Apabetalone, an Inhibitor of BET Proteins, Downregulates Alkaline Phosphatase and Improves Cardiovascular Risk

Dean Gilham[1], Laura M. Tsujikawa[1], Li Fu,[1], Sylwia Wasiak[1], Chris Halliday[1], Chris D. Sarsons[1], Phoebe Ho[1], Stephanie C. Stotz[1], Brooke D. Rakai[1], Ken Lebioda[1], Ravi Jahagirdar[1], Mike Sweeney[2], Jan O. Johansson[2], Norman C.W. Wong[1], Kamyar Kalantar-Zadeh[3], Mathias Haarhaus[4], Ewelina Kulikowski[1]

Resverlogix Corp. [1]Calgary, Canada and [2]San Francisco, USA, [3]University of California, Irvine, USA, [4]Karolinska University Hospital, Stockholm, Sweden

Abstract

Background: Elevated serum alkaline phosphatase (ALP) predicts major adverse cardiac events (MACE). ALP is associated with vascular calcification (VC), inflammation & endothelial dysfunction in patients with cardiovascular disease (CVD) &/or chronic kidney disease (CKD). Apabetalone is an inhibitor of BET proteins - epigenetic readers modulating gene expression in pathological VC & inflammation. We studied apabetalone's impact on tissue non-specific ALP (TNALP) expression in cell culture, then analyzed serum ALP in phase 2 trials.

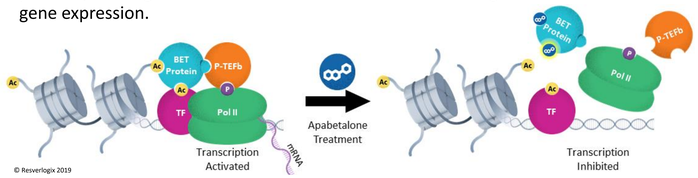
Methods: Expression of TNALP (gene symbol *ALPL*) was measured in primary hepatocytes, HepaRG, HepG2, primary human renal mesangial cells (MC), vascular smooth muscle cells (VSMC) & vascular endothelial cells by q-PCR. TNALP was assessed by immunoblot & flow cytometry, ALP activity by enzymatic assays. Serum ALP was measured in CVD patients in phase 2 trials (ASSERT, SUSTAIN & ASSURE). Subpopulations had CKD (eGFR<60).

Results: Apabetalone downregulated *ALPL* expression in liver cells by 60-80%. HepG2s had lower TNALP protein >55%, enzyme activity >40% & TNALP positive cells 15-30%; renal MCs had >90% decreases in *ALPL* expression & TNALP enzyme activity (p<0.001). *ALPL* was suppressed 50-70% in 3 vascular endothelial cell types with apabetalone. In VSMCs, apabetalone lowered *ALPL* expression, TNALP protein, enzyme activity & extracellular calcium deposition.
In ASSERT, apabetalone dose dependently reduced serum ALP (p<0.001). In combined phase 2 analysis, apabetalone lowered ALP (p<0.001), including patients in the CKD subgroup (p=0.008). Notably, the apabetalone-mediated decreases in serum ALP in phase 2 correlated with reduced MACE at 12-14 weeks (HR 0.64 per 1-SD in ALP, 95% CI 0.46–0.90 p=0.009 1-SD=13U/L); similar associations were observed at 24–26 weeks (HR 0.66 per 1-SD ALP 95% CI 0.43–0.99 p=0.045; 1-SD=14U/L).

Conclusions: Apabetalone lowers serum ALP, consistent with reduced hepatic, renal & vascular TNALP production. Modulation of ALP by apabetalone may affect pathogenetic processes to lower cardiovascular risk. This study provides insight to MACE reductions in phase 2 clinical trials.

Apabetalone Mechanism of Action

BET proteins control gene transcription through interactions with acetylated histones and transcription factors that promote 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.



