### Title

SO078APABETALONE, A BROMODOMAIN AND EXTRA-TERMINAL (BET) PROTEIN INHIBITOR, REDUCES ALKALINE PHOSPHATASE IN CVD PATIENTS, IN MICE, AND IN CELL CULTURE SYSTEMS




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### Authors

Gilham, Dean
Tsujikawa, Laura
Wasiak, Sylwia
et al.



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# ASPECTS OF CKD PATHOPHYSIOLOGY 1

## SO078 APABETALONE, A BROMODOMAIN AND EXTRA-TERMINAL (BET) PROTEIN INHIBITOR, REDUCES ALKALINE PHOSPHATASE IN CVD PATIENTS, IN MICE, AND IN CELL CULTURE SYSTEMS

Dean Gilham[1], Laura Tsujikawa[1], Sylwia Wasiak[1], Ravi Jahagirdar[1], Li Fu[1], Phoebe Ho[1], Christopher Halliday[1], Christopher Sarsons[1], Stephanie Stotz[1], Brooke Rakai[1], Kenith Lebioda[1], Jan Johansson[2], Michael Sweeney[2], Norman Wong[1], Kamyar Kalantar-Zadeh[3], Mathias Haarhaus[4], Ewelina Kulikowski[1]

*[1]Resverlogix, Calgary, Canada, [2]Resverlogix, San Francisco, United States of America, [3]University of California Irvine, Irvine, United States of America and [4]Karolinska University Hospital, Stockholm, Sweden*

**Background and Aims:** Elevated serum alkaline phosphatase (ALP) independently predicts major adverse cardiac events (MACE) by contributing to vascular calcification and endothelial dysfunction arising in chronic kidney disease (CKD) and cardiovascular disease (CVD). Apabetalone is an orally active inhibitor of bromodomain and extra-terminal (BET) proteins – epigenetic readers that modulate gene expression involved in vascular inflammation and calcification. Here we examined apabetalone's effects on ALP post-hoc in recent clinical trials, then performed mechanistic studies into apabetalone's impact on tissue non-specific ALP (TNALP) expression in mice and cell culture.

**Method:** Serum ALP was determined in CVD patients in phase 2 trials (3 month ASSERT and 6 month SUSTAIN & ASSURE) and in the phase 3 BETonMACE CVD outcomes trial, including subpopulations with CKD (eGFR$<$60 mL/min/1.73m$^2$). Apabetalone's effect on expression of TNALP (gene symbol *ALPL*) was examined in mice, cultured primary human hepatocytes (PHH), HepaRG, HepG2, vascular smooth muscle cells (VSMCs), and vascular endothelial cells by real-time PCR. TNALP protein levels were assessed by immunoblots and flow cytometry. ALP enzyme activity was measured in enzymatic assays.

**Results:** In phase 2 trials, baseline serum ALP independently predicted MACE (hazard ratio [HR] 1.6, 95% CI 1.2-2.2, p=0.001). In the 3 month ASSERT trial, apabetalone dose dependently reduced serum ALP ($p<0.001$ vs placebo). Prominent reductions in ALP were apparent in patients on apabetalone (n=331) vs placebo (n=166) in combined analysis of the ASSURE & SUSTAIN trials (median % change -11 vs -3.2; $p<0.001$). In the subset with CKD, patients on apabetalone (n=69) had greater reduction in serum ALP than placebo (n=22; p=0.008). Strikingly, ALP reductions in phase 2 correlated with reduction in MACE (HR 0.58, 95% CI 0.44-0.77, $p<0.001$). Consistent with phase 2, BETonMACE saw serum ALP reduced by 6.8 U/L with apabetalone (n=1082) vs placebo (n=1070; $p<0.001$) at 24 weeks. At the conclusion of BETonMACE, fewer MACE occurred in the CKD subgroup with apabetalone (n=124) vs placebo (n=164; HR 0.50 95% CI 0.26-0.96 p=0.032). Neither apabetalone nor statins that control low-density lipoprotein cholesterol inhibited recombinant TNALP enzyme activity, implying that decreased serum ALP activity in patients reflected reduction in TNALP production rather than inhibition of the enzyme.

Liver-derived TNALP accounts for ≈50% of circulating ALP. In the liver of mice on high fat diet, apabetalone or JQ1 (BET inhibitors with different chemical scaffolds) reduced *Alpl* mRNA ($p<0.001$) with corresponding trends in TNALP activity. In PHH, HepaRG, & HepG2 cells, apabetalone dose dependently suppressed *ALPL* expression by 60-80%. In HepG2 cells, apabetalone reduced TNALP protein (>55%, $p<0.001$), enzyme activity (> 40%; $p<0.001$), and % of TNALP positive cells (15-30%; $p<0.001$). MZ1, which promotes degradation of BET proteins, downregulated *ALPL* / TNALP similar to apabetalone. In VSMCs, apabetalone or JQ1 suppressed *ALPL* gene expression, TNALP protein levels, and enzyme activity, leading to decreases in extracellular calcium deposition. In addition, apabetalone downregulated *ALPL* expression in human aortic, umbilical vein, and brain microvascular endothelial cells by 50-70%.

**Conclusion:** Apabetalone lowers serum ALP in clinical trials, which is consistent with reduced hepatic production of TNALP - the most abundant ALP isoform. Further, apabetalone downregulates *ALPL* gene expression in vascular cell types while reducing calcification. Together, BET-dependent epigenetic modulation of ALP by apabetalone can affect several pathogenetic processes, and thereby improve cardiovascular outcomes. This study provides insights to the CVD event reductions observed in the CKD subpopulation in the BETonMACE Phase 3 trial.