Apabetalone (RVX-208) attenuates inflammatory milieu underlying adhesion of monocytes to endothelial cells in T2DM with CVD patients

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ABSTRACT

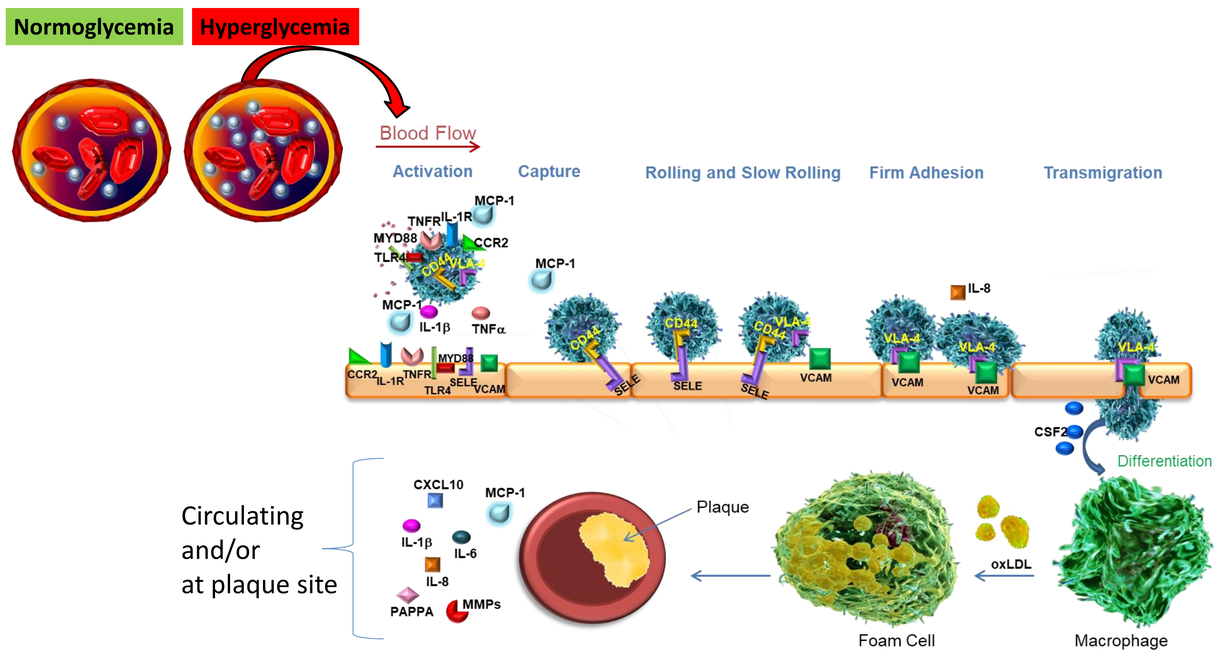
Aim: To explore mechanisms behind the 57% relative risk reduction of major adverse cardiovascular events (MACE) in patients (pts) with type 2 diabetes mellitus (T2DM) and CVD given 200 mg apabetalone (APL, RVX-208, inhibitor of bromodomain and extra-terminal [BET] proteins that are epigenetic readers of histone acetylated lysine).

Method: SOMAscan proteomics of patient plasma given APL (n=25) or placebo (n=30) and cultured monocyte (THP-1) or endothelial (HUVEC) cells.

Results: Plasma proteomics from CVD+/-T2DM pts given APL or placebo showed changes in 4 well-known pathologic pathways and inflammation triggered by TNFα underpinning CVD. Proteins induced by TNFα ($p<0.001$; z-score = 2.270) were attenuated by APL ($p<0.001$; z-score = -2.308). To replicate this inflammatory milieu, TNFα (10 ng/ml) or high glucose (HG, 25.6 mM) was added to co-cultures of THP-1 and HUVEC leading to enhanced adhesion 12- and 2.4-fold, respectively but inhibited by APL (44-32%). Very Late Antigen-4 (VLA-4) a THP-1 adhesion mRNA rose 1.3-fold in HG and APL suppressed it >50%. Similarly, E-selectin, MCP-1, and MYD88 mRNAs that mediated adhesion rose by 2-, 2- and 1.3-fold, respectively in HUVECs exposed to HG while APL attenuated (30-90%). Furthermore, Nanostring data from HUVECs showed HG induced many inflammatory genes underlying CVD but APL blocked ~90% of these. Gene Set Enrichment and functional Gene Ontology Analysis showed many inflammatory and immunoregulatory genes were positively impacted by HG but negatively affected by APL.

