Hepatic Expression of C-Reactive Protein is Epigenetically Regulated by BET Proteins and Inhibited by Apabetalone (RVX-208) in Vitro and in CVD Patients

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ABSTRACT

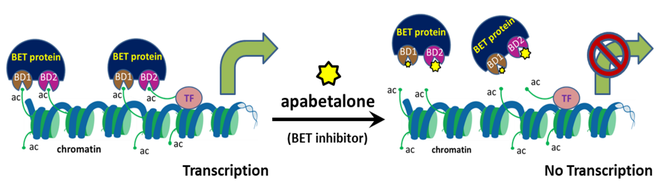
Chronic inflammation contributes to cardiovascular disease (CVD) and is characterized by elevated plasma levels of IL-6, IL-1β and C-reactive protein (CRP). CRP serves as a CVD stratification marker as it correlates with major adverse cardiac events (MACE). Here we show that hepatic induction of CRP in response to chronic cytokine signaling is regulated by epigenetic mechanisms in vitro and in patients.

Apabetalone is a small molecule inhibitor of epigenetic readers called bromodomain and extraterminal (BET) proteins that bind to acetylated DNA-associated proteins to regulate inflammatory gene transcription. In vitro, apabetalone attenuated CRP gene and protein expression under basal conditions in cultured primary human hepatocytes (PHH). Moreover, IL-6 and IL-1β mediated induction of CRP expression was also suppressed by apabetalone in both PHH and the HepaRG hepatic cell line. In HepaRG, PROTAC MZ-1 targeted degradation of BET proteins also reduced cytokine mediated CRP expression, demonstrating that inflammatory expression of CRP is BET-dependent. Short-term cytokine treatment increased occupancy of the BET family member BRD4 on the CRP promoter, which was countered by either apabetalone or a structurally unrelated BET inhibitor JQ1, as shown by chromatin immunoprecipitation (ChIP). These data directly link BRD4 to CRP transcription.

In a pooled analysis of phase 2 trials ASSERT, SUSTAIN and ASSURE, treatment with apabetalone resulted in a 62% relative risk reduction in MACE in patients with coronary artery disease (CAD) and elevated CRP (>2mg/L). In both ASSERT (12 weeks; n=55) and ASSURE (26 weeks; n=94), a comparison of baseline and end-of-study plasma proteome (SOMAscan 1.3K platform) detected a downregulation of inflammatory mediators, including CRP, in apabetalone treated patients versus placebo. Consequently, bioinformatics analysis of the proteomics data predicted an apabetalone-driven downregulation of inflammatory pathways.

MECHANISM OF ACTION

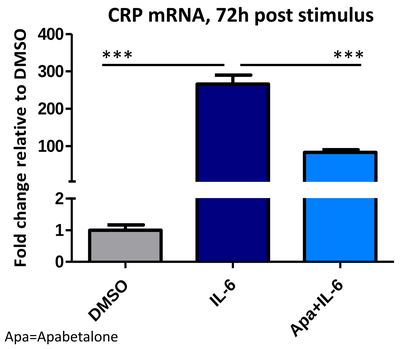
Apabetalone targets bromodomains in BET proteins, causing release from chromatin and downregulation of BET sensitive gene expression.

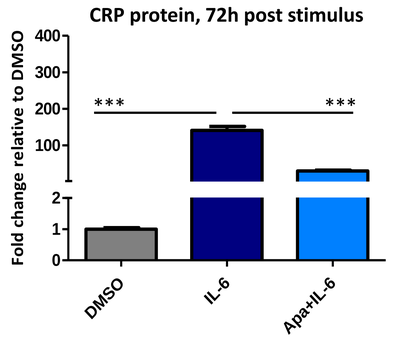


BET: bromodomain and extraterminal proteins
ac: acetylated lysine on DNA associated proteins
TF: transcription factor
BD: bromodomain
Yellow star size indicates selectivity of apabetalone for BD2

Apabetalone suppresses CRP expression *in vitro*

Primary human hepatocytes: Following 72h treatment with DMSO (vehicle) or 30 μM apabetalone ± 10 ng/mL IL-6, mRNA was analyzed by rtPCR; protein secretion in the final 24h of the experiment was examined by ELISA. 1-way ANOVA (Tukey's), *** p<0.001.

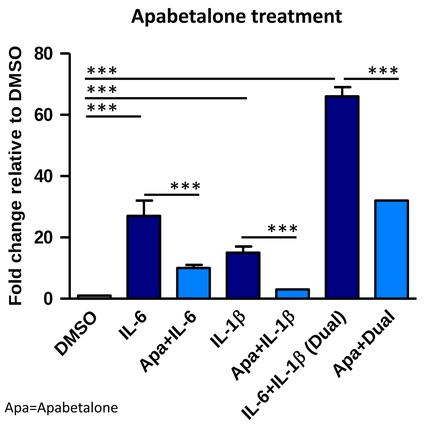




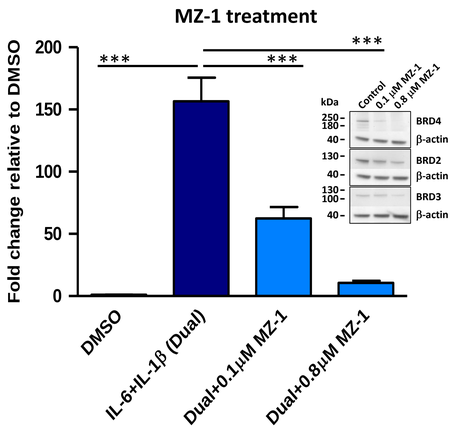
Apa=Apabetalone

CRP gene induction by cytokines is BET-dependent

HepaRG cells: Apabetalone counters early CRP induction by cytokines. 1h pre-incubation with DMSO or 25 μM apabetalone, followed by a 2h incubation with cytokines (10 ng/mL); mRNA analysis by rtPCR. 1-way ANOVA (Tukey's), *** p<0.001.

