Apabetalone Downregulates Alkaline Phosphatase and Improves Cardiovascular Risk

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Abstract

Background: Apabetalone is an inhibitor of BET proteins - epigenetic readers modulating gene expression. In phase 2 trials, apabetalone reduced major adverse cardiac events (MACE) in patients with cardiovascular disease (CVD) & improved eGFR in those with chronic kidney disease (CKD). Elevated serum alkaline phosphatase (ALP) is a risk factor for MACE, as it contributes to inflammation, vascular calcification & endothelial dysfunction. We examined apabetalone-mediated effects on ALP in CVD patients post-hoc & determined apabetalone's impact on tissue non-specific ALP (TNAP) expression in cell culture systems.

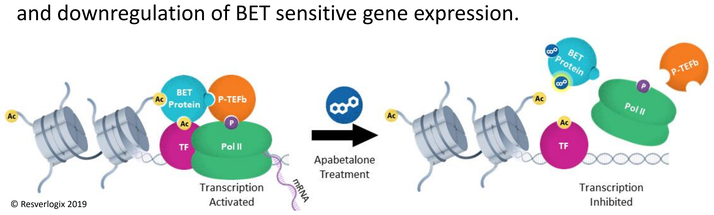
Methods: Circulating ALP was measured in CVD patients receiving apabetalone in the 3-month (ASSERT) and 6-month (SUSTAIN & ASSURE) trials. Apabetalone's effect on expression of TNAP (gene symbol *ALPL*) was determined in cultured primary human hepatocytes (PHH), HepaRG, HepG2, calcifying vascular smooth muscle cells (VSMCs) & vascular endothelial cells. Protein abundance & ALP enzyme activity were also measured.

Results: In phase 2 trials, baseline serum ALP correlated with MACE (R^2=0.87). In ASSERT, apabetalone dose dependently reduced serum ALP (p<0.001 vs placebo). In ASSURE & SUSTAIN, patients on apabetalone (n=331) had greater reduction in serum ALP than placebo (n=166; median % change -3.2 vs -11; p<0.001), including those with CKD, i.e. eGFR<60 (apabetalone n=35 placebo n=13 median % change -6.3 vs -14; p<0.02). In vitro, apabetalone suppressed *ALPL* expression in PHH, HepaRG & HepG2 cells by 60-80%. Trans-differentiation of VSMCs to calcifying cells resulted in 2.5-fold increase in *ALPL* gene expression. Apabetalone countered calcium deposition & suppressed *ALPL*/TNAP gene expression, protein levels & enzyme activity. Apabetalone also downregulated *ALPL* in aortic endothelial cells, umbilical vein endothelial cells & brain microvascular endothelial cells 50-70%.

Conclusions: In phase 2 trials, apabetalone lowered serum ALP. Mechanistically, apabetalone downregulates *ALPL*/TNAP expression in multiple cell types, which may contribute to reductions in MACE observed in patients. The impact of apabetalone on biomarkers, renal function & CVD outcomes is being evaluated in the phase 3 BETonMACE trial.

Apabetalone Mechanism of Action

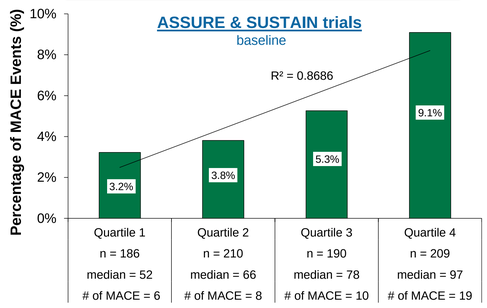
BET proteins control gene transcription through interactions with acetylated histones and transcription factors that promotes recruitment of RNA polymerase II. Apabetalone, an orally available small molecule, binds to bromodomains in BET proteins, causing their release from chromatin and downregulation of BET sensitive gene expression.