Summary: APL lowers MACE in T2DM and CVD pts by attenuating monocyte adhesion to endothelial cells and thereby possibly reducing atheroma formation.

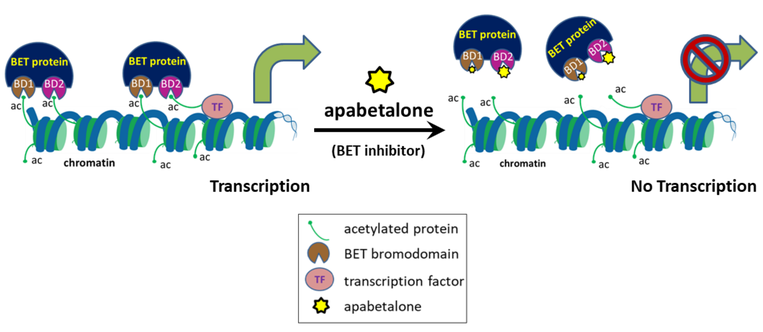
SUMMARY



- Apabetalone suppresses all pro-atherogenic mediators shown above
- 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 in in post-Acute Coronary Syndrome patients with CVD, diabetes mellitus and low HDL-c.

RESULTS

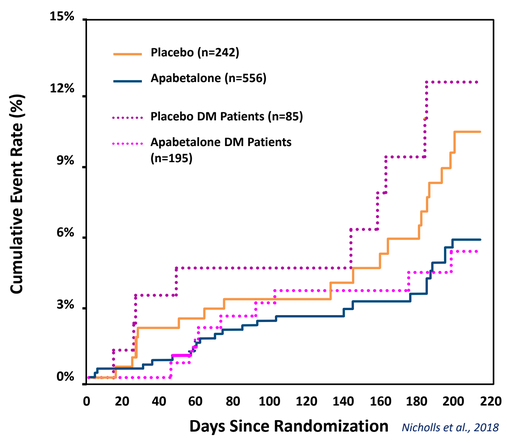
Mechanism of Action



BET: Bromodomain and extra-terminal protein

Yellow star indicates selectivity of apabetalone for BD2.

Apabetalone reduces MACE, phase 2b post-hoc analysis



MACE: **M**ajor **A**dverse **C**ardiac **E**vents including: death, myocardial infarction, stroke, coronary revascularization, hospitalization for acute coronary syndrome or heart failure

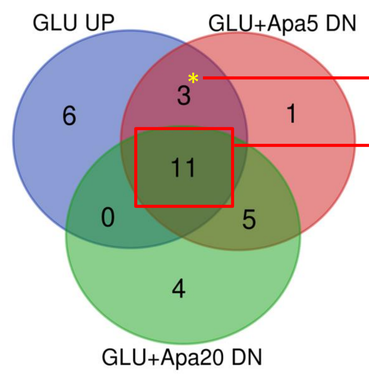
Apabetalone reverses high glucose impact on major inflammatory gene sets in endothelial cells

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

GO: Gene ontology →molecular function (MF)

GSEA

Overlap of the top 20 gene sets with a FDR qvalue < 1 x 10-18 for each treatment group are compared



Unique low dose apabetalone effects
GO_CELLULAR_RESPONSE_TO_CYTOKINE_STIMULUS
GO_CYTOKINE_RECEPTOR_BINDING
GO_RESPONSE_TO_BIOTIC_STIMULUS

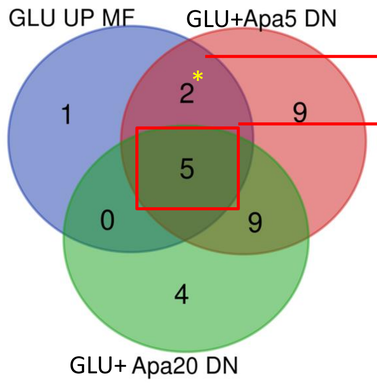
Top 5/11: Glucose induced gene sets suppressed by both low and high dose apabetalone
GO_IMMUNE_SYSTEM_PROCESS
GO_IMMUNE_RESPONSE
GO_DEFENSE_RESPONSE
GO_CELLULAR_RESPONSE_TO_ORGANIC_SUBSTANCE
GO_POSITIVE_REGULATION_OF_RESPONSE_TO_STIMULUS

