Epigenetic Reader Inhibitor Apabetalone (RVX-208) Counters Proinflammatory Hyperactivation of CD14+ Monocytes from Patients with Type 2 Diabetes and Cardiovascular Disease

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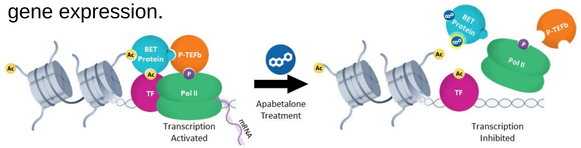
Background and Rationale

Monocytes from patients with type 2 diabetes (DM2) and cardiovascular disease (CVD) display proinflammatory behavior such as enhanced cytokine production and vascular wall invasion, which promote atherosclerosis. This dysregulation is partly caused by epigenetic reprogramming which can be countered by epigenetic drugs.

Apabetalone Mechanism of Action

Apabetalone binds competitively to bromodomains in histone acetylation "readers" termed BET proteins, causing their release from chromatin and downregulation of BET sensitive gene expression.



BET: bromodomain and extraterminal proteins; ac: acetylated lysine on DNA associated proteins; TF: transcription factor; BD: bromodomain; Yellow star size indicates selectivity of apabetalone for BD2

Study Design

14 Subjects with DM2 and Stable CVD (DM2+CVD)
(prior MI, PCI, CABG, unstable angina, TIA, CVA, PAD ≥ 3 months ago)
on Statin and Insulin Therapy
versus
12 Matched Control Subjects

Blood Collection

CD14+ MonocyteIsolation

***Ex vivo* 25 μM Apabetalone or 0.025% DMSO ± 25 U/mL IFNγ**

1. Gene Expression Analysis (4h) with Nanostring® Innate Immune Panel (109 genes)

2. Protein Secretion Analysis (24h) with Milliplex® Immuno Profiling (42 cytokines)

3. Bioinformatics with Ingenuity® Pathway Analysis (IPA®) Upstream Regulators Analytic Tool

DM2+CVD Monocytes *versus* Controls: Baseline Analysis
Apabetalone Attenuates Pro-Inflammatory Activation *Ex Vivo*

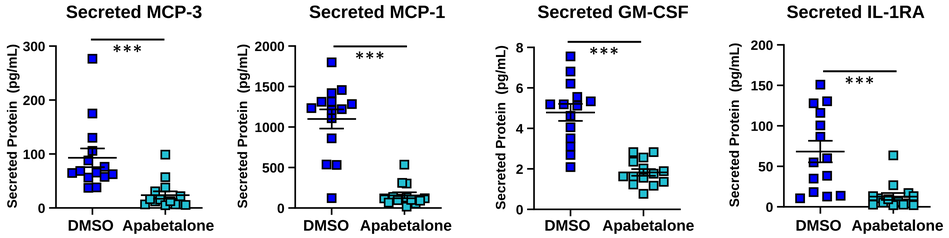
A. Monocytes from DM2+CVD patients have higher expression of pro-inflammatory genes and proteins at baseline. This "activation" is reversed by ex vivo apabetalone treatment.

Gene Name	Function	Fold Difference at Baseline DM2+CVD *vs.* Control	Gene Expression (4h) in DM2+CVD % Suppression by Apabetalone
IL1B	Pro-inflammatory cytokine IL-1β	3.2	No change
IL1A	Pro-inflammatory cytokine IL-1α	2.4	-67%
FCAR	IgA receptor	1.7	-65%
CXCL8	Chemokine IL-8	1.5	-61%
MARCO	Scavenging receptor	0.6	-75%
MS4A4A	M2 macrophage marker	0.6	-76%
SF3A3	Splicing Factor	0.8	-15%

Secreted Protein	Fold Difference at Baseline DM2+CVD *vs.* Control	Protein Secretion (24h) in DM2+CVD % Suppression by Apabetalone
Cytokine IL-8	1.67	-32%
Cytokine GROα	1.68	-55%

Statistics: Two-Way Repeated Measures ANOVA, Bonferroni's test (in-between group comparisons) or Tukey's test (within-group comparisons). Significant p<0.05, NS: not significant.

