Apabetalone (RVX-208) attenuates an inflammatory milieu that enhances adhesion of monocytes to endothelial cells in type 2 diabetes mellitus with cardiovascular disease patients

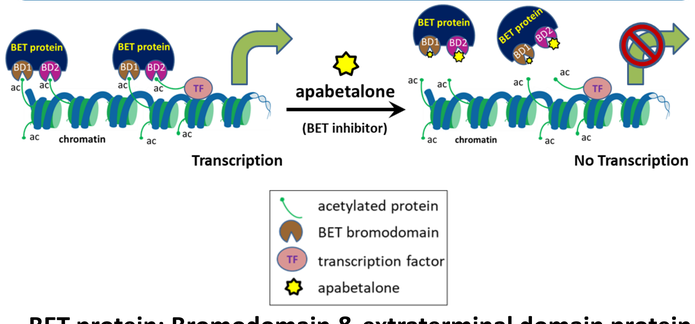
Laura M. Tsujikawa[1], Brooke D. Rakai[1], Shovon Das[1], Christopher Halliday[1], Li Fu[1], Dean Gilham[1], Christopher D. Sarsons[1], Sylwia Wasiak[1], Stephanie C. Stotz[1], Kristina D. Rinker[3], Michael Sweeney[2], Jan O. Johansson[2], Norman C. Wong[1] and Ewelina Kulikowski[1]

Resverlogix Corp. [1]Calgary, Canada, [2]San Francisco, USA, and [3]Department of Chemical and Petroleum Engineering, University of Calgary, Calgary, Canada

ABSTRACT

To explore mechanisms underlying a 57% relative risk reduction of major adverse cardiovascular events (MACE) in type 2 diabetes mellitus (T2DM) patients (pts) with CVD given 200 mg apabetalone (Apa, RVX-208) orally. Apa is a small molecule that inhibits bromodomain and extra-terminal (BET) proteins which are epigenetic readers of acetylated lysine in histones contained within actively transcribing chromatin. Inhibiting BET proteins attenuate transcribed genes that are upregulated in disease states. Studies here used SOMAscan proteomics of patient plasma given placebo (n=30) or Apa (n=25) and cultured monocyte (THP-1) or endothelial (HUVEC) cells. Results showed differences in the proteome spanning 4 CVD pathways; acute phase response, intrinsic prothrombin activation, leukocyte extravasation signaling and coagulation. This data showed TNFα target genes were preferentially up regulated in T2DM+CVD ($p<0.001$; z-score = 2.270) vs euglycemic pts suggesting an inflammatory state that was attenuated by Apa ($p<0.001$; z-score = -2.308). To mimic inflammation in vitro, TNFα (10 ng/ml) added to co-culture of THP-1 & HUVEC induced adhesion 12-fold in euglycemia but Apa inhibited it by 44%. HUVECs exposed to TNFα induced; IL-1β, E-selectin, VCAM1 and IL-6 mRNA by 1685, 1164, 196 & 9-fold respectively while Apa inhibited (50-99%). In THP-1, TNFα induced IL-1β, MYD88 and CD-44 mRNA by 3.5, 2.6, and 1.8-fold respectively but inhibited by Apa (39-84%). To mimic T2DM, high glucose (HG, 25.6 mM) induced adhesion of THP-1 to HUVEC by 2.4-fold was blocked by Apa. In HG, Very Late Antigen-4 (VLA-4) mRNA, a THP-1 adhesion gene, rose 1.3-fold while Apa blocked it >50%. HG induced adhesion genes in HUVECs; E-selectin & MYD88 by 2- & 1.3-fold was lowered by Apa. In summary Apa lowers MACE in pts with T2DM+CVD by possibly attenuating monocyte adhesion to endothelial cells. Apa acts by inhibiting BET proteins that facilitate transcription of genes mediating adhesion of monocytes to endothelial cells. The clinical relevance of the Apa effect is being tested in a phase 3 trial called BETonMACE in pts with T2DM+CVD.