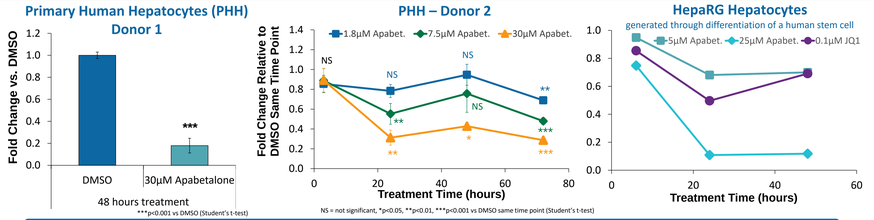
BET: bromodomain and extraterminal proteins
Ac: acetylated lysine residue on DNA associated proteins
BD: bromodomain
TF: transcription factor
Yellow halo indicates selectivity of apabetalone for bromodomain 2 within BET proteins

Haarhaus et. al 2019 Curr Opin Nephol Hyperten

Disclosures: All authors are employees of Resverlogix or clinical steering committee members

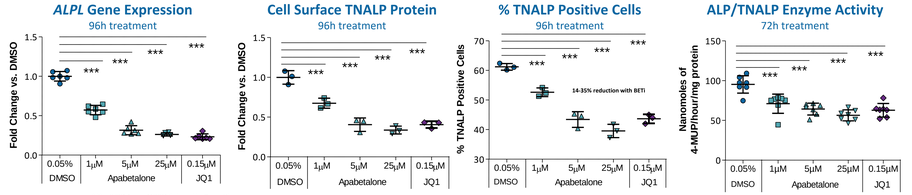
Results: Mechanistic Cell Culture Studies of *ALPL* Gene Transcription, TNALP Protein Abundance, TNALP Activity and Calcium Deposition

Liver is a major source of serum alkaline phosphatase (TNALP 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

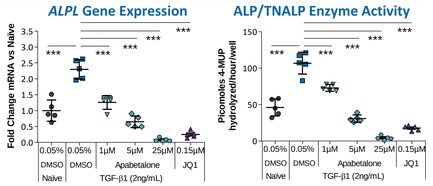
Apabetalone reduces *ALPL* gene expression, TNALP protein and enzyme activity in HepG2 hepatocytes



***p<0.001 1-way ANOVA followed by Dunnett's

TNALP protein assessed by immunofluorescent flow cytometry. Enzyme activity using 4-methylumbeliferyl phosphate (4-MUP) as substrate.
Legend: ALP = alkaline phosphatase **TNALP** = tissue non-specific ALP isoform ***ALPL*** = gene symbol for TNALP **BETi** = BET inhibitor

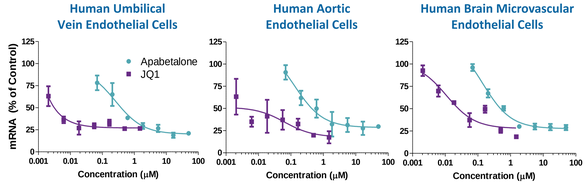
Apabetalone downregulates *ALPL* gene expression and ALP enzyme activity in human renal mesangial cells (MC)



***p<0.001 1-way ANOVA followed by Dunnett's

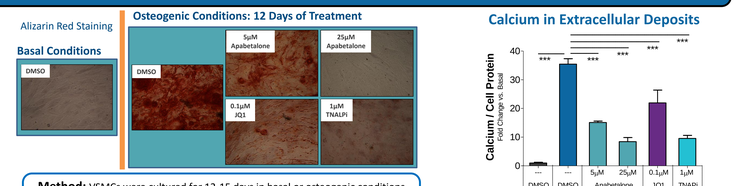
Method: MC treated 48h. Gene expression by real-time PCR. Enzyme activity using 4-MUP as substrate.

