Apabetalone, an Epigenetic BET Inhibitor in a Phase 3 Trial, Inhibits Vascular Inflammation and Cellular Adhesion Leading to Beneficial Outcomes in CVD Patients

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Abstract

Background: Apabetalone (RVX-208) is a small molecule bromodomain & extraterminal (BET) protein inhibitor, targeting the second bromodomain (BD2) of BET proteins. In cardiovascular disease (CVD) patients enrolled in phase 2 trials apabetalone treatment reduced the relative risk of a CV event by 44% (Nicholls 2017). Elevated cytokines, such as TNFα, promote vascular inflammation (VI) and monocyte adhesion in CVD and Diabetes Mellitus, driving atherosclerosis. Here we test the impact of apabetalone on cell types that contribute to atherosclerosis.

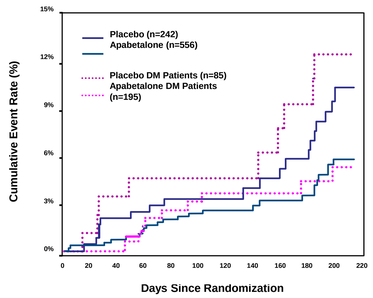
Methods: Human endothelial cells (HUVECs) and THP-1 monocytes were stimulated with TNFα and treated with apabetalone or MZ-1 PROTAC. mRNA (qPCR, Nanostring) and protein levels (FACS, western blot) were compared. HUVEC-THP-1 adhesion assays assessed the functional consequences of TNFα stimulation and apabetalone treatment. The phase 2 ASSURE CVD patient plasma proteome (SOMAscan®) was analyzed using Ingenuity® Pathway Analysis (IPA®) to predict canonical and upstream regulator pathways impacted by apabetalone.

Results: Apabetalone repressed transcription of inflammatory and adhesion genes in TNFα-stimulated HUVEC and THP-1 cells. Corresponding HUVEC protein abundance was also reduced. MZ-1 BET protein degradation blocked TNFα responses, indicating BET-dependency. Functionally, apabetalone suppressed monocytic THP-1 cell adhesion to inflamed endothelial cells. CVD patient plasma proteome analysis revealed that apabetalone reduced key players in adhesion (VCAM-1, ICAM-1) and plaque stability (MMP-3, MMP12). IPA® analysis of the clinical proteome data predicted that apabetalone inhibits pro-atherogenic mediators and inflammatory pathways.

Conclusion: Apabetalone attenuates VI through the epigenetic regulation of inflammatory and adhesion gene transcription. Downregulation of VI by apabetalone may contribute to the reduction in CVD events observed in phase 2 studies. The ability of apabetalone to prevent major adverse cardiac events in post-acute coronary syndrome patients with type 2 diabetes mellitus (DM) and low HDL-C is being assessed in phase 3 trial (BETonMACE).

Results

Apabetalone lowers MACE, phase 2b analysis



Apabetalone counters pro-inflammatory gene expression

mRNA transcripts of key HUVEC and THP-1 cytokine, chemokine, TLR Signaling, and adhesion molecules are induced by TNFα/stimulants (4h co-treatment), and reduced by apabetalone (1h pre-treatment+ 4hr co-treatment).

