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

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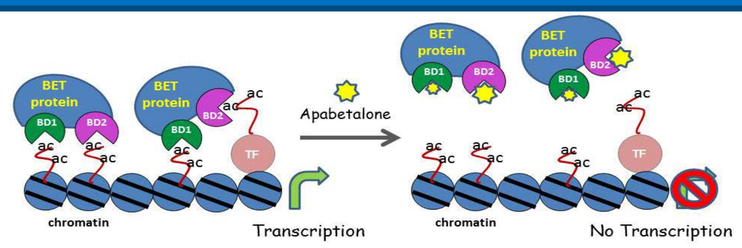
Abstract

INTRODUCTION: 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 (CAVSMCs) 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 effects of apabetalone were examined in vitro. 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 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 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



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

Acknowledgment: Contributions to this figure by Dr. Eric Campeau, Dr. Olesya Kharenko & Dr. Sylwia Wasiak

Results: Clinical Trials

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

*p<0.05, **p<0.01,***p<0.001 vs placebo (Van Elteren test adjusted by study)

Phase 2, Placebo Controlled, Double Blinded Trials: Patients with Cardiovascular Disease on Standard of Care

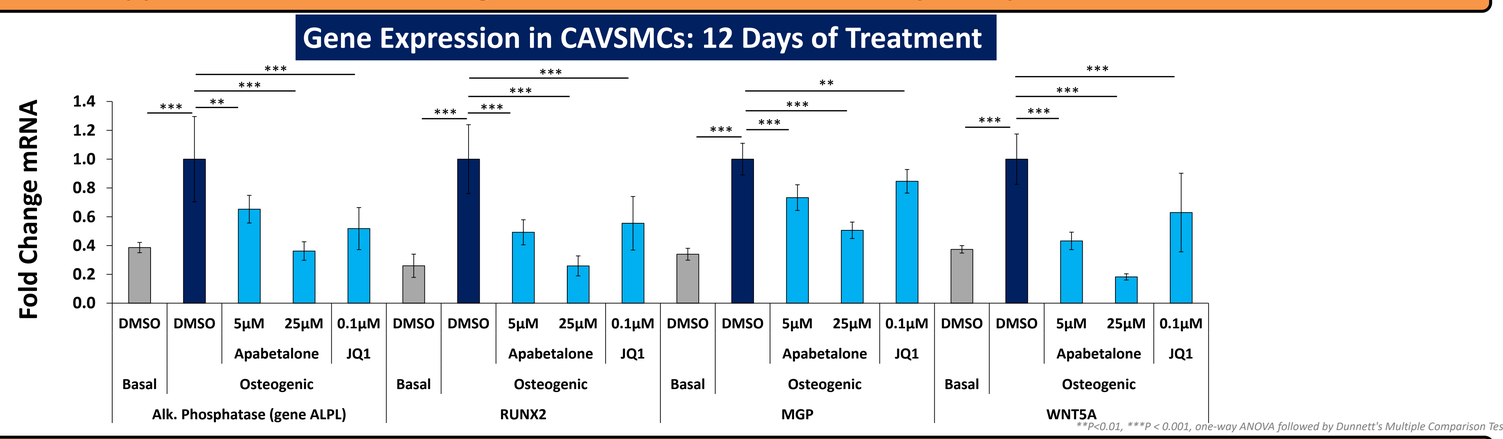
2. Plasma proteomics show pathways associated with VC are elevated in patients with stage 4 or 5 CKD versus matched controls. Apabetalone downregulates these pathways in CKD patients

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

Bioinformatics (IPA) Analysis of the Plasma Proteome (SOMAscan™)				
Pathways associated with VC are elevated in CKD vs matched controls at baseline			Apabetalone downregulates pathways associated with VC in CKD patients 12 hr post-dose	
Pathway	**Regulation in CKD vs controls**	***P*-value**	**Response to apabetalone in CKD patients**	***P*-value**
BMP signaling pathway	IPA z-score: +2.12	0.0000062	IPA z-score: -2.67	0.000000102
RANK signaling in osteoclasts	IPA z-score: +1.89	0.00033	IPA z-score: -4.36	3.53×10^{-10}
z-score negative = downregulation ; z-score positive = upregulation			no modulation of either pathway by apabetalone in control subjects.	