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

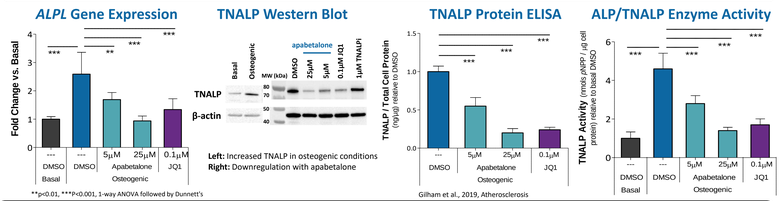
Apabetalone suppresses extracellular calcium deposition in human coronary artery vascular smooth muscle cells (VSMC)



***p<0.001 1-way ANOVA followed by Dunnett's
Gilham et al., 2019, Atherosclerosis

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

Apabetalone reduces *ALPL*/TNALP expression and enzyme activity in calcifying VSMC



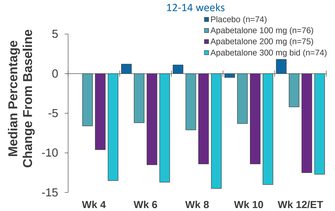
Left: Increased TNALP in osteogenic conditions
Right: Downregulation with apabetalone

**p<0.01, ***P<0.001, 1-way ANOVA followed by Dunnett's
Gilham et al., 2019, Atherosclerosis

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

Apabetalone dose dependently reduces serum ALP

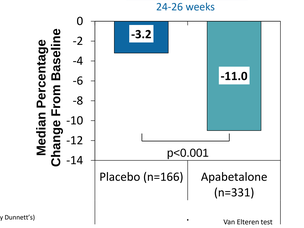
ASSERT trial
12-14 weeks



Haarhaus et al., 2019 Atherosclerosis
p<0.001 vs placebo at all doses of apabetalone (ANCOVA followed by Dunnett's)

Apabetalone-mediated serum ALP reductions sustained up to 6 months

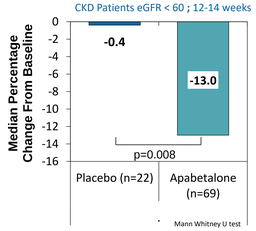
ASSURE & SUSTAIN
24-26 weeks



Van Elteren test

Apabetalone reduced serum ALP in CKD patients

ASSERT, ASSURE & SUSTAIN
CKD Patients eGFR < 60 ; 12-14 weeks

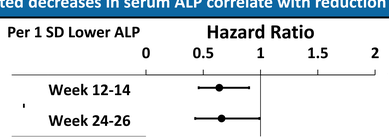


Mann Whitney U test

Apabetalone-mediated decreases in serum ALP correlate with reduction in MACE

Relationship between MACE and 1-SD decrease of ALP from baseline in patients receiving apabetalone examined in Cox proportional hazard models adjusted for sex, age, race, established cardiovascular risk factors, CKD, study, treatment modality, baseline ALP, CRP and liver function tests.

12-14 weeks: patients in the ASSERT, SUSTAIN & ASSURE trials.
24-26 weeks: SUSTAIN & ASSURE



	HR	95% CI	SD ALP (U/L)	*p*
Week 12-14	0.64	0.46-0.90	13	0.009
Week 24-26	0.66	0.43-0.99	14	0.045

Haarhaus et. al 2019, Atherosclerosis

MACE: Major Adverse Cardiac Events including: death, myocardial infarction, coronary revascularization, hospitalization for cardiovascular causes

Summary and Conclusions

- Apabetalone downregulates *ALPL* (TNALP) gene expression & protein levels in multiple cell types.
- Apabetalone reduces *ALPL/TNALP* expression in renal mesangial cells. Apabetalone treatment is associated with improved eGFR in phase 2 trials (Kulikowski et al. 2018, Kidney Blood Press Res).
- TNALP is a mediator of endothelial dysfunction. Apabetalone downregulates *ALPL* expression in primary human endothelial cells.
- Calcification of VSMCs is countered by apabetalone, suggesting reduced pathological vascular calcification may occur in patients.
- In phase 2 clinical trials, apabetalone dose dependently reduced serum ALP, a risk factor for major adverse cardiac events (MACE) and a biomarker of all-cause mortality.
- Apabetalone reduces MACE in patients with CVD. The magnitude of benefit in MACE correlated with the extent of ALP reduction.
- Effects of apabetalone on MACE and ALP in the phase 3 BETonMACE trial are presented in ASN Abstract SA-OR40 Oct. 24, 5-7pm