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FP294 INHIBITION OF BET PROTEINS WITH APABETALONE REDUCES MEDIATORS OF VASCULAR CALCIFICATION IN VITRO AND IN CKD PATIENTS

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INTRODUCTION AND AIMS: Bromodomain and extraterminal (BET) proteins, such as BRD4, modulate gene expression by bridging 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 subpopulation with chronic kidney disease (CKD) in phase 2 trials. In CKD patients, vascular calcification (VC) increases CVD risk & is a predictor of all-cause mortality. Because VC is associated with MACE, effects of BET inhibition on processes associated with VC were examined.

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 (VSMCs) were used to assess expression of VC markers, trans-differentiation in osteogenic conditions, and extracellular mineralization that leads to pathology. ChIP-seq examined BRD4 assembly on chromatin during osteogenic trans-differentiation 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 (OPG), & 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 effects of apabetalone were examined in vitro. Trans-differentiation of VSMCs with osteogenic conditions induced expression of ALP, OPG, RUNX2 & WNT5A, which was suppressed by apabetalone. Further, apabetalone dose dependently countered extracellular calcium deposition. Compared to basal conditions, trans-differentiation to a calcifying phenotype promoted re-distribution of BRD4 on chromatin, resulting in fewer enhancers (118 in osteogenic, 288 in basal). 38 unique enhancers were generated in osteogenic conditions, several of which were in proximity to genes associated with calcification. 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.

CONCLUSIONS: In clinical trials, apabetalone mediates reduction of factors & pathways associated with VC. Involvement of BRD4 in VSMC trans-differentiation & calcification is a novel discovery. BRD4 ChIP-seq identified novel factors associated with trans-differentiation, and thus potential targets to oppose VC. Inhibition of BRD4 by apabetalone resulted in fewer BRD4 containing enhancers, and reduced expression of genes that promote (a) trans-differentiation & (b) extracellular calcium deposition. The impact of chronic treatment with apabetalone on biomarkers, renal function and CVD outcomes in patients with impaired kidney function is being studied in the phase 3 BETonMACE outcomes trial.