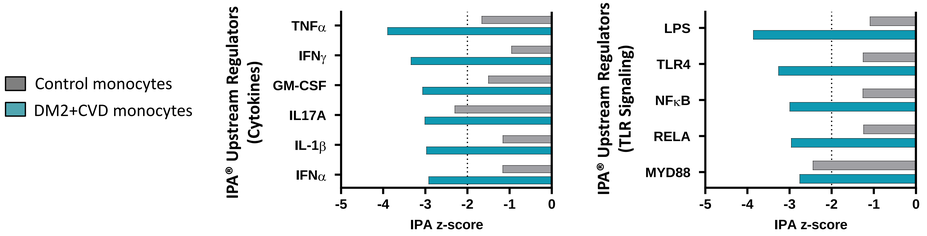
B. Apabetalone attentuates cytokine secretion (24h) in monocytes from DM2+CVD patients.



Statistics: Two-Way Repeated Measures ANOVA, Tukey's test for multiple comparisons. *** p-value<0.001

C. Apabetalone downregulates cytokine and TLR gene targets more robustly in monocytes from DM2+CVD patients as compared to controls (4h).

IPA® z-score computes directional changes in gene expression to predict overall pathway activation. z-score < -2 = inhibition.



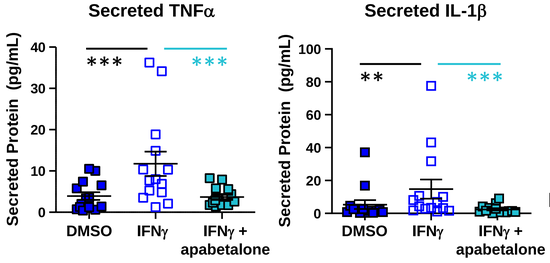
DM2+CVD Monocytes *versus* Controls: IFNγ Stimulation
Apabetalone Counters Monocyte Hyperactivation *Ex Vivo*

A. DM2+CVD monocytes have a greater transcriptional response to IFNγ stimulation (4h) and a greater sensitivity to apabetalone inhibition

Gene Name	Function	DM2+CVD *vs.* Control: IFNγ: Fold Difference	Controls % Suppression by Apabetalone (4h)	DM2+CVD % Suppression by Apabetalone (4h)
CCL7	Chemokine MCP-3	2.0	-93%	-90%
CCL8	Chemokine MCP-2	1.7	-83%	-85%
TNF	Cytokine TNFα	1.7	No change	-33%
RELA	NF-κB complex	1.3	-18%	-42%
MYD88	NF-κB signaling adaptor	1.3	-22%	-40%
IFITM1	Viral response	0.7	-66%	-72%

Statistics: Two-Way Repeated Measures ANOVA, Bonferroni's test (in-between group comparisons) or Tukey's test (within-group comparisons). Significance defined as p-value <0.05.

B. Apabetalone counters IFNγ-induced cytokine secretion in monocytes from DM2+CVD patients



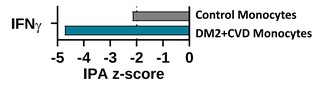
Statistics: Two-Way Repeated Measures ANOVA, Tukey's test for multiple comparisons. ** p<0.01, *** p-value<0.001

C. Apabetalone downregulates IFNγ targets

DM2+CVD monocytes + IFNγ (4h)			
Chemokines		**Pattern Recognition**	
CCL2	-91%	*TLR8*	-68%
CCL7	-90%	*LY96*	-67%
CXCL1	-88%	*TLR1*	-60%
CCL8	-85%	*FPR2*	-58%
CXCL9	-80%	*TICAM2*	-44%
CCR1	-79%	*RELA*	-42%
CXCL10	-61%	*MYDD88*	-40%
oxLDL Receptor		**ROS Production**	
MSR1	-79%	*CYBB*	-42%

Statistics: Two-Way RM ANOVA, Tukey's test. Significant p<0.05

D. IFNγ signature is preferentially inhibited by apabetalone in DM2+CVD monocytes



Summary and Conclusions

- Monocytes from DM2+CVD patients show a hyperactive pro-inflammatory phenotype *ex vivo*, in non-stimulated and stimulated conditions, despite standard of care therapy.
- Apabetalone reverses this hyperactivation by downregulating key inflammatory genes and secreted cytokines.
- Monocytes from DM2+CVD patients show greater gene sensitivity to BET inhibitor treatment. Data suggests a greater transcriptional dependency on BET proteins in diseased conditions.
- Findings support the development of apabetalone as a therapy for high risk CVD patients with epigenetic dysregulation of the innate immune response.

†**Disclosure:** Authors are employed by Resverlogix & hold stock and stock options