Inhibition of BET Proteins with Apabetalone Reduces Mediators of Vascular Calcification In Vitro and in CKD Patients

Dean Gilham1, Laura Tsujikawa3, Sylwia Wasiak1, Chris Halliday1, Chris Sarsons1, Stephanie Stotz1, Kamyar Kalantar-Zadeh2, Ravi Jahagirdar1, Jan O. Johansson3, Norman CW Wong4, Mike Sweeney2, Richard Robson5, Ewelina Kulikowska1

Reverslogix Corp. 1Calgary, Canada and 3San Francisco, USA, 2University of California, Irvine, USA, 4Christchurch Clinical Studies Trust, Christchurch, New Zealand

Abstract

INTRODUCTION: Bromodomain and extranuclear (BET) proteins, such as BRD4, module gene expression by binding acetylated histones & transcription factors with transcriptional regulators. Apabetalone, an orally active BET inhibitor, reduced the incidence of major adverse cardiac events (MACE) in patients with CVD and improved eGFR in a subgroup with chronic kidney disease (CKD) in phase 2 trials. In CKD patients, vascular calcification (VC) increases VC risk & is a predictor of all-cause mortality. Because VC is associated with MACE, effects of BET inhibition on processes associated with VC were explored.

METHODS: Proteomic profiling of plasma was conducted in CVD patients receiving apabetalone in the 3 month (ASSERT) and 6 month (SUSTAIN & ASSURE) phase 2 trials, as well as in patients with stage 4/5 CKD receiving a single dose in a phase 1 pharmacokinetic study. Human coronary artery vascular smooth muscle cells (CASMVCs) were used to assess expression of VC markers, transdifferentiation in osteogenic conditions, and extracellular mineralization that leads to pathology. ChIP-seq examined BRD4 assembly on chromatin during osteogenic transdifferentiation and the effects of apabetalone.

RESULTS: Apabetalone reduced circulating levels of proteins associated with VC in phase 2 trials in CVD patients including osteopontin, osteoprotegerin, & alkaline phosphatase (ALP). Proteomic assessment of plasma from CKD patients vs matched controls demonstrated activation of molecular pathways driving VC including BMP-2 signaling and RANK signaling in osteoclasts. Both pathways were downregulated by apabetalone 12 hours post dose in the CKD cohort. Mechanistic understanding of apabetalone were examined in vitro. Transdifferentiation of CASMVCs with osteogenic conditions induced expression of ALP, RUNX2, MGP, & WNT5A, which was suppressed by apabetalone. Further, apabetalone dose dependently countered extracellular calcium deposition. Compared to basal conditions, transdifferentiation to a calcifying phenotype promoted re-distribution of extracellular calcium deposition. Compared to basal conditions, several of which were in proximity to genes associated with calcification or atherosclerosis. Apabetalone dose dependently reduced levels of BRD4 on many of these enhancers, which correlated with decreased expression of the associated gene. Genome wide, apabetalone decreased the size of BRD4 containing enhancers, consistent with its mechanism of action.

Mechanism of Action

1. Apabetalone decreases circulating levels of proteins associated with VC in patients with cardiovascular disease

2. Phase I, Safety & PK Study: Subjects with Severe Renal Impairment (CKD), 12 hours post single 100mg dose (n=8)

3. Apabetalone opposes induction of osteogenic markers in human coronary artery vascular smooth muscle cells

4. Apabetalone suppresses extracellular calcium deposition in human CASMVCs

5. ChIP-seq identifies fewer BRD4-rich enhancers in CASMVCs cultured in osteogenic vs basal conditions. Apabetalone reduces number (table) & size (graphs) of enhancers, consistent with displacement of BRD4 from chromatin

6. ChIP-seq identifies genes associated with BRD4-rich enhancers

7. Increased expression of genes with BRD4-rich enhancers. Apabetalone reduces levels of BRD4, which correlates with decreased gene expression

Results: In Vitro

In vitro, apabetalone interferes with transdifferentiation of CAVSMCs with osteogenic conditions induced expression of ALP, RUNX2, MGP, & WNT5A, which was suppressed by apabetalone. Further, apabetalone dose dependently countered extracellular calcium deposition. Compared to basal conditions, transdifferentiation to a calcifying phenotype promoted re-distribution of extracellular calcium deposition. Compared to basal conditions, several of which were in proximity to genes associated with calcification or atherosclerosis.

Apabetalone dose dependently reduced levels of BRD4 on many of these enhancers, which correlated with decreased expression of the associated gene. Genome wide, apabetalone decreased the size of BRD4 containing enhancers, consistent with its mechanism of action.

Summary and Conclusions

In clinical trials, apabetalone mediated reduced risk of end points & pathways associated with vascular calcification (VC).

- Apabetalone reduced expression of markers of transdifferentiation & genes that promote calcification of CASMVCs.

- Apabetalone reduced the number and size of BRD4-rich enhancers, consistent with displacement of BRD4 from chromatin.

- BRD4 ChIP-seq identified unique genes associated with CASMVC transdifferentiation & calcification.

- Inhibition of BRD4 in CASMVC transdifferentiation & calcification is a novel discovery.

- The impact of BRD4 on biomarkers, renal function, and CVD outcomes in patients with impaired kidney function is being evaluated in the phase 3 BETonMACE trial.