Apabetalone Downregulates Alkaline Phosphatase and Improves Cardiovascular Risk

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Background: Apabetalone is an inhibitor of BET proteins - epigenetic readers modulating gene expression. In phase 2 trials, apabetalone reduced major adverse cardiovascular 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 & ASSERT) trials. Apabetalone’s effect on expression of TNAP (gene symbol ALP) was determined in cultured primary human hepatocytes (PHH), HepaRG, HepG2, calving 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 (R2=0.87). In ASSERT, apabetalone dose dependently reduced serum ALP (p<0.001 vs placebo). In ASSERT & 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 ALP expression in PHH, HepaRG & HepG2 cells by 60-80%. Trans-differentiation of VSMCs to calcifying cells resulted in 2.5-fold increase in ALP gene expression. Apabetalone counteracted calcium deposition & suppressed ALP/TNAP gene expression, protein levels & enzyme activity. Apabetalone also downregulated ALP 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 ALP/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 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.

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

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

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 ALP (gene expression & protein production in multiple cell types.
- Calculation of VSMCs is countered by apabetalone. The translational implication is reduction in pathological vascular calcification that leads to cardiovascular events in patients.
- TNAP is a mediator of endothelial dysfunction. Apabetalone downregulates ALP/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).