Top GSEA gene sets impacted are GO biological processes

Performed GO analysis based on molecular function to find contributors driving the effects on GO biological processes

GO

Overlap of the gene sets with ≥ 20 fold enrichment with $p < 0.05$

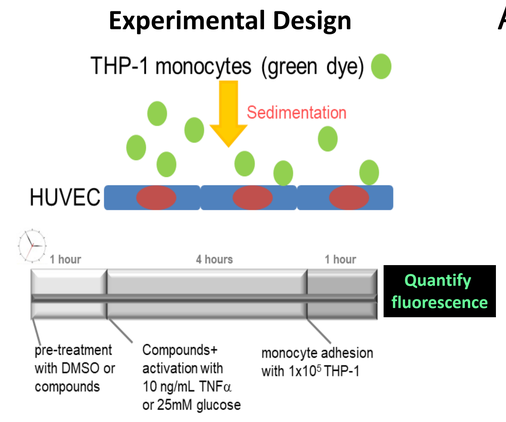


Unique low dose apabetalone effects
pattern recognition receptor activity (GO:0038187)
signaling pattern recognition receptor activity (GO:0008329)

Top 5/5: Glucose induced gene sets suppressed by both low and high dose apabetalone
chemokine activity (GO:0008009)
chemokine receptor binding (GO:0042379)
cytokine activity (GO:0005125)
icosanoid receptor activity (GO:0004953)
cytokine receptor binding (GO:0005126)

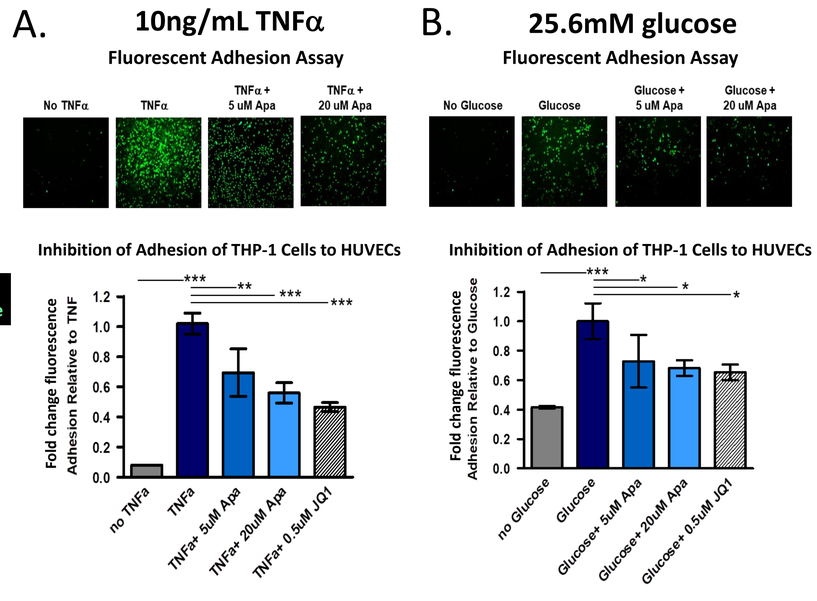
Nanostring gene expression data from the human inflammation gene panel was uploaded into GSEA and GO →cutoff = 10%

Apabetalone suppresses monocyte adhesion to endothelial cells



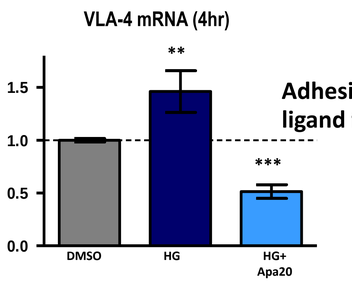
Statistics: One way ANOVA followed by Tukey's multiple comparison test

Apa = Apabetalone

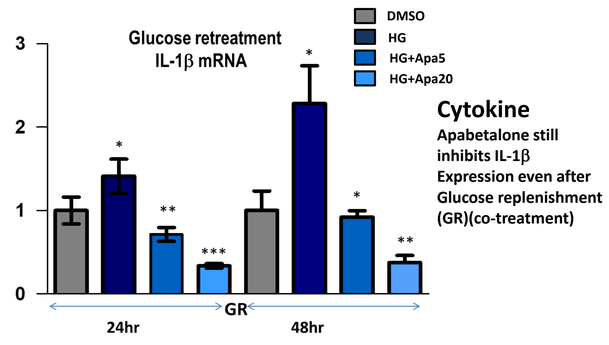


Apabetalone inhibits high glucose induced pro-atherogenic gene expression in monocytes and endothelial cells