HUVEC

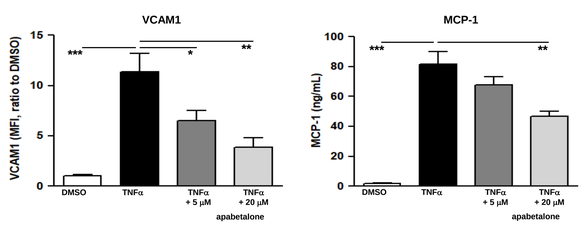
Simulation:		TNFα			IL-1β			LPS	
		Fold induction	% reduction		Fold induction	% reduction		Fold induction	% reduction
	Gene	Control	Apabetalone 5 μM	Apabetalone 20 μM	Control	Apabetalone 5 μM	Apabetalone 20 μM	Control	Apabetalone 5 μM	Apabetalone 20 μM
Cytokines	COX2	4	NS	-86	19	-46	-85	1	-42	-83
	CSF2	945	-82	-98	8096	-59	-91	9	-64	-85
	IL-1β	1685	-90	-99	ND	ND	ND	ND	ND	ND
	IL-6	9	-51	-91	191	-54	-84	1.6	-67	-69
	IL-8	26	ND	-48	ND	ND	ND	ND	ND	ND
	OPG	43	-95	-99	142	-96	-99	1.4	-71	-84
Chemokine	MCP-1	4	-21	-71	44	-35	-62	4	-50	-82
TLR signaling	MYD88	1	NS	-56	1	-30	-66	1.6	-44	-38
Adhesion molecules	CD44	2	NS	-34	3	NS	NS	1	-33	-34
	SELE	1164	NS	-54	368	-17	-40	11	-51	-76
	VCAM1	196	-59	-83	96	-72	-91	6	-73	-96

THP-1

		TNFα		
		Fold induction	% reduction	
	Gene	Control	Apabetalone 5 μM	Apabetalone 20 μM
Cytokines	IL-1β	3.5	-75	-84
	TNFα	3.8	NS	-54
Chemokines	CCR1	1.4	-51	-85
	CCR2	0.5	-50	-92
	MCP-1	3.7	-77	-91
TLR signaling	MYD88	2.6	-39	-71
	TLR4	0.7	NS	-51
Adhesion molecules	CD44	1.8	-26	-39
	VLA-4	0.9	-35	-61

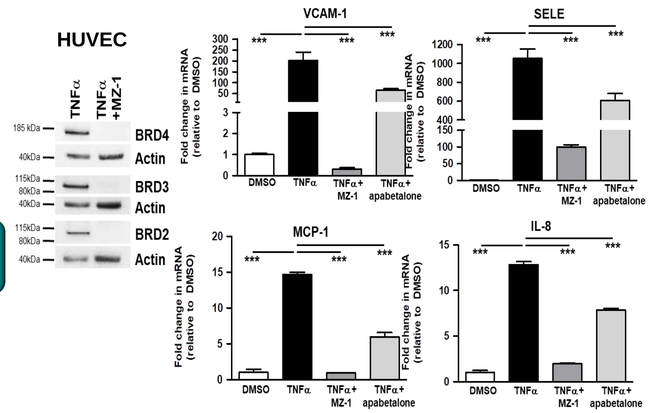
Apabetalone suppresses protein expression of VCAM1 and MCP-1 in endothelial cells.

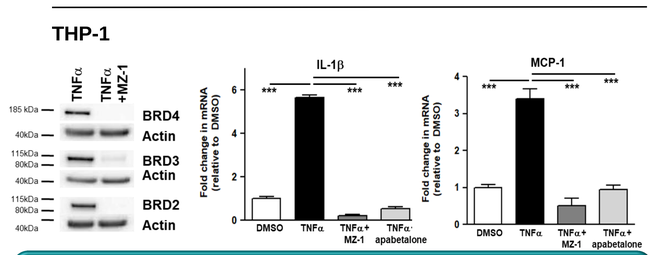
4 hour TNFα stimulation induced HUVEC VCAM1 surface expression (FACS) and MCP-1 secretion (BD™ cytometric bead array). Co-treatment with apabetalone reduced VCAM1 and MCP-1 protein levels. Statistical analysis: 1-way ANOVA, Dunnett's Multiple Comparison Test



