerase II-dependent transcription^[12,13]. Overall, *in vitro* studies have been revealed that the BET proteins (predominantly BRD4) were powerful epigenetic co-activators of inflammatory response and atherogenesis via involvement in the JNK / STAT5 transcriptional activity^[14,15].

First BET protein inhibitors were developed as anticancer drugs that have exhibited an ability not only to suppress expression of many oncogenic proteins (c-MYC and cyclin D1) by interplay with histone deacetylases, but upregulate pro-apoptotic proteins and down-regulate anti-apoptotic proteins (i.e., surviving)^[16,17]. New era in clinical implementation of the BET protein inhibitors have become after description of the role of key elongation factors, such as P-TEFb, in pathogenesis of microvascular inflammation and atherosclerosis^[18]. Moreover, the results of CANTOS trial have ascertained that directly targeting inflammation could be therapeutic strategy in CV diseases, because this approach was associated with MACEs reduction^[19]. The aim of the mini review is summarize knowledge for BET protein inhibitors in prevention of poor clinical outcomes in patients with atherosclerosis and coronary artery disease (CAD).

AN IMPACT OF BET INHIBITORS ON SURROGATE END POINTS IN CAD

Apabetalone (RVX-208) is a selective BET inhibitor which

modulates lipid and inflammatory pathways implicated in CAD^[20]. There is wide range of evidence regarding that the apabetalone having an ability to regulate inflammation-driven gene expression has ensured down-regulated expression of adhesion molecules (ICAM-1, VCAM-1, PECAM, and E-selectin), lowered endothelial cell-to-cell activation, suppressed macrophage inflammatory responses, declined circulating levels of thrombin, C-reactive protein, TNF-alpha, IL-1beta, activated complement fragments C5a, C3b, and C5b-C6, and decreased sub-intimal oxidized lipids accumulation in atherosclerosis^[21-23].

Administration of apabetalone in the ASSERT (Clinical Trial for Dose Finding and Safety of RVX000222 in Subjects With Stable Coronary Artery Disease) study for 12 weeks led to increase in the levels of apo A-I, HDL cholesterol, and large HDL particles^[24]. Thus, apabetalone appeared to be an effective oral inducer of apolipoprotein A-I synthesis, the potent capability of which in CAD statin-treated patients had been established in three respectively small clinical trials (Table 1). The results of the ASSURE (ApoA-1 Synthesis Stimulation and intravascular Ultrasound for coronary atheroma Regression Evaluation) trial have revealed that BET inhibitor apabetalone has demonstrated a significant reduction in coronary atherosclerotic plaque length and that the changes in plaque structure were strongly associated with on-treatment concentration of high-density lipoprotein (HDL) particles, but not HDL cholesterol or apolipoprotein A-I^[25]. Apabetalone has exhibited an ability to stimulate an expression of apolipoprotein AI gene that was associated with atherosclerotic plaque volume regression through improvements in the plasma HDL profile in the SUSTAIN (Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation) study^[26,27].

In general, apabetalone was well tolerated, and the treatment with apabetalone did not impact on the total rate of infections or infestations in patients, as well as transient and reversible elevations in liver transaminases 3 times and more the upper limit of normal ranges were found rarely and did not relate to bilirubin elevation^[28].

BET INHIBITORS FOR LONG-TERM PROGNOSIS OF CV DISEASES AND EVENTS

Recently performed pool analysis of three previously performed clinical trials dedicated of apabetalone administration in comparison

with placebo has yielded significant decrease in MASEs (5.9% versus 10.4%; $p = 0.02$)^[29]. Moreover, this effect was found to be prominent in patients with diabetes mellitus (5.4% versus 12.7%; $p = 0.02$), with baseline HDL cholesterol less than 39 mg/dl (5.5% versus 12.8%; $p = 0.01$), and with elevated high-sensitivity CRP levels (5.4% versus 14.2%; $p = 0.02$)^[29]. Ongoing phase III BETonMACE (Effect of RVX000222 on Time to Major Adverse Cardiovascular Events in High-Risk T2DM Subjects With CAD, NCT02586155) clinical trial ($n = 2425$) that is currently being investigated to elucidate whether apabetalone may have serious clinical implications in diabetic patients with CAD. High intensity statin therapy is determined as co-medication for this study. MACEs are primary clinical outcomes and several biomarkers, such as CRP, alkaline phosphatase, fasting glucose, are considered as secondary outcomes to assay safety and tolerability. As it is expected the trial may help receiving strong evidence regarding of viability of new conception of the treatment of CAD and atherosclerosis. However, the patients who are being enrolled in the BETonMACE have to have established CAD according to prior myocardial infarction, percutaneous coronary intervention or visualization procedure confirmation. Therefore, diagnosis of diabetes mellitus should be verified according to new criteria of ADA. Finally, it is not clear whether patients with diabetes mellitus and CAD having contra-indications to intensive statin therapy will not be able to enroll in the study due to sophisticated study design and protocol and that it will not be influence on the frequency of MACEs in the active treatment group.

CONCLUSIONS

Early clinical studies have shown positive effects of apabetalone on the levels of Apo A-I and HDL cholesterol that was associated with significant declining of the systemic and local inflammatory reaction. There is evidence regarding that the apabetalone induced reverse of the atherosclerotic plaque volume. Whether this approach based on anti-inflammatory properties of apabetalone will be represented as potential brand new strategy to reduce CV risk is not clear and this assumption is addressed to the on-going BETonMACE study.

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