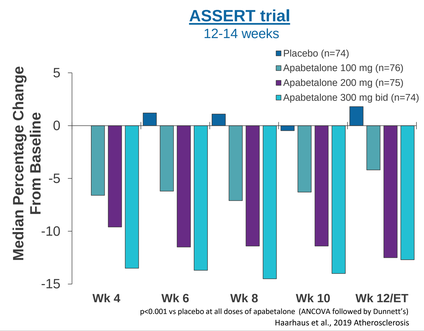
BET: bromodomain and extraterminal proteins
ac: acetylated lysine residue on DNA associated proteins
BD: bromodomain
TF: transcription factor
Yellow halo indicates selectivity of apabetalone for bromodomain 2 within BET proteins

Results: Serum ALP in CVD Patients in Phase 2, Placebo Controlled, Double Blind Clinical Trials on Top of Standard of Care

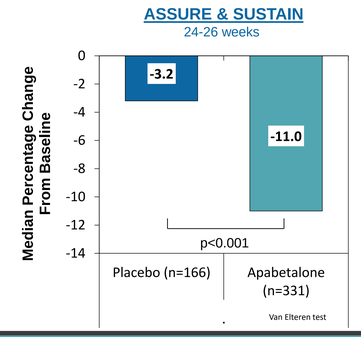
Baseline serum ALP correlates with rate of cardiac events (MACE)



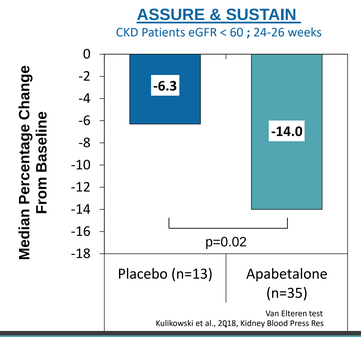
Apabetalone dose dependently reduces serum ALP



Apabetalone-mediated serum ALP reductions sustained up to 6 months

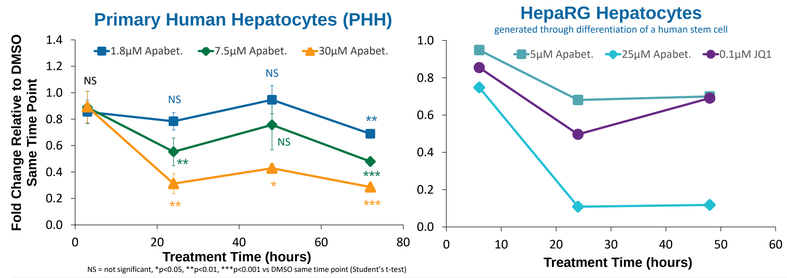


Apabetalone reduced serum ALP in CKD patients



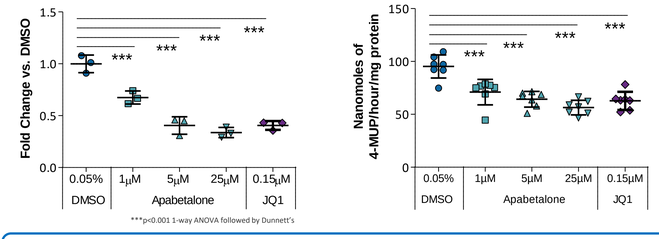
Results: Mechanistic Cell Culture Studies of *ALPL* Gene Transcription, TNAP Protein Abundance, TNAP Activity, & Calcium Deposition

Liver is a major source of serum alkaline phosphatase (TNAP isoform, gene symbol *ALPL*) Apabetalone downregulates *ALPL* gene expression in cultured human hepatocytes



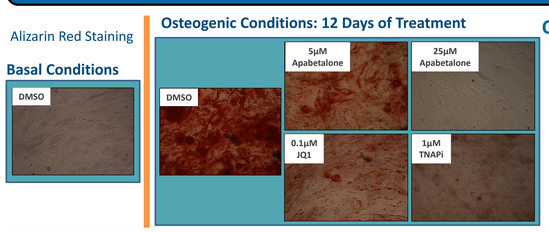
Method: Hepatocytes were cultured with apabetalone or comparator BET inhibitor JQ1. Gene expression was assessed by real-time PCR. Apabet. = Apabetalone.