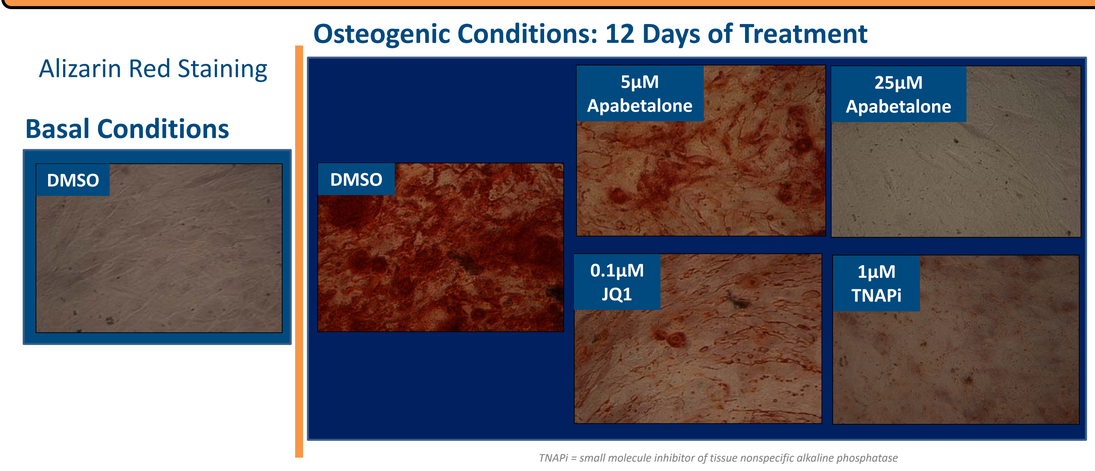
Results: *In Vitro*

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

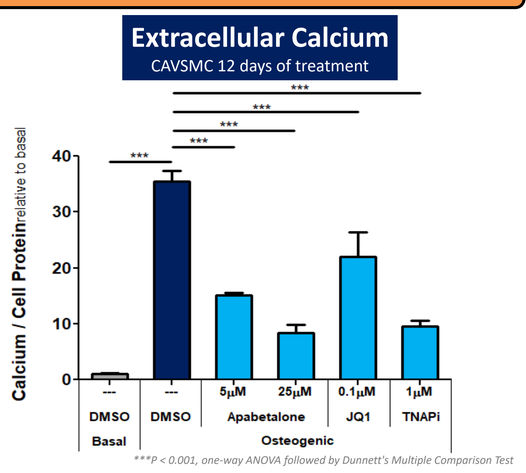


**P<0.01, ***P < 0.001, one-way ANOVA followed by Dunnett's Multiple Comparison Test

4. Apabetalone suppresses extracellular calcium deposition in human CAVSMCs



TNAPi = small molecule inhibitor of tissue nonspecific alkaline phosphatase

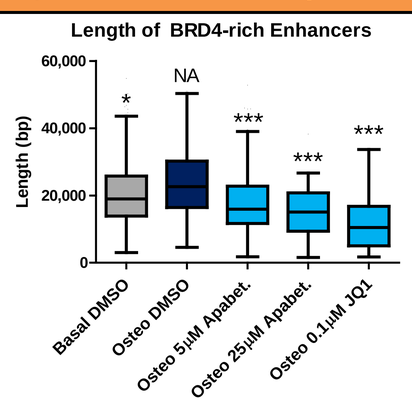


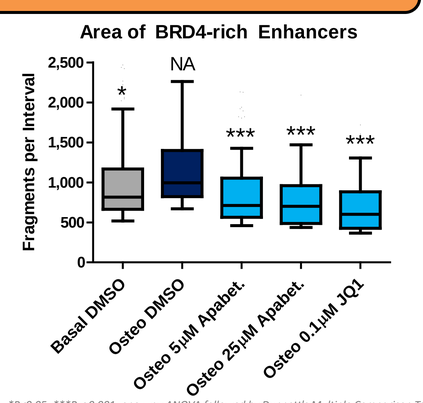
***P < 0.001, one-way ANOVA followed by Dunnett's Multiple Comparison Test

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

# of BRD4-rich Enhancers				
Basal	**Osteogenic**			
		Apabetalone		**JQ1**
DMSO	**DMSO**	**5μM**	**25μM**	**0.1μM**
288	188	92	44	57

Method: CAVSMCs were cultured for 12 days in basal or osteogenic conditions ± apabetalone or comparator BET inhibitor JQ1. BRD4 ChIP-seq identified the number, genomic location, and size of BRD4-rich enhancers.

