Apabetalone (RVX-208) inhibits key drivers of vascular inflammation, calcification, and plaque vulnerability through a BET-dependent epigenetic mechanism

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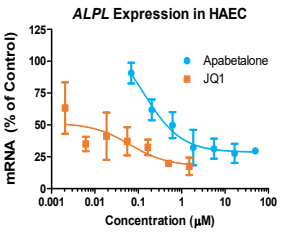
ABSTRACT

Apabetalone (RVX-208) is an orally available small molecule bromodomain & extraterminal (BET) protein inhibitor that targets the second bromodomain (BD2) of BET proteins. Apabetalone returns dysregulated BET-dependent transcription toward normal physiological levels. In phase 2 trials, apabetalone treatment reduced the incidence of major adverse cardiac events by 44% in CVD patients and by 57% in diabetic CVD patients. Previous studies have highlighted apabetalone's positive impact on vascular calcification (VC) and inflammation (VI) marker expression in vitro, as well as its ability to lower serum alkaline phosphatase (ALP) levels, and improve atherosclerotic plaque stability parameters in treated patients. In CVD, elevated inflammatory mediators and cell surface adhesion molecules drive VI, resulting in leukocyte adhesion, infiltration, uptake of oxLDL, and ultimately plaque formation. Here we show in vitro that THP-1 monocyte adhesion to human aortic endothelial cells (HAECs) increases with TNFα stimulation and is attenuated by apabetalone treatment, with fewer monocytes attaching to HAECs under flow conditions. This functional outcome is attributed to apabetalone's reduction of key endothelial adhesion genes, VCAM-1 (50%, p=0.0001) and SELE (37%, p=9 x 10^{-5}). Apabetalone also prevents TNFα induction of endothelial recruitment genes (MCP-1; 75%, p=0.0002) and genes involved in plaque rupture (IL8; 24%, p=2x10^{-5}). Basal HAEC ALP expression, a potential contributor to endothelial dysfunction and VC, also decreases with apabetalone treatment (70%, p=0.005). Induction of VI genes by TNFα is BET-dependent as degradation of BET proteins by MZ-1 prevents an increase in transcripts in response to TNFα treatment. Ingenuity® Pathway Analysis (IPA®), GSEA, and GO analysis of HAEC gene expression data predicts apabetalone inhibition of pro-atherogenic pathways, gene sets, and upstream regulators induced by TNFα. These include cytokine and chemokine, Toll-Like Receptor (TLR), NFκB, Interferon and TNFα signaling. In addition, IPA® disease and biological function analysis predicts inhibition of immune cell activation and recruitment by apabetalone. Plasma proteomics (SOMAscan®) and IPA® analysis from apabetalone-treated CVD patients in ASSERT and ASSURE phase 2 trials indicate that apabetalone inhibits pro-atherogenic upstream regulators (IL-6 and IFNγ), canonical pathways, and diseases and functions. Serum ALP also decreases dose dependently with apabetalone treatment (ASSERT). Epigenetic inhibition of VI and VC driven atherogenesis likely contributes to the reduction in MACE observed in phase 2 apabetalone treated patients. The ongoing phase 3 post-acute coronary syndrome (ACS) clinical trial in T2DM patients, BETonMACE, is currently testing this

Apabetalone inhibits alkaline phosphatase expression in HAEC

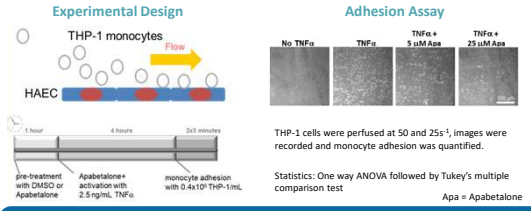


HAECs were treated with BET inhibitors for 4 hours, followed by analysis of alkaline phosphatase expression (gene symbol *ALPL*) by real-time PCR.

JQ1: a pan selective BET inhibitor with a chemical scaffold different from apabetalone, used as a comparator molecule.

Conclusion: Apabetalone maximally reduced *ALPL* mRNA by 70% (p<0.001, Student's t-test versus cells treated with solvent only)

Apabetalone suppresses monocyte adhesion to endothelial cells



THP-1 cells were perfused at 50 and 25s^{-1}, images were recorded and monocyte adhesion was quantified.

