Apabetalone Downregulates Factors and Pathways Associated with Vascular Calcification

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Abstract

Background: Apabetalone, an oral small molecule BET inhibitor, reduced the incidence of major adverse cardiac events (MACE) in patients with CVD and improved eGFR in a subgroup with CDK in phase 2 trials. Because vascular calcification (VC) is associated with MACE, effects of apabetalone on processes associated with VC were examined.

Methods: Plasma proteomic analysis was conducted in CVD patients receiving 200mg of apabetalone daily in the 3 month (ASSERT) and 6 month (SUSTAIN & ASSURE) phase 2 trials, as well as patients with stage 4/5 CDK that received a single 100mg dose. Co-vascular artery VSMCs were used to examine effects of apabetalone on transdifferentiation & calcium deposition.

Results: Apabetalone significantly reduced circulating levels of VC markers in CVD patients in phase 2 trials, including alkaline phosphatase, osteopontin and osteoprotegerin. Plasma proteomics of CKD patients (n=8) indicated activation of molecular pathways driving VC including IL-6 signaling, BMP-2 signaling & RANK signaling in osteoclasts. Downregulation of these pathways by apabetalone was predicted in the CKD cohort 12hrs post-dose.

In VSMCs cultured in osteogenic conditions, apabetalone opposed induction of transdifferentiation markers & inhibited calcium deposition. BRD4 is a transcriptional regulator & target of apabetalone. ChIP-seq showed transdifferentiation of VSMCs to a calcifying phenotype promoted re-distribution of BRD4 on chromatin, resulting in fewer BRD4-rich enhancers (118 in osteogenic, 288 in basal). 38 genes were uniquely associated with BRD4-rich enhancers in osteogenic vs. basal conditions; several of the genes have been linked to calcification. Apabetalone reduced BRD4 on many of these enhancers, which correlated with decreased gene expression. Bioinformatics indicated BRD4 may cooperate with specific transcription factors to promote calcification.

Conclusions: Involvement of BRD4 in VSMC transdifferentiation & calcification is a novel discovery. Further assessment of apabetalone as a therapeutic for VC is warranted. The impact of apabetalone on biomarkers, renal function & CVD outcomes in patients with impaired kidney function is being evaluated in a subgroup of the phase 3 BETonMACE trial.

Mechanism of Action

BET proteins, such as BRD4, bind acetylated lysine (ac) on histones or transcription factors (TF) via bromodomains (BD), and recruit transcriptional machinery to drive expression of BET sensitive genes. Apabetalone targets bromodomains in BET proteins, causing release from chromatin and downregulation of BET sensitive gene expression.

Yellow star size: selectivity of apabetalone for BD2

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Results: CVD or CKD Patients in Phase 1 or 2 Clinical Trials

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

2. Plasma proteomic analysis demonstrates pathways associated with VC are elevated in patients with stage 4S/5 CDK vs. matched controls. Apabetalone downregulates these pathways.

Phase 1 Safety & PK: Subjects with severe renal impairment (CDK); 12 hours post single 100mg dose (n=8)

Bioinformatics (IPA) Analysis of the Plasma Proteome (SOMAscan™)

Pathways associated with VC are elevated in CVD vs. matched controls at baseline 

Pathway Regulation in CVD vs. controls P-value Response to apabetalone in CVD patients P-value

IL-6 signaling IPA z-score: +2.12 9.0x10-10 IPA z-score: -3.46 2.9x10-10

BMP signaling pathway IPA z-score: +2.12 6.1x10-10 IPA z-score: -2.45 2.7x10-10

RANK signaling in osteoclasts IPA z-score: +1.89 3.3x10-10 IPA z-score: -2.65 1.8x10-10

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

6. A) Number of genes associated with BRD4-rich enhancers B) Increased expression with BRD4-rich enhancers; apabetalone reduces BRD4, which correlates with decreased gene expression.

Conclusion: Apabetalone reduced circulating proteins & pathways associated with vascular calcification (VC).

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

Involvement of BRD4 in VSMC transdifferentiation & calcification is a novel discovery.

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

The impact of apabetalone on biomarkers, renal function, and CVD outcomes in patients with impaired kidney function is being evaluated in the phase 3 BETonMACE trial (ClinicalTrials.gov Identifier: NCT02586155).

Summary and Conclusions

- In clinical trials, apabetalone reduced circulating proteins & pathways associated with vascular calcification (VC).
- Apabetalone reduced expression of markers of transdifferentiation & genes that promote calcification of VSMCs.
- Involvement of BRD4 in VSMC transdifferentiation & calcification is a novel discovery.
- Apabetalone reduced number & size of BRD4-rich enhancers, consistent with BRD4 displacement from chromatin.
- The impact of apabetalone on biomarkers, renal function, and CVD outcomes in patients with impaired kidney function is being evaluated in the phase 3 BETonMACE trial (ClinicalTrials.gov Identifier: NCT02586155).

- Legend: Genes with links to calcification or atherosclerosis with BRD4-rich enhancers in osteogenic conditions (red font) were measured for expression by real-time qPCR. The BRD4 CHIP-seq (black dots) corresponding to the chosen genes were measured by bioinformatics analysis of the plasma proteome (SOMAscan).