Mechanism of Action

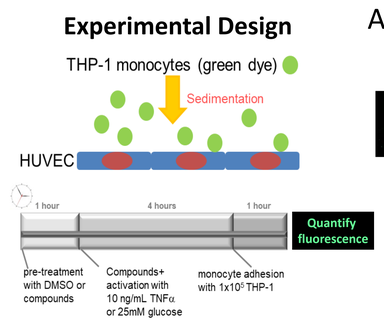


BET protein: Bromodomain & extraterminal domain protein

Yellow star indicates selectivity of apabetalone for BD2

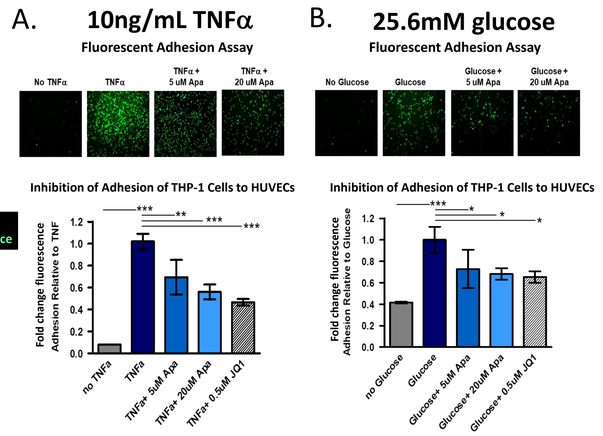
†**Disclosure:** Authors were employed by Resverlogix & held stock options With the exception of K. Rinker.

Apabetalone suppresses monocyte adhesion to endothelial cells



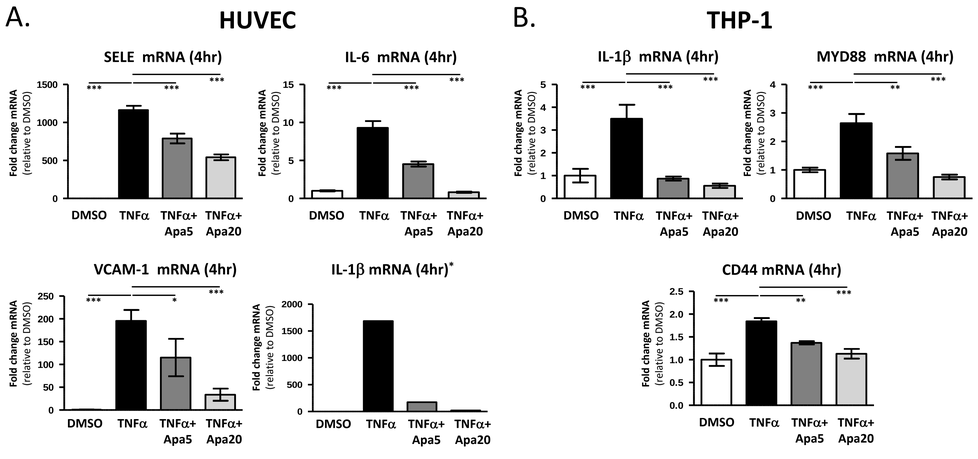
Statistics: One way ANOVA followed by Tukey's multiple comparison test

Apa = Apabetalone



Apabetalone inhibits TNFα and high glucose induced pro-atherogenic gene expression in monocytes and endothelial cells

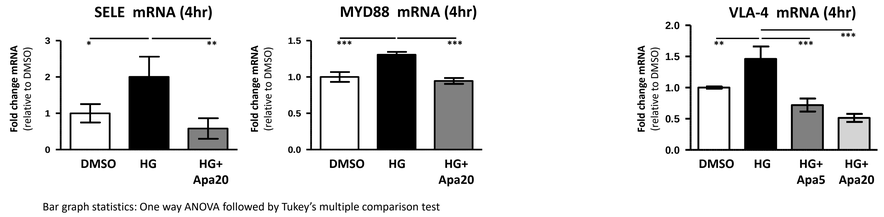
TNFα (10ng/μL)



