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 CKD in phase 2 trials. Because vascular calcification (VC) is associated with MACE, effects of apabetalone on processes associated with VC were examined.

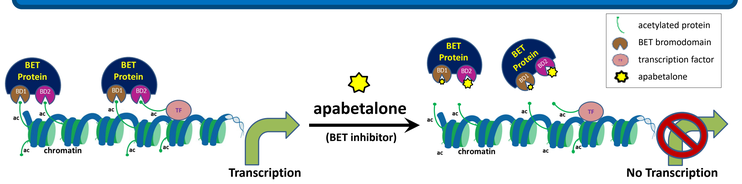
Methods: Plasma proteomic analysis was conducted in CVD patients receiving 200mg of apabetalone daily in the 3 month (ASSERT) and 6 month (SUSTAIN & ASSURE) phase 2 trials, as well as patients with stage 4/5 CKD that received a single 100mg dose. Coronary artery VSMCs were used to examine effects of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



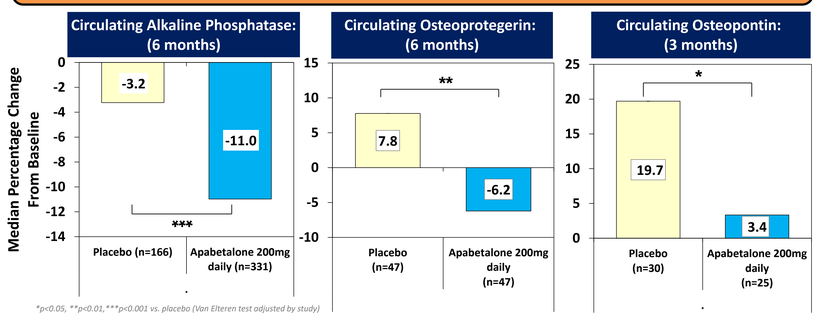
BET proteins, such as BRD4, bind acetylated lysine (ac) on histones or transcription factors (TF) via bromodomains (BD), and recruit transcriptional machinery to drive expression of BET sensitive genes. Apabetalone targets bromodomains in BET proteins, causing release from chromatin and downregulation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

Yellow star size: selectivity of apabetalone for BD2

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

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.



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

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

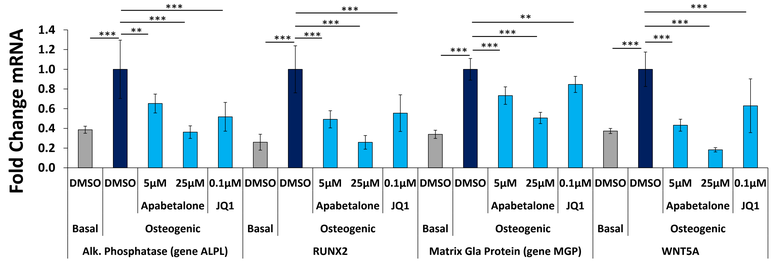
Phase I Safety & PK : Subjects with severe renal impairment (CKD); 12 hours post single 100mg dose (n=8)

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 hours post-dose	
Pathway	**Regulation in CKD vs. controls**	***P*-value**	**Response to apabetalone in CKD patients**	***P*-value**
IL-6 signaling	IPA z-score: +3.21	$9.0x10^{-10}$	IPA z-score: -3.46	$2.9x10^{-8}$
BMP signaling pathway	IPA z-score: +2.12	$6.1x10^{-6}$	IPA z-score: -2.45	$2.7x10^{-4}$
RANK signaling in osteoclasts	IPA z-score: +1.89	$3.3x10^{-4}$	IPA z-score: -2.65	$1.8x10^{-4}$
z-score positive = upregulation ; z-score negative = downregulation			no modulation of these pathway by apabetalone in control subjects	

Results: Cell Studies

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

Gene Expression in VSMCs: 12 Days of Treatment

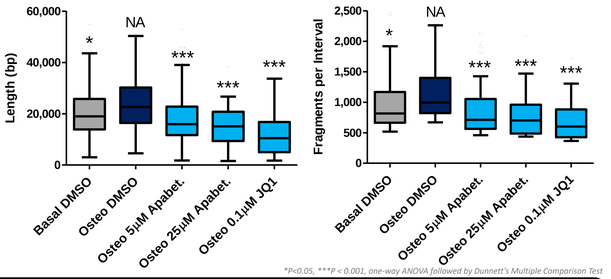


**P<0.01, ***P < 0.001, one-way ANOVA followed by Dunnett's Multiple Comparison Test

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.