Pro-inflammatory gene expression is BET-dependent

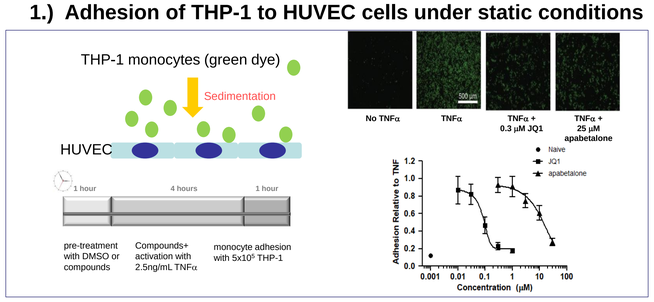
MZ-1 and apabetalone (4hr pre-treatment) prevent TNFα (2hr co-treatment) induction of key pro-inflammatory markers through the degradation or inhibition of BET proteins



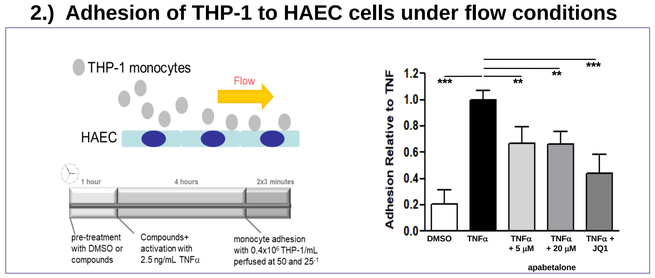


Apabetalone suppressed monocytic THP-1 cell adhesion to inflamed endothelial cells under both static and flow conditions.

1.) Adhesion of THP-1 to HUVEC cells under static conditions



2.) Adhesion of THP-1 to HAEC cells under flow conditions



Apabetalone suppresses pro-atherogenic and plaque rupture mediators in CVD patient plasma

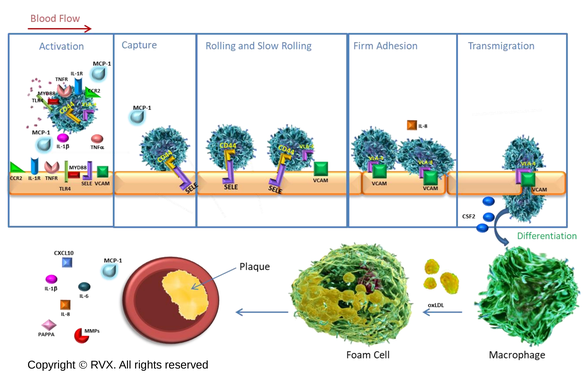
SOMAscan® CVD Patient Plasma Protein Apabetalone (n=47) vs. Placebo (n=47)			
Target Gene	Molecule type	% change vs. placebo	p-value
VCAM-1	Adhesion	-12.2	0.005
ICAM-1	Adhesion	-10.9	0.006
MMP-3	Enzyme	-26.8	0.005
MMP-12	Enzyme	-24.6	0.003

Ingenuity Pathway Analysis (IPA®) of SOMAscan® CVD Patient Plasma Protein Apabetalone vs. Placebo			
Upstream Regulator	Molecule type	z-score/direction	p-value
IL1B	cytokine	-2.8	<0.001
IL2	cytokine	-2.7	<0.001
CSF2	cytokine	-2.6	<0.001
IL6	cytokine	-2.5	<0.001
Ifn gamma	complex	-2.4	<0.001
NFKBIB	transcription regulator	-2.4	<0.001
TNF	cytokine	-2.1	<0.001

Ingenuity Pathway Analysis (IPA®) of SOMAscan® CVD Patient Plasma Protein Apabetalone vs. Placebo			
Canonical Pathway	z-score	p-value	Contributors
HMGB1 Signaling	-1.7	<0.001	TLR4, VCAM1, ICAM1, IL5, PTPN11, GRB2, OSM, IL17F, IL17B, TNFRSF11B

Summary

Apabetalone downregulates pro-atherogenic gene expression in endothelial and monocytic cells.



BET-dependent downregulation of vascular inflammation and subsequent cell adhesion by apabetalone may contribute to the reduction in MACE, a hypothesis currently being tested in the phase 3 cardiovascular outcomes trial BETonMACE in patients with CVD, diabetes mellitus and low HDL-c.

†**Disclosure:** Resverlogix employees received salaries & stock options from RVX.