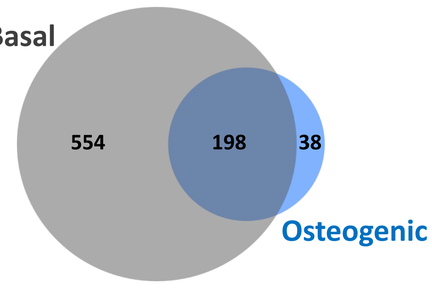




*P<0.05, ***P < 0.001, one-way ANOVA followed by Dunnett's Multiple Comparison Test

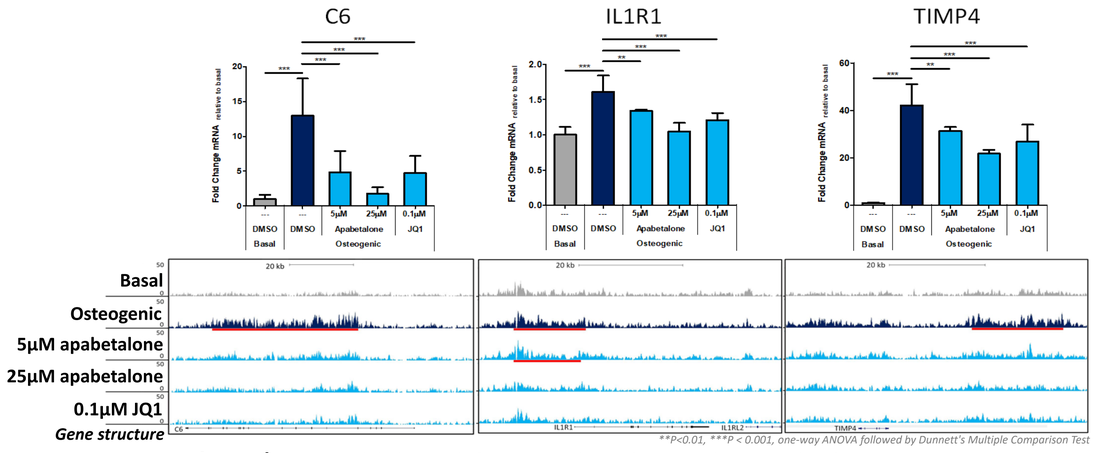
6. ChIP-seq identifies genes associated with BRD4-rich enhancers

VENN diagram: Number of genes within 25,000bp of BRD4-rich enhancers in CAVSMC



Legend: 38 genes were associated with BRD4-rich enhancers in osteogenic, but not basal conditions, including C6, IL1R1 and TIMP4 (see section 7).

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



**P<0.01, ***P < 0.001, one-way ANOVA followed by Dunnett's Multiple Comparison Test

Legend: Genes with links to calcification or atherosclerosis with BRD4-rich enhancers in osteogenic conditions (red lines) were examined for expression by real-time PCR (top) BRD4 ChIP-seq tracks (bottom).

Summary and Conclusions

- In clinical trials, apabetalone mediated reduction of factors & pathways associated with vascular calcification (VC).
- Apabetalone reduced expression of markers of transdifferentiation & genes that promote calcification of CAVSMCs.
- Apabetalone reduced the number and size of BRD4-rich enhancers, consistent with displacement of BRD4 from chromatin.
- BRD4 ChIP-seq identified 38 unique genes associated with CAVSMC transdifferentiation and calcification.
- Involvement of BRD4 in CAVSMC transdifferentiation & calcification is a novel discovery.
- 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.