Apabetalone (RVX-208) inhibits key pro-atherogenic mediators and pathways in diabetes and inflammatory conditions; in vitro and in patients

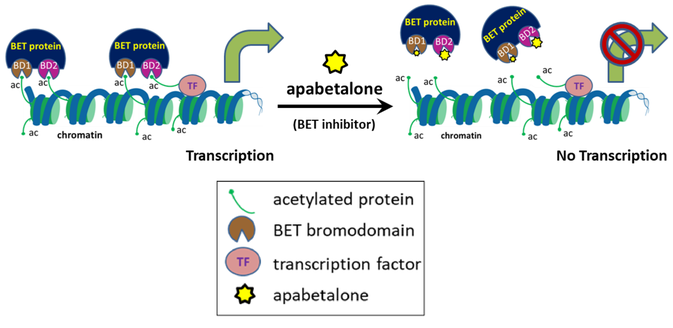
Laura M. Tsujikawa[1], Brooke D. Rakai[1], Shovon Das[1], Christopher Halliday[1], Li Fu[1], Dean Gilham[1], Chris Sarsons[1], Sylwia Wasiak[1], Stephanie C. Stotz[1], Kristina D. Rinker[3], Deborah Studer[3], Michael Sweeney[2], Jan O. Johansson[2], Norman C. Wong[1] and Ewelina Kulikowski[1]

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

ABSTRACT

Apabetalone (RVX-208) is a small molecule bromodomain & extraterminal (BET) protein inhibitor that targets the second bromodomain (BD2) within BET proteins. In phase 2 trials, apabetalone treatment reduced relative risk of MACE events by 57% in patients with cardiovascular disease (CVD) and type II diabetes (T2DM). In both CVD and T2DM, elevated circulating glucose, inflammatory mediators, and cell surface adhesion molecules drive vascular inflammation (VI) resulting in the recruitment, adhesion, and infiltration of leukocytes to the atherosclerotic plaque. Continued inflammation promotes cytokine production, immune cell infiltration, and plaque rupture, which accounts for 67% of fatal myocardial infarctions (MIs) and sudden cardiac deaths. Here we show in vitro that TNFα and high glucose treatment induced significant adhesion of THP-1 monocytes to endothelial cells, an outcome inhibited by apabetalone treatment. Apabetalone suppressed the transcription of critical drivers of pro-inflammatory signaling (RELA), immune cell activation and recruitment (MCP-1), and plaque rupture (IL-8). Ingenuity® Pathway Analysis (IPA®), GSEA, and GO analysis of human umbilical vein endothelial cell (HUVEC) gene expression data predicted that apabetalone would inhibit pro-atherogenic pathways, gene sets, and upstream regulators. These include cytokine and chemokine signaling, immune and inflammatory response, Toll-Like Receptor (TLR) signaling, and TNFα signaling. In addition, IPA® disease and biological function analysis predicted inhibition of immune cell recruitment and activation by apabetalone. These in vitro effects are consistent with plasma proteomic results (SOMAscan®) from apabetalone-treated CVD&T2DM patients, which demonstrated inhibitory effects on TNFα signaling, acute phase response, intrinsic prothrombin activation, leukocyte extravasation signaling and coagulation. Amelioration of diabetes and inflammation driven atherogenesis by apabetalone treatment likely contributes to the reduction in MACE observed in phase 2. The ongoing phase 3 post-ACS clinical trial in T2DM patients, BETonMACE, is investigating the effect of apabetalone on MACE reduction and will report in 2019.

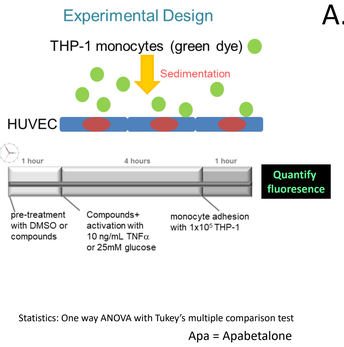
MECHANISM OF ACTION



BET protein: Bromodomain & extraterminal domain protein

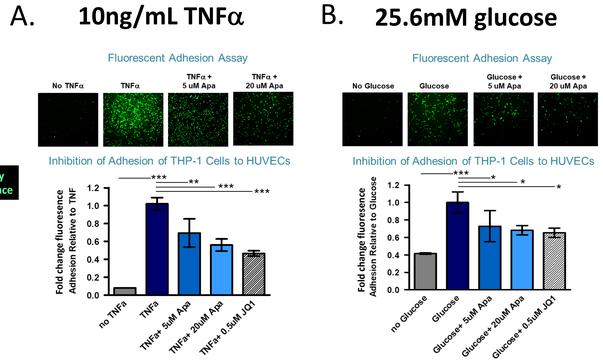
Yellow star indicates selectivity of apabetalone for BD2

Apabetalone suppresses monocyte adhesion to endothelial cells



Statistics: One way ANOVA with Tukey's multiple comparison test

Apa = Apabetalone



IPA® predicts apabetalone inhibition of TNFα-activated pro-inflammatory pathways, regulators, and diseases in endothelial cells

HUVEC Nanostring gene expression data (1.3x cutoff) was uploaded into IPA® (Ingenuity Pathway Analysis).

