



Abstract Presentation by Dr. Norman Wong at Keystone Symposia, Seattle, WA, Feb. 2, 2017: Epigenetics and Human Disease: Progress from Mechanisms to Therapeutics.

**Selective BET inhibitors are useful for normalizing inflammation leading to reduced cardiovascular disease (CVD) in humans and in an animal model of rheumatoid arthritis.**

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Apabetalone (RVX-208) a bromodomain extra-terminal (BET) inhibitor selectively binds the 2nd ligand domain within a BET protein thus displacing it from acetylated lysine marks on histone tails. Data from ~1000 patients, many (n=499) of whom had CVD when given 200 mg/d RVX-208 lead to a 55% relative risk reduction in major adverse cardiac events (MACE) vs placebo. This benefit of RVX-208 may stem from BET inhibition (BETi) in calming perturbations in inflammatory, metabolic, coagulation and complement pathways with well-known roles in CVD risk. This data added to others detailing RVX-208 effects on risks underpin our phase 3 CVD events trial BETonMACE. Of major interest is the anti-inflammatory (AI) actions seen in our clinical data showing BETi lowers CRP by 28% in RVX-208 treated patients (n=331). RVX-208 lowers IL-6 and MCP-1 in LPS treated U937 cells in a dose and time dependent manner by 90 and 85%, respectively within 24 hrs. RVX-208 displaced BET proteins BRD2-4 from chromatin. These AI effects prompted us to study another potent selective BETi called RVX-297 which lowered IL-6 and MCP-1 in LPS stimulated U937 cells by 95 and 80% respectively within 24 hrs. In ChIP assays, RVX-297 displaced the BET protein BRD4 and pol II from promoters of cytokine genes IL-6 and IL-1 $\beta$  that mediate inflammation. Whether RVX-208 (150 mg/kg/d) had AI effects was tested in a collagen induced rat model of rheumatoid arthritis (RA). In treated rats, ankle swelling was markedly reduced by 60% in line with histologically normal synovial lining vs severe deterioration in controls. RVX-297 treated rats ambulated normally while controls did not. Plasma IL-6 levels were 52% lower in RVX-297 treated rats. Together, data from studying RVX-208 and -297 show that they dissociate BET proteins from promoter DNA which control expression of inflammatory genes (IGs) with key roles in CVD and RA. BETi displacement of proteins from DNA is important when added to the fact that cellular response to inflammation requires immediate and robust expression of defined IGs. For this rapid transcriptional response, the cell places chromatin structures upstream of IGs called super-enhancers (SE) that act as molecular sinks for attracting BET proteins such as BRD4. The placement of a SE adjacent to a promoter that controls a IG recruits this gene to the response against an inflammatory insult. Thus targeting BET proteins including BRD4 with selective BETi may have broad effects on many genes or pathways by crippling SE mediated cellular response to the inflammatory component of CVD, RA and many other diseases.

