Apabetalone (RVX-208), a Selective BET Protein Inhibitor, Reduces Expression of Acute Phase Response Markers In Vitro and in Patients with Cardiovascular Disease and Chronic Kidney Disease

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Abstract

Apabetalone (RVX-208) is an inhibitor of the epigenetic readers bromodomain and extraterminal (BET) proteins, currently in a phase 3 outcomes trial in patients with cardiovascular disease (CVD) and diabetes mellitus. A post hoc analysis of phase 2b trials demonstrated a 55% relative risk reduction in major adverse cardiac events (MACE) in CVD patients. Elevated inflammatory markers correlate with CVD. Inflammation also accompanies chronic kidney disease (CKD) and CKD patients are at risk of CVD. Previous research has shown that apabetalone modulates pathways that contribute to chronic inflammation, including the acute phase response (APR). Here, pathway analysis of gene microarrays showed downregulation of APR by apabetalone in primary human hepatocytes (PHH). Real-time PCR and ELISA analysis of RVX-208 treated PHHs confirmed that APR genes that correlate with CVD are suppressed by 20 to 95%, including CRP, ceruloplasmin (CP), serum amyloid P (SAP), PAI-1, alpha 2-macroglobulin (A2M), complement C2, C3 and C5, MBL2, serum amyloid A and interleukin 18. Apabetalone decreased IL-6-induced expression of CP, SAP and A2M, with most striking effects on CRP (-75%). Apabetalone also decreased LPS-induced expression of SAP in a mouse endotoxemia model. To assess effects of apabetalone on inflammatory mediators in CVD patients, SOMAscan™ 1.3K proteomic analysis was performed on plasma from phase 2b ASSERT (12 weeks; n=25) and ASSURE (26 weeks; n=47) clinical trials. This approach identified APR as the top downregulated pathway by apabetalone in both trials. APR biomarkers are elevated in CKD patients where they correlate with disease progression. To gain insight into the pharmacodynamics of the APR response to apabetalone, stage 4 CKD patients (n=8) received a single dose of the drug followed by plasma proteomics at several time points. At 12h post dose, APR was significantly downregulated by apabetalone. Of note, CRP was decreased in CKD patients after 12h of treatment (-7%, p=0.04) versus baseline, as well as in ASSERT (-43%, p=0.01) and ASSURE (-21%, p=0.02) trials versus placebo. Downregulation of the APR pathway by apabetalone may lead to reduced chronic inflammation in CVD and CKD patients and contribute to the reduction in MACE in patients with high residual CVD risk.

Results

1A. Apabetalone reduces expression of the acute phase response (APR) pathway in primary human hepatocytes

Bioinformatics Analysis of Gene Expression in PHH (GSEA) Rank and Normalized Enrichment Score (NES)	
Gene Microarray	**Acute Phase Response Pathway**
Hepatocyte Donor 1	Rank: 2nd out of 1330 pathways NES = -2.3 (predicted downregulation)
Hepatocyte Donor 2	Rank: 23rd out of 1330 pathways NES = -1.9 (predicted downregulation)

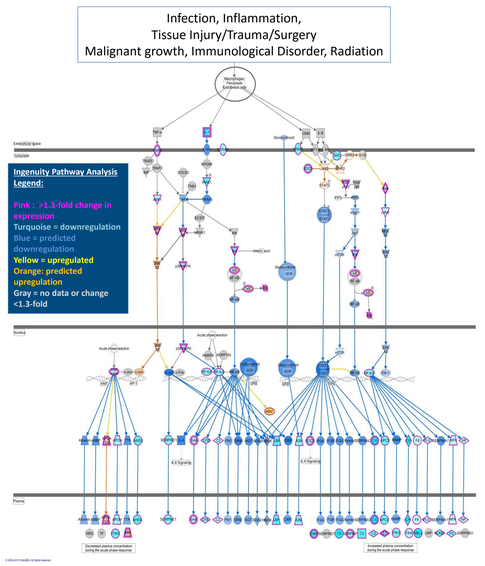
p-value<0.05

1B. Apabetalone reduces expression of APR genes in primary human hepatocytes (PHH)