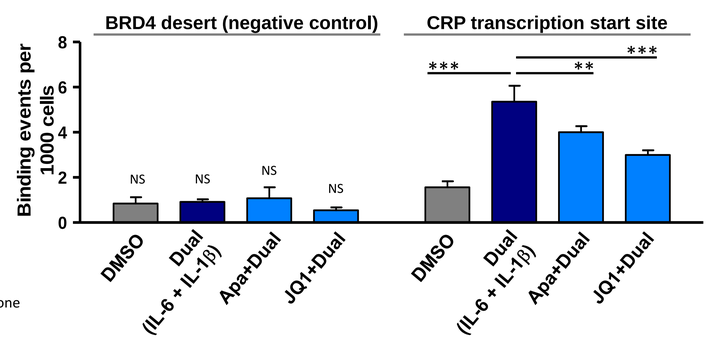


BET degrading PROTAC MZ-1 (24h pre-incubation) suppresses CRP gene induction in response to a 2h treatment with IL-6 and IL-1β; mRNA analysis by rtPCR. 1-way ANOVA (Tukey's),*** p<0.001.



Apa=Apabetalone

Apabetalone counters BRD4 recruitment to the CRP transcription start site during cytokine stimulation. ChIP from HepaRG cells pre-treated for 1h with DMSO, 25 μM apabetalone or 0.5 μM JQ1, folllowed by a 2h incubation with cytokines (10 ng/mL). 1-way ANOVA (Tukey's), ** p<0.01, *** p<0.001, NS, not significant.



Apa=Apabetalone

Apabetalone reduces plasma CRP and inflammation pathways in CVD patients

SOMAscan™ Proteomics: % Change in Plasma C-Reactive Protein Abundance Phase 2 Clinical Trials in CVD Patients Receiving Standard of Care

Study	# Patients in Study		Apabetalone vs. Placebo	p-value vs. Placebo
	Apabetalone	Placebo		
ASSERT (3 months)	n=25	n=30	-42.7 %	0.01
ASSURE (6 months)	n=47	n=47	-21.3 %	0.02

Apabetalone downregulates inflammatory pathways in CVD patients

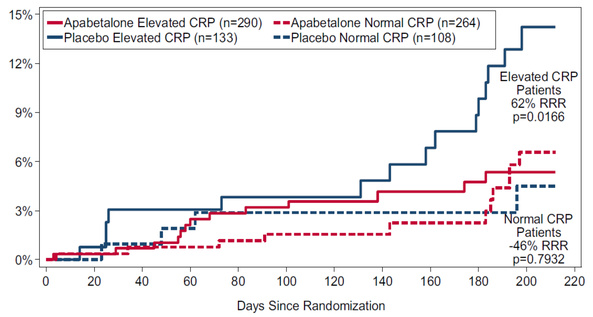
Bioinformatics (IPA®) Analysis of the Plasma Proteome (SOMAscan™) from the ASSERT Phase 2 Trial

Ingenuity® Pathway Analysis (IPA®)	Pathway/ Regulator#	Activation z-score*	p-value of overlap§
Canonical Pathway	Acute Phase Response Signaling	-2.1	$2.4x10^{-10}$
Upstream Regulators	Lipopolysaccharide	-3.1	$8.9x10^{-13}$
	Interleukin 6	-2.6	$7.1x10^{-15}$
	Interferon γ	-2.6	$2.5x10^{-10}$
	Oncostatin M	-2.0	$6.5x10^{-7}$
	Interleukin 1α	-2.0	$2.0x10^{-6}$
	Nuclear factor κB subunit 1	-1.8	$5.2x10^{-5}$

Plasma proteins affected by apabetalone treatment by more than 10% (*versus* placebo, $p<0.05$) were analyzed with the IPA® Canonical Pathway and Upstream Regulator analytics.
*IPA® z-score compares the observed differential regulation of a gene in the dataset to changes predicted by the literature which can be either "activating" or "inhibiting". z-score <-2 predicts downregulation within a gene set associated with a transcriptional regulator.
§The overlap p-value measures whether there is a statistically significant overlap between the dataset genes and the genes that are regulated by a transcriptional regulator. It is calculated using Fisher's Exact Test, and significance is attributed to *p*-values<0.01.

Apabetalone reduces MACE in CAD patients with elevated CRP at baseline (> 2 mg/L)

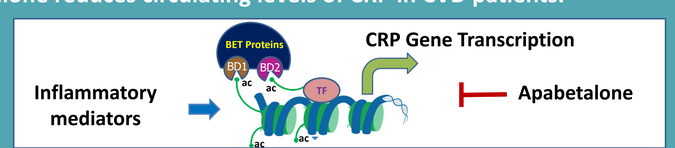
- Pooled analysis of CAD patients (n=798) who received apabetalone over 3 to 6 month period (phase 2 trials ASSERT, SUSTAIN and ASSURE).
- MACE defined as death, myocardial infarction, coronary revascularization and hospitalization for CVD causes.
- Log-Rank test was used to compare MACE between two groups.



Nicholls et al., 2017, *Am. J. Cardiovasc. Drugs*

SUMMARY

- **Apabetalone reduces CRP expression in resting and cytokine-treated human hepatocytes.**
- **BET proteins drive inflammatory CRP expression in human hepatocytes.**
- **Apabetalone reduces circulating levels of CRP in CVD patients.**



- **Apabetalone-mediated downregulation of inflammatory pathways in CVD patients may contribute to reduction in MACE observed in clinical trials.**

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