Tables consist of Z-scores which ≥ ~2 = activation and ≤ ~-2 = inhibition

Canonical Pathway	TNFα	TNFα+Apa 5uM	TNFα+Apa 20uM
TREM1 Signaling	3.8	-1.8	-3.1
Acute Phase Response Signaling	3.8	-1.6	-3.1
IL-6 Signaling	3.7	-0.8	-2.2
Dendritic Cell Maturation	3.7	-2.2	-4.0
Renin-Angiotensin Signaling	3.5	0.4	-2.1
HMGB1 Signaling	3.4	-1.1	-2.2
NF-κB Signaling	3.4	-1.4	-2.9
IL-8 Signaling	3.4	0.4	-2.7
Type I Diabetes Mellitus Signaling	3.0	-2.1	-3.1

P<0.01

Diseases and Bio Functions	TNFα	TNFα+Apa 5uM	TNFα+Apa 20uM
Migration of cells	6.0	-1.4	-4.1
Cell movement	5.9	-1.2	-4.2
Cell movement of phagocytes	5.4	-1.1	-3.4
Activation of cells	5.3	-2.2	-3.4
Homing of cells	5.2	-1.0	-3.5
Cell movement of myeloid cells	5.2	-0.8	-3.4
Activation of leukocytes	5.2	-1.7	-3.0

P< 1x10^-17

Upstream Regulators	TNFα	TNFα+Apa 5uM	TNFα+Apa 20uM
lipopolysaccharide	6.3	-3.3	-4.3
TNF	6.1	-2.6	-4.2
NFkB (complex)	5.9	-2.7	-3.5
IL1B	5.4	-1.9	-3.3
poly rI:rC-RNA	5.4	-3.9	-4.1
TLR4	5.2	-3.3	-4.4
TICAM1	5.1	-2.7	-4.5
MYD88	5.1	-3.1	-4.1
TLR3	5.0	-3.3	-3.5
IL1A	4.9	-2.2	-1.8
RELA	4.8	-1.9	-2.7

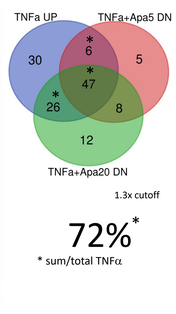
P< 1x10^-17

Apabetalone reverses the TNFα induced activation of pro-inflammatory and pro-atherogenic pathways, upstream regulators, and diseases and biological functions

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

A. 10ng/mL TNFα: HUVEC cells were pre-treated with DMSO or Apabetalone for 1hr then co-treated with TNFα for 4hrs→ RNA

Gene	Fold Change TNFα	%change TNFα+Apabetalone 5uM	20uM
Cytokines & Chemokines			
CSF2	155	-76	-93
CXCL10	86	-78	-84
IL1A	14	-73	-88
IL1B	3	-64	-66
IL1R1	3	-40	-82
IL6	10	-59	-90
IL8	185	-47	-79
LTB	119	-77	-99
MCP-1	133	-18	-65
Interferon signaling			
IFIT2	11	-23	-15
IRF1	8	-26	-42
TLR signaling			
TLR1	3	-39	-81
TLR2	29	-52	-78
NOD2	6	-65	-82
TLR/NFkB signaling			
RIPK2	12	-22	-57
NFkB signaling			
NFKB1	6	-17	-26
RELA	2	-4	-36
RELB	14	-15	-30
TNF signaling			
TNF	15	-93	-93
TNFAIP3	81	-79	-51

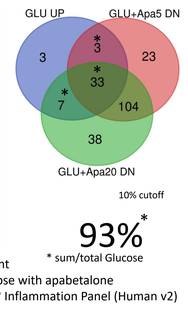


72%*

* sum/total TNFα

B. 25mM glucose: HUVEC cells were co-treated with 25mM glucose and Apabetalone for 24hrs→ RNA

Gene	Fold Change Glucose	%change Glucose+Apabetalone 5uM	20uM
Cytokines & Chemokines			
CCL20	1.7	-20	-44
CSF3	1.4	-33	-25
CXCL10	1.4	-33	-44
CYSLTR2	1.4	-69	-91
IL8	1.3	-31	-24
LTB	2.3	-45	-55
LTB4R	1.3	-47	-71
MCP-1	2.0	-51	-92
TLR signaling			
TLR3	1.3	-88	-89
TLR7	1.7	-20	-25
TLR9	1.4	-32	-39
NFkB signaling			
RELA	1.2	-15	-48
TGFb signaling			
TGFB2	3.1	-24	-83
TGFB3	1.2	-66	-59
TNF signaling			
TNFAIP3	1.2	-32	-39
TRADD	1.2	-19	16
Inflammasome			
NLRP3	1.7	-20	-44



93%*

* sum/total Glucose

Yellow = upregulated (UP) fold change from no stimulant

Blue = downregulated (DN) % change from TNFα/Glucose with apabetalone

Bolded genes highlighted genes in abstract , nCounter® Inflammation Panel (Human v2)