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

Apa = Apabetalone

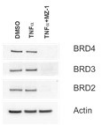
Apabetalone inhibits pro-atherogenic gene expression in endothelial cells in a BET-dependent manner by reducing BRD4 occupancy at gene loci

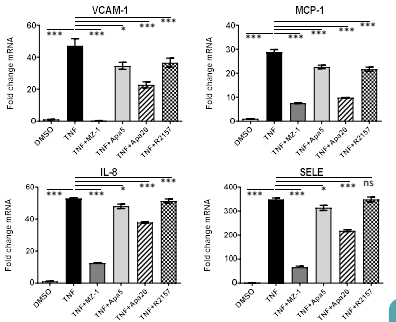
MZ-1 degradation of BET proteins

HAEC cells were pretreated with apabetalone (5 or 20 μM), RVX2157 (10 μM), MZ-1 (0.5 μM), or vehicle (DMSO) for 4 hours, with additional TNFα (10 ng/ml) stimulation for 2 hours before cells were harvested for real time-PCR or Western blotting

Apa = Apabetalone

R2157 = RVX2157 10 μM, a BD2 specific compound





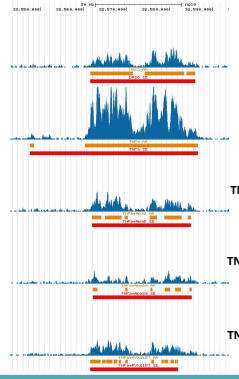
Bar graph statistics: One way ANOVA followed by Tukey's multiple comparison test (n=3) *** = p<0.001, * = p<0.05

BRD4 ChIP-seq on MCP-1

HAEC cell treatment: apabetalone (5 or 20 μM), RVX2157 (10 μM), or DMSO for 1 hour then addition of TNFα (10 ng/ml) for 1 hour

Orange bar = Active region (AR)

Red bar = Super enhancer (SE)



BET-dependency demonstrated by:

1. MZ-1 and BETi inhibition of expression
2. Loss of BRD4 occupancy on MCP-1 locus

Bioinformatics of HAEC RNA-seq data: IPA®

Upstream Regulators		TNFα	TNFα +Apa5	TNFα +Apa20	TNFα +R2157
TNF signaling	TNF	13.6	-2.1	-4.1	-4.1
	Tnf (family)	5.7	-1.7	-1.6	-1.8
TLR signaling	LPS	11.9	-5.0	-3.4	-4.8
	MYD88	6.1	-3.2	-2.8	-1.4
	TLR4	6.6	-3.4	-2.9	-3.1
NFκB signaling	NFkB (complex)	10.5	-5.3	-3.5	-3.0
	RELA	7.3	-1.8	-1.1	-0.1
IL1 signaling	IL1	7.1	-2.2		-1.0
	IL1B	10.2	-4.4	-3.6	-3.0
Interferon signaling	IFNG	9.9	-5.7	-3.5	-4.2
	IRF7	6.0	-4.4	-3.6	-3.8
STAT Transcription	STAT1	6.9	-4.9	-2.8	-3.3
	STAT4	6.8	-2.7	0.9	-1.4
Other cytokines	CSF2	6.6	-2.6	-1.9	-1.4
	OSM	6.6	-1.4	-3.2	-0.9
	RANKL	6.9	-2.6	-2.6	-1.5
	TGFB1	6.6	-2.1	0.8	-1.8
	F2	7.8	-3.2	-0.9	-1.5
	Ige	7.1	-2.9	-2.7	-2.1
	Nfat (family)	6.6	-2.3	-2.0	-1.6

Diseases and Functions		TNFα	TNFα +Apa5	TNFα +Apa20	TNFα +R2157
Immune cell activation and recruitment	Migration of cells	6.6	-2.7	-3.5	-4.3
	Cell movement of blood cells	6.6	-2.7	-2.7	-4.4
	Leukocyte migration	4.4	-3.0		-2.9
	Recruitment of blood cells	4.3	-2.9		-2.8
	Activation of leukocytes	4.2	-2.3	-2.4	-2.9
	Transcription	5.5	-2.1	-3.7	-2.4
	Transcription of RNA	5.0	-2.1	-3.4	-2.2
	Activation of DNA endogenous promoter	4.1	-2.3	-1.9	-1.5
Endothelial	Angiogenesis	5.4	-2.0	-1.5	-1.8
	Development of vasculature	5.4	-2.0	-1.5	-1.8
	Cell movement of endothelial cells	4.6	-1.9	-1.7	-1.9