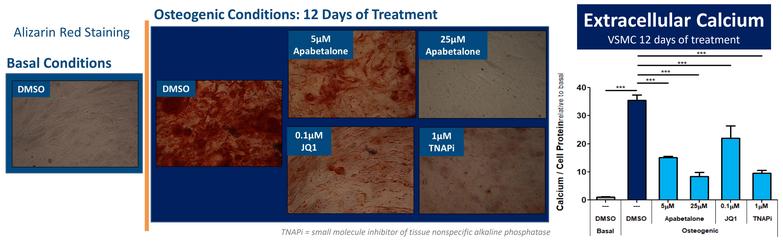
# of BRD4-rich Enhancers				
Basal	Osteogenic			
		Apabetalone		JQ1
DMSO	DMSO	5μM	25μM	0.1μM
288	188	92	44	57

Method: VSMCs were cultured for 12 days in basal or osteogenic conditions with apabetalone or comparator BET inhibitor JQ1. BRD4 ChIP-seq identified the number, length, and area of BRD4-rich enhancers.



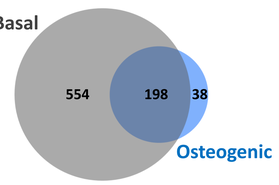
*P<0.05, ***P < 0.001, one-way ANOVA followed by Dunnett's Multiple Comparison Test

4. Apabetalone suppresses extracellular calcium deposition in human coronary artery vascular smooth muscle cells.



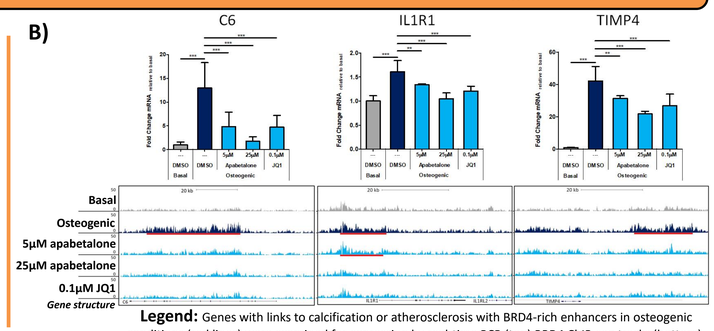
6. A) Number of genes associated with BRD4-rich enhancers B) Increased expression with BRD4-rich enhancers; apabetalone reduces BRD4, which correlates with decreased gene expression.

A) VENN diagram: Number of genes within 25,000bp of BRD4-rich enhancers in VSMC.



38 genes were associated with BRD4-rich enhancers in osteogenic, but not basal conditions, including C6, IL1R1 and TIMP4.

B)



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).

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

7. Transcription factors (TF) that may cooperate with BRD4 to promote calcification.

	5μM Apabetalone		25μM Apabetalone		0.1μM JQ1	
TF / Upstream Regulator	**IPA *p*-value**	**TF motif e-value**	**IPA *p*-value**	**TF motif e-value**	**IPA *p*-value**	**TF motif e-value**
SMAD4	<0.05	$2.5x10^{-24}$	<0.001	$1.1x10^{-44}$	<0.01	$3.0x10^{-29}$
Smad2/3-Smad4	<0.01	$2.5x10^{-24}$	<0.01	$1.1x10^{-44}$	<0.01	-
FOS	<0.01	$9.3x10^{-25}$	<0.01	-	<0.001	$2.0x10^{-51}$
ATF3	<0.05	$5.9x10^{-36}$	<0.05	-	-	$4.4x10^{-126}$
TWIST2	<0.05	$1.3x10^{-35}$	<0.01	$2.1x10^{-61}$	<0.05	$2.8x10^{-51}$
TBX2	<0.01	$8.9x10^{-29}$	<0.01	$1.8x10^{-24}$	-	$5.8x10^{-50}$
MEIS1	-	-	<0.05	$5.9x10^{-26}$	-	$2.2x10^{-27}$

Legend: Bioinformatic analysis of BRD4 ChIP-seq revealed TFs that associate with BETi sensitive BRD4 assemblies on chromatin in osteogenic conditions. De novo motif discovery analysis and Ingenuity Pathway Analysis® were applied. Seven TFs were identified by both methods; all except MEIS1 have previously been linked to calcification. Disruption of BRD4-TF associations with BETi could regulate expression of genes that drive transdifferentiation & calcification.

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.
- BRD4 ChIP-seq identified 38 unique genes associated with VSMC transdifferentiation and calcification.
- Bioinformatic analysis of BRD4 ChIP-seq indicate 7 TFs may cooperate with BRD4 to promote VSMC calcification.
- 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).