Apabetalone (RVX-208) inhibits key drivers of vascular inflammation, calcification, and plaque vulnerability through a BET-dependent epigenetic mechanism

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ABSTRACT

Apabetalone (RVX-208) is an orally available small molecule bromodomain & extraterminal (BET) protein inhibitor that targets the second bromodomain (BD2) of BET proteins. Apabetalone returns dysregulated BET-dependent transcription toward normal physiological levels. In phase 2 trials, apabetalone treatment reduced the incidence of major adverse cardiac events by 44% in CVD patients and by 57% in diabetic CVD patients. Previous studies have highlighted apabetalone's positive impact on vascular calcification (VC) and inflammation (VI) marker expression in vitro, as well as its ability to lower serum alkaline phosphatase (ALP) levels, and improve atherothrombotic plaque stability parameters in treated patients. In CVD, elevated inflammatory mediators and cell surface adhesion molecules drive VI, resulting in leukocyte adhesion, infiltration, uptake of oxLDL, and ultimately plaque formation. Here we show in vitro that TNFα monocyte adhesion to human aortic endothelial cells (HAECs) increases with TNFα stimulation and is attenuated by apabetalone treatment, with fewer monocytes attaching to HAECs under flow conditions. This functional outcome is attributed to apabetalone’s reduction of key endothelial adhesion genes, VCAM-1 (50%, p=0.0001) and SELE (37%, p=9 x 10⁻²). Apabetalone also prevents TNFα induction of pro-atherogenic endothelial recruitment genes (MCP-1, 75%, p=0.0002) and genes involved in plaque rupture (ILB, 24%, p=2x10⁻¹). Basal HAEC ALP expression, a potential contributor to endothelial dysfunction and VI, also decreases with apabetalone treatment (70%, p=0.005). Induction of VI genes by TNFα is BET-dependent as degradation of BET proteins by MZ-1 prevents an increase in transcripts in response to TNFα treatment. Ingenuity® Pathway Analysis (IPA®), GSEA, and GO analysis of HAEC gene expression data predicts apabetalone inhibition of proatherogenic pathways, gene sets, and upstream regulators induced by TNFα. These include cytokine and chemokine, Toll-Like Receptor (TLR), NFκB, interferon and TNFα signaling. In addition, IPA® disease and biological function analysis predicts inhibition of immune cell activation and recruitment by apabetalone. Plasma proteomics (SOMAscan®) and IPA® analysis from apabetalone-treated CVD patients in ASSERT and ASSURE phase 3 trials indicate that apabetalone inhibits pro-atherogenic upstream regulators (IL-6 and IFNγ), canonical pathways, and diseases and functions. Serum ALP also decreases dose-dependently with apabetalone treatment (ASSERT). Epigenetic inhibition of VI and VC driven atherogenesis likely contributes to the reduction in MACE observed in phase 2 apabetalone treated patients. The ongoing phase 3 post-acute coronary syndrome (ACS) clinical trial in T2DM patients, BETonMACE, is currently testing this APABETALONE INHIBITION OF PRO-ATHEROGENIC GENES IN ENDOTHELIAL CELLS IN A BET-DEPENDENT MANNER BY REDUCING BRD4 OCCUPANCY AT GENE LOCI

**Bioinformatics of HAEC RNA-seq data:**

- **IPA® Analysis of Phase II Trial Plasma Proteome (SOMAscan®)**
  - Activation score: increased (yellow); decreased (blue)
  - p-value: increased (yellow); decreased (blue)
  - Disease and Function Analysis: increased (yellow), decreased (blue)

- **Upstream Regulator Analysis:**
  - Activation score: increased (yellow); decreased (blue)
  - p-value: increased (yellow); decreased (blue)

**Betependency demonstrated by:**
1. MZ-1 and BETi inhibition of expression
2. Loss of BRD4 occupancy on MCP-1 locus

**Bioinformatics of HAEC RNA-seq data:**

**GSEA and Gene Ontology (GO) Analysis**

**APABETALONE INHIBITION OF PRO-ATHEROGENIC GENES IN ENDOTHELIAL CELLS IN A BET-DEPENDENT MANNER BY REDUCING BRD4 OCCUPANCY AT GENE LOCI**

**Apabetalone inhibits alkaline phosphate expression in HAEC**

**Apabetalone suppresses monocyte adhesion to endothelial cells**

**Apabetalone inhibits pro-atherogenic gene expression in endothelial cells in a BET-dependent manner by reducing BRD4 occupancy at gene loci**

**SUMMARY**

1. Apabetalone inhibits the expression of pro-atherogenic genes in a BET-dependent manner resulting in the inactivation of pathologic pathways and regulators.
2. BET-dependent downregulation of vascular inflammation, cell adhesion, and ALP by apabetalone may contribute to the reduced incidence of MACE (phase 2), a hypothesis currently being tested in the phase 3 cardiovascular outcomes trial, BETonMACE.