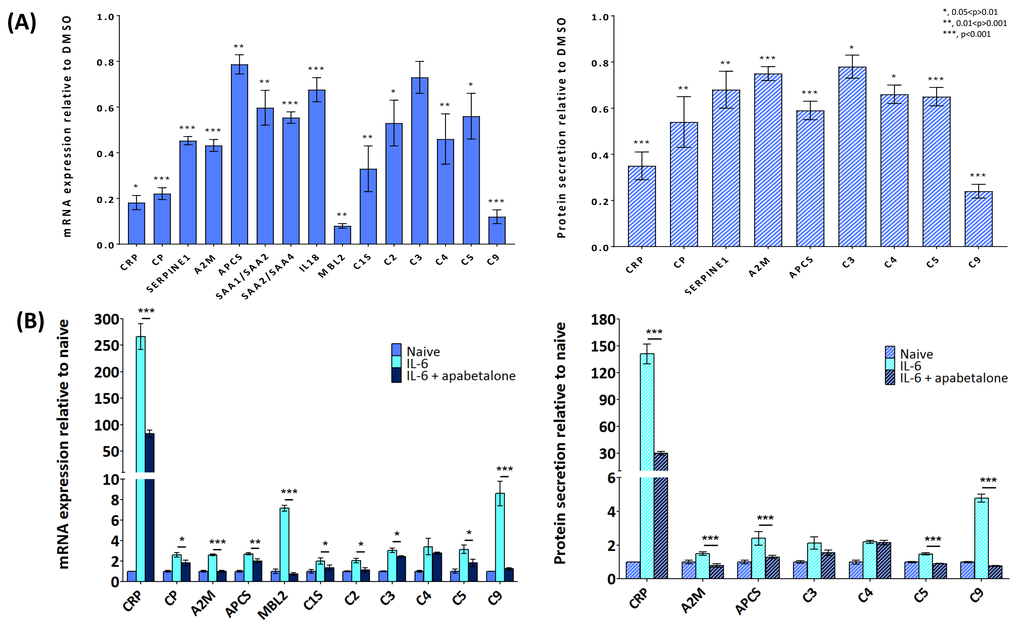
Gene Expression Microarray from Primary Human Hepatocytes (Fold Change)			
Gene Name	**Gene Symbol**	**Donor 1**	**Donor 2**
Mannose-binding lectin	MBL2	**0.09**	**0.25**
Complement component 9	C9	**0.11**	**0.21**
Ceruloplasmin	CP	**0.19**	**0.29**
Interleukin 1 receptor antagonist	IL1RN	**0.33**	**0.61**
Complement component 4A	C4A /C4B	**0.34**	**0.48**
Complement component 5	C5	**0.47**	**0.78**
Alpha-2-HS-glycoprotein	AHSG	**0.47**	**0.29**
Kallikrein B	KLKB1	**0.48**	1.04
Complement component 1s	C1S	**0.5**	**0.75**
Amyloid P component, serum	APCS	**0.5**	**0.57**
Thrombin	F2	**0.54**	**0.86**
Plasminogen Activator Inhibitor	SERPINE1	**0.54**	**0.37**
Complement component 2	C2	**0.55**	**0.86**
Alpha-2-macroglobulin	A2M	**0.56**	**0.6**
TNF receptor superfamily 1B	TNFRSF1B	**0.66**	**0.78**
Complement component 3	C3	**0.7**	1.08
Serum amyloid A2/A4	SAA2/SAA4	**0.73**	**0.57**
Haptoglobin	HP	**0.73**	**0.76**
TNF receptor superfamily 1A	TNFRSF1A	0.74	**0.74**
Serum amyloid A1/A2	SAA1/SAA2	0.8	**0.78**
Orosomucoid 1	ORM1	**0.81**	0.94
Interleukin 18	IL18	0.85	**0.35**
Osteoprotegerin	TNFRSF11B	0.85	**0.64**
Histidine-rich glycoprotein	HRG	1.04	**2.08**
C-reactive protein	CRP	1.07	**0.54**
Transthyretin	TTR	1.26	**1.4**
Apolipoprotein A-I	APOA1	1.94	**2.31**

Legend: Expression relative to DMSO-treated controls (1);
blue = downregulated; white = no change; yellow = upregulated;
bold: p-value<0.05.