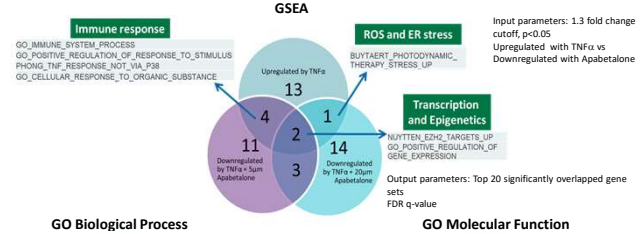
Canonical Pathways	TNFα	TNFα +Apa5	TNFα +Apa20	TNFα +R2157
Cardiac Hypertrophy Signaling (Enhanced)	4.2	-2.9	-3.3	-4.2
Dendritic Cell Maturation	4.0	-3.4	-2.3	-2.6
HMGB1 Signaling	3.9	-1.6	-2.3	-1.3
Production of Nitric Oxide and ROS in Macrophages	3.8	-2.6	-2.5	-2.0
IL-8 Signaling	3.7	-1.7	-2.6	-2.7
TREM1 Signaling	3.6	-1.9	-3.0	-1.7
IL-6 Signaling	3.6	-2.3	-2.8	-1.3
Type I Diabetes Mellitus Signaling	3.3	-3.0	-1.6	-1.0
iNOS Signaling	3.0	-2.1	-2.2	-1.3
Role of Pattern Recognition Receptors	2.8	-1.9	-4.4	-2.8
NF-κB Activation by Viruses	2.7	-2.1	-3.9	-2.8
Type II Diabetes Mellitus Signaling	2.6	-2.4	-2.9	-1.5
STAT3 Pathway	2.6	-2.5	-2.5	-2.8
Acute Phase Response Signaling	2.6	-2.4	-1.9	-0.2
RANK Signaling in Osteoclasts	2.4	-2.0	-3.3	-1.7
Thrombin Signaling	1.9	-2.0	-3.3	-3.9

Input parameters: 1.3 fold change cutoff, p<0.05 R2157 = RVX2157 10uM

Z score ≥ 2 = activation (yellow); Z score ≤ -2 = inactivation (blue)

Apabetalone and R2157 inhibit TNFα activated pro-atherogenic upstream regulators, pathways, and diseases and functions

Bioinformatics of HAEC RNA-seq data: Gene Set Enrichment Analysis (GSEA) and Gene Ontology (GO) Analysis

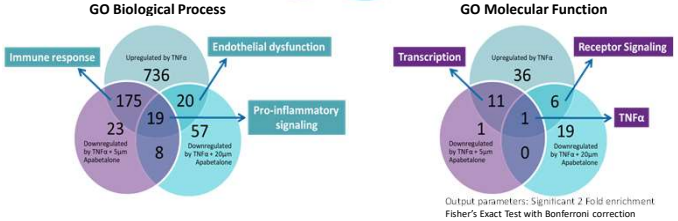


Input parameters: 1.3 fold change cutoff, p<0.05

Upregulated with TNFα vs Downregulated with Apabetalone

Output parameters: Top 20 significantly overlapped gene sets

FDR q-value



Output parameters: Significant 2 Fold enrichment

Fisher's Exact Test with Bonferroni correction

Apabetalone opposes TNFα activated gene sets: Pro-inflammatory, immune response, endothelial dysfunction

Apabetalone inhibits pro-atherogenic drivers and processes and downregulates ALP in CVD patients

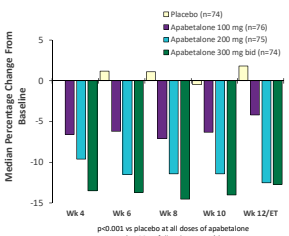
IPA® Analysis of Phase II Trial Plasma Proteome (SOMAscan™)

ASSERT: apabetalone (n=25) vs. placebo (n=30)		Activation z-score	p-value of overlap
Canonical Pathway	Leukocyte Extravasation Signaling	-2.000	<0.001
Upstream Regulators	IFNG	-2.749	<0.001
	IL6	-2.548	<0.001
Diseases and Functions	Activation of leukocytes	-2.847	<0.001

ASSURE: apabetalone (n=47) vs. placebo (n=47)		Activation z-score	p-value of overlap
Canonical Pathway	HMGB1 Signaling	-2.646	<0.001
Upstream Regulators	IFNG	-2.182	<0.001
	IL6	-2.036	<0.001
Diseases and Functions	Binding of leukocytes	-2.450	<0.001

Plasma proteins cutoff = 10% Δ (*vs. placebo,* p<0.05). IPA® z-score ≤-2 predicts inhibition based on target gene modulation. p-value = Fisher's Exact Test.

ASSERT trial
Circulating Alkaline Phosphatase:



p<0.001 vs placebo at all doses of apabetalone (ANCOVA follow by Dunnett's)

Patients with cardiovascular disease received apabetalone or placebo (on top of standard of care)

Apabetalone dose dependently reduced circulating ALP (p<0.001 median change vs. placebo on all three doses)

SUMMARY

1. Apabetalone inhibits the expression of pro-atherogenic genes in a BET-dependent manner resulting in the inactivation of pathologic pathways and regulators.
2. BET-dependent downregulation of vascular inflammation, cell adhesion, and ALP by apabetalone may contribute to the reduced incidence of MACE (phase 2), a hypothesis currently being tested in the phase 3 cardiovascular outcomes trial, BETonMACE.