Apabetalone suppresses 72% and 93% of all TNFα and high glucose induced pro-inflammatory nanostring panel genes respectively

IPA® predicts apabetalone inhibtion 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

Ingenuity® Pathway Analysis	Regulator/Pathway	Activation z-score*	p-value of overlap§
Upstream Regulator+	TNFα	-2.31	<0.001
Canonical Pathway++	Intrinsic Prothrombin Activation Pathway	-2.23	<0.001
	Acute Phase Response Signaling	-2.12	<0.001
	Coagulation System	-2.00	<0.001
	Leukocyte Extravasation Signaling	-2.00	0.002

Plasma proteins input cutoff = 10% (*vs. placebo, p*<0.05) were analyzed with the IPA®

*IPA® z-score compares the observed regulation of genes in the dataset to changes observed in the literature. z-score <-2 predicts inhibition

§The p-value of overlap is calculated using Fisher's Exact Test.

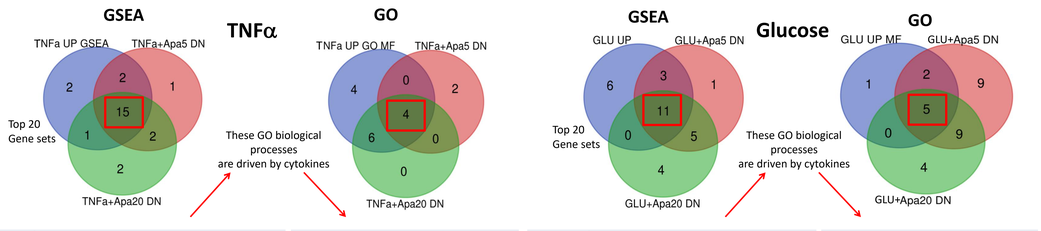
+Apabetalone treated CVD patients +T2DM (n=7)vs. placebo treated CVD patients +T2DM (n=5).

++Apabetalone treated patients (n=25) vs. all placebo treated patients (n=30)both CVD and CVD+T2DM.

Apabetalone reverses TNFα and glucose impact on major inflammatory gene sets in endothelial cells: GSEA and GO Analysis

GSEA: Broad Institute software using both pathways and gene sets: Hallmark, Curated (Biocarta, KEGG, Reactome), GO (biological process/molecular function/cellular compartment), and immunologic gene sets

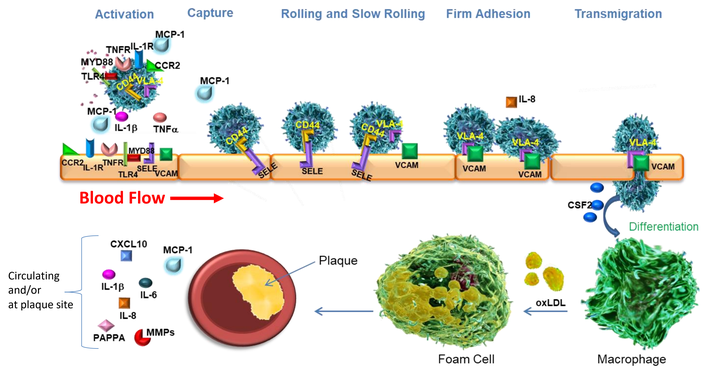
GO: Focus on molecular function: Defines concepts/classes used to describe activities of a gene product at the molecular level.



Top 5/15: TNFa induced GSEA gene sets suppressed by low and high dose apabetalone	TNFa induced molecular function GO gene sets suppressed by low and high dose apabetalone
GO_POSITIVE_REGULATION_OF_RESPONSE_TO_STIMULUS	chemokine activity (GO:0008009)
GO_CELLULAR_RESPONSE_TO_ORGANIC_SUBSTANCE	chemokine receptor binding (GO:0042379)
GO_IMMUNE_SYSTEM_PROCESS	cytokine activity (GO:0005125)
GO_DEFENSE_RESPONSE	cytokine receptor binding (GO:0005126)
GO_RESPONSE_TO_CYTOKINE	

Top 5/11: Glucose induced GSEA gene sets suppressed by low and high dose apabetalone	TNFa induced molecular function GO gene sets suppressed by low and high dose apabetalone
GO_IMMUNE_SYSTEM_PROCESS	chemokine activity (GO:0008009)
GO_IMMUNE_RESPONSE	chemokine receptor binding (GO:0042379)
GO_DEFENSE_RESPONSE	cytokine activity (GO:0005125)
GO_CELLULAR_RESPONSE_TO_ORGANIC_SUBSTANCE	icosanoid receptor activity (GO:0004953)
GO_POSITIVE_REGULATION_OF_RESPONSE_TO_STIMULUS	cytokine receptor binding (GO:0005126)

SUMMARY



1. **Apabetalone suppresses all pro-atherogenic mediators shown 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.**

†**Disclosure:** Authors were employed by Resverlogix & held stock options