Bar graph statistics: One way ANOVA followed by Tukey's multiple comparison test

* = Data from cDNA samples in singlicate prepared for human Nanostring Inflammation panel v2

High Glucose (HG;25.6mM)



Bar graph statistics: One way ANOVA followed by Tukey's multiple comparison test

IPA® predicts apabetalone inhibition of TNFα and pro-atherogenic pathways in CVD patient proteome

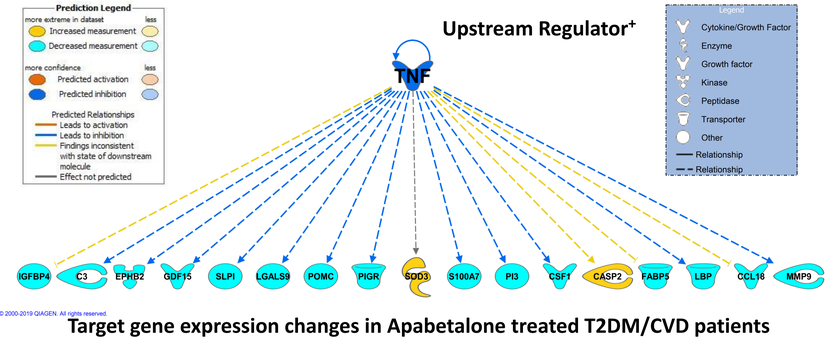
Bioinformatics (IPA®) Analysis of the Plasma Proteome (SOMAscan™) ASSERT phase II trial
Apabetalone treatment vs. placebo – All patient comparison

Ingenuity® Pathway Analysis	Pathway	Activation z-score	p-value of overlap
Canonical Pathway⁺⁺	Intrinsic Prothrombin Activation Pathway	-2.236	<0.001
	Acute Phase Response Signaling	-2.121	<0.001
	Coagulation System	-2.000	<0.001
	Leukocyte Extravasation Signaling	-2.000	0.002

T2DM+CVD vs. CVD – Baseline & Apabetalone treatment comparison

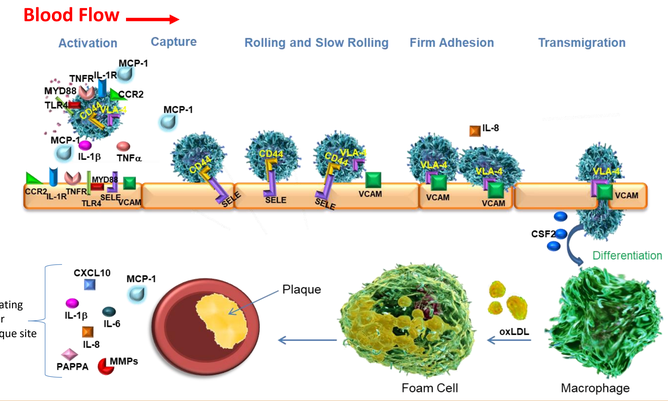
	Regulator	T2DM/CVD vs CVD Baseline Z-score	T2DM/CVD Apabetalone z-score	p-value of overlap
Upstream Regulator⁺	TNFα	2.270	-2.308	<0.001

Plasma proteins cutoff = 10% (*vs. placebo, p*<0.05). *IPA® z-score <-2 predicts inhibition; p-value = Fisher's Exact Test.
⁺⁺Apabetalone treated patients (n=25) vs. all placebo treated patients (n=30) both CVD and T2DM+CVD.
⁺Apabetalone treated T2DM+CVD patients (n=7) vs. placebo treated T2DM+CVD patients (n=5).



Target gene expression changes in Apabetalone treated T2DM/CVD patients

SUMMARY



1. **Apabetalone suppresses pro-atherogenic mediators (above)**
2. **BET-dependent downregulation of vascular inflammation and cell adhesion by apabetalone may contribute to the reduction in MACE, a hypothesis currently being tested in the phase 3 cardiovascular outcomes trial, BETonMACE.**