THP-1



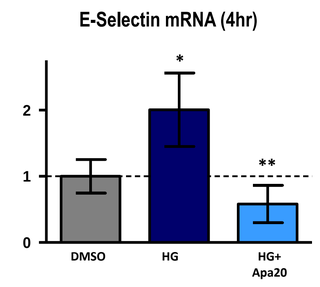
Adhesion molecule: ligand for VCAM-1



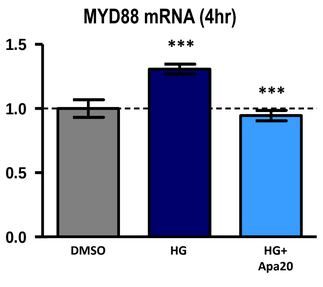
Cytokine

Apabetalone still inhibits IL-1β Expression even after Glucose replenishment (GR)(co-treatment)

HUVEC

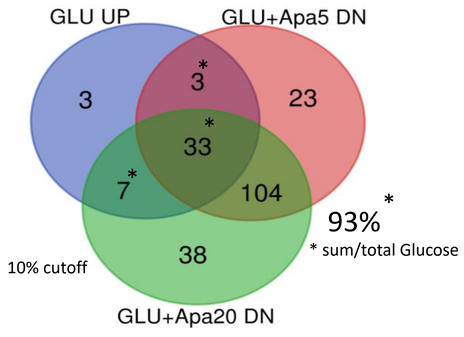


Adhesion molecule



TLR signaling

24hr co-treatment	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

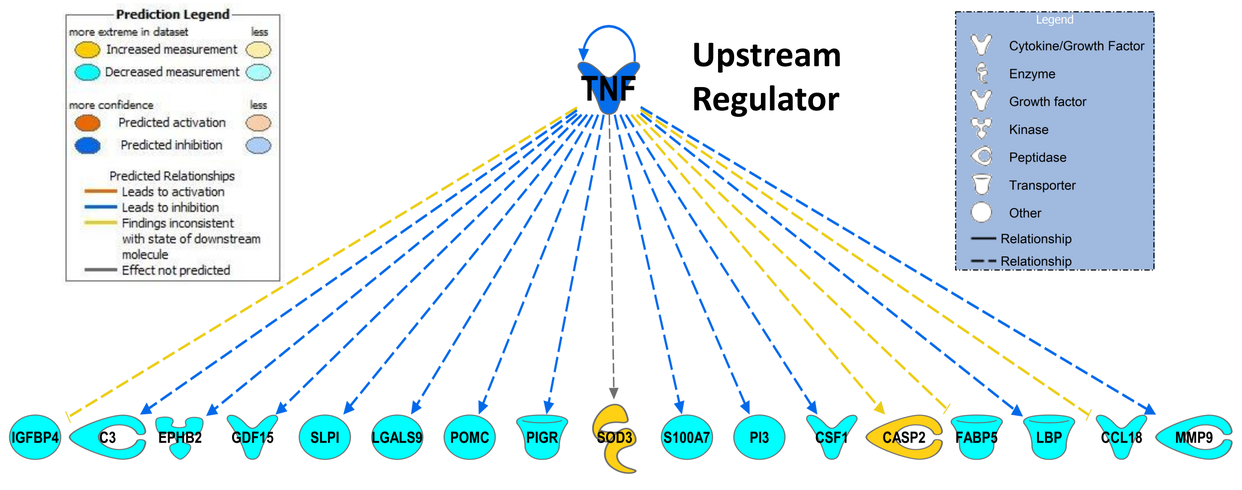


Real time pcr statistics: One way ANOVA followed by Tukey's multiple comparison test
Y axis = Fold change mRNA relative to DMSO
Yellow (Table) = upregulated fold change from no glucose
Blue (Table) = downregulated % change from glucose with apabetalone
Bolded gene: highlighted genes in abstract
nCounter® Inflammation Panel (Human v2)

IPA® predicts apabetalone inhibition of TNFα and pro-atherogenic pathways in ASSERT 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 cutoff = 10% (*vs. placebo, p*<0.05). *IPA® z-score <-2 predicts inhibition;; p-value = 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.



Target gene expression changes

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