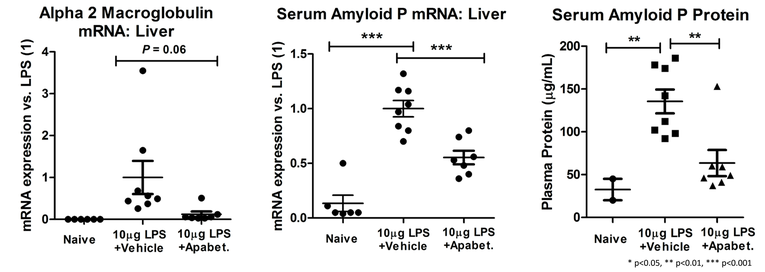
Ingenuity Pathway Analysis (IPA): Acute Phase Response Pathway
Fold changes in mRNA abundance in apabetalone vs. DMSO-treated primary human hepatocytes



2. Apabetalone downregulates APR expression in PHH at steady state (A) and in inflammatory conditions (B)



3. Apabetalone reduces expression of APR genes in a mouse model of inflammation



4. Apabetalone downregulates the APR pathway in CKD or CVD patients

Bioinformatics (IPA) Analysis of the Plasma Proteome (SOMAscan™)	
Trial	**Acute Phase Response Pathway**
Phase I, Safety & PK in Subjects with Severe Renal Impairment (CKD) 12 hours post single 100mg dose (n=8)	Rank: 6th out of 327 pathways IPA z-score: -5.6 (predicted downregulation)
ASSERT phase 2b trial 12 weeks of apabetalone treatment of patients with CVD 200mg daily (n=25) vs. placebo (n=30)	Rank: 2nd out of 320 pathways IPA z-score: -2.1 (predicted downregulation)
ASSURE phase 2b trial 26 weeks of apabetalone treatment of patients with CVD 200mg daily (n=47) vs. placebo (n=47)	Rank: 1st out of 351 pathways IPA z-score: -2.0 (predicated downregulation)

5. Apabetalone reduces levels of circulating CRP in CVD and CKD patients

SOMAscan™ Proteomics Data: % Change in Plasma C-Reactive Protein Abundance in Phase I PK Trial in CKD Patients or Matched Controls Without CKD			
Study	**# of Patients in Study**	**Apabetalone vs. baseline 12 hr post single dose**	**p-value vs. baseline**
Cohort 1- End stage renal disease not on dialysis; eGFR < 30 mL/min/1.73m^2	n=8	-7.3 %	0.04
Cohort 2- Matched subjects, no kidney disease	n=8	-12.6 %	0.15

SOMAscan™ Proteomics Data: % Change in Plasma C-Reactive Protein Abundance in Phase 2 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

Summary

- Acute phase response (APR) is amongst the top pathways downregulated by apabetalone in primary human hepatocytes and in plasma from treated patients with cardiovascular disease (CVD) or chronic kidney disease (CKD).
- Apabetalone reduces expression of APR genes linked to CVD risk in resting and cytokine-treated primary human hepatocytes.
- Apabetalone downregulates expression of APR genes in a mouse model of inflammation and endotoxemia.
- In three clinical trials, apabetalone reduces circulating levels of C-reactive protein (CRP), an APR protein that correlates with inflammation and independently predicts adverse cardiovascular events.
- Apabetalone-mediated downregulation of the APR pathway in CVD patients may contribute to reductions in MACE observed in clinical trials.

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