Apabetalone reduces TNAP protein and enzyme activity in HepG2 hepatocytes



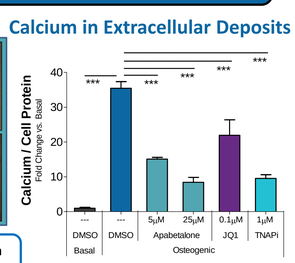
TNAP protein assessed by flow cytometry. Activity using 4-methylumbeliferyl phosphate (4-MUP) as substrate.
Legend: ALP = alkaline phosphatase **TNAP** = tissue non-specific ALP isoform **ALPL** = gene symbol for TNAP

Apabetalone suppresses extracellular calcium deposition in human coronary artery vascular smooth muscle cells (VSMC)

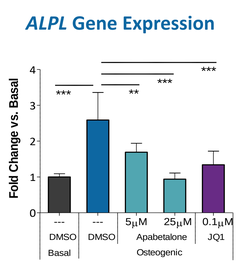


Method: VSMCs were cultured for 12-15 days in basal or osteogenic conditions with apabetalone, comparator BET inhibitor JQ1, or small molecule inhibitor of TNAP (TNAPi)

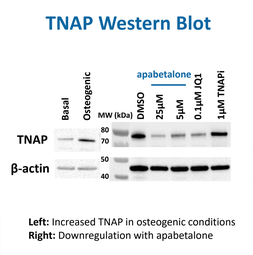
Calcium in Extracellular Deposits



ALPL Gene Expression

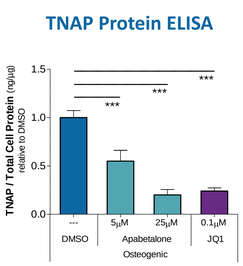


TNAP Western Blot

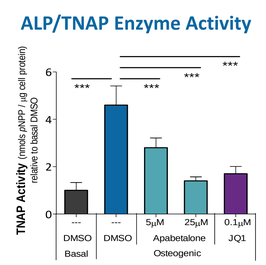


Left: Increased TNAP in osteogenic conditions
Right: Downregulation with apabetalone

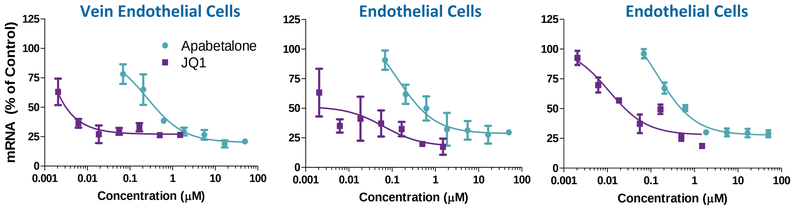
TNAP Protein ELISA



ALP/TNAP Enzyme Activity



Apabetalone downregulates *ALPL* expression in primary endothelial cells



Method: *ALPL* gene expression by real-time PCR in response to 4 hours treatment with BET inhibitors

Summary and Conclusions

- In phase 2 clinical trials, apabetalone reduced serum ALP, a risk factor for cardiovascular events and a biomarker that correlates with all cause mortality in subjects with CKD.
- Apabetalone downregulates TNAP (*ALPL*) gene expression & protein production in multiple cell types.
- Calcification of VSMCs is countered by apabetalone. The translational implication is reduction in pathological vascular calcification that leads to cardiac events in patients.
- TNAP is a mediator of endothelial dysfunction. Apabetalone downregulates *ALPL*/TNAP gene expression in primary human endothelial cells.
- The phase 3 BETonMACE trial will evaluate the impact of apabetalone on CVD outcomes (ClinicalTrials.gov Identifier: NCT02586155. Results Q4 2019).