Apabetalone (RVX-208) Reduces Expression of Acute Phase Response Markers in Vitro and in Patients with Cardiovascular Disease

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Introduction

Apabetalone (RVX-208) is an inhibitor of the epigenetic regulators 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. In vitro, apabetalone modulates expression of genes that underlie CVD, including those in the acute phase response pathway (APR).

Hypothesis

Since APR proteins are inflammatory markers known to correlate with CVD outcomes, and BET inhibitors have anti-inflammatory properties, apabetalone treatment may downregulate APR expression in vitro and in patients.

Methods

Microarrays, real-time PCR and ELISA were performed on primary human hepatocytes (PHH). SOMAscan™ proteomic analysis was performed on plasma from the phase 2b ASSERT (12 weeks; n=55) and ASSURE (26 weeks; n=94) clinical trials. SOMAscan™ uses aptamers (short DNA sequences with "protein-like" side chains), each of which is highly specific for its cognate protein, to measure approximately 1300 protein analytes.

Results

Microarrays of apabetalone treated PHH showed downregulation of the APR pathway (**Fig. 1**). APR genes that correlate with CVD and MACE were suppressed by 20 to 95%, including C-reactive protein (CRP), ceruloplasmin (CP), serum amyloid P (APCS), plasminogen activator inhibitor (SerpinE1), alpha 2-macroglobulin (A2M), complement C2, C3 and C5, MBL2, serum amyloid A (SAA) and interleukin 18 (**Fig. 2 and 3A**). Apabetalone decreased the IL-6-induced expression of CP, SAP and A2M, with most striking effects on CRP (75% reduction) (**Fig. 3B**). Interestingly, in PHH, CRP mRNA was upregulated two-fold by the pro-inflammatory metabolite trimethylamine N-oxide (TMAO). Apabetalone not only countered this increase, but also decreased the expression of the TMAO producing enzyme FMO3 by 40% (**Fig. 3C**). Consistent with the findings above, plasma proteomics analysis identified APR as the top downregulated pathway by apabetalone in both clinical trials (**Fig. 4**). Circulating levels of C2, C3, C5, and SAP were significantly decreased, and CRP was downregulated by 43% (p=0.01) and 21% (p=0.02) versus placebo in ASSERT and ASSURE, respectively (**Fig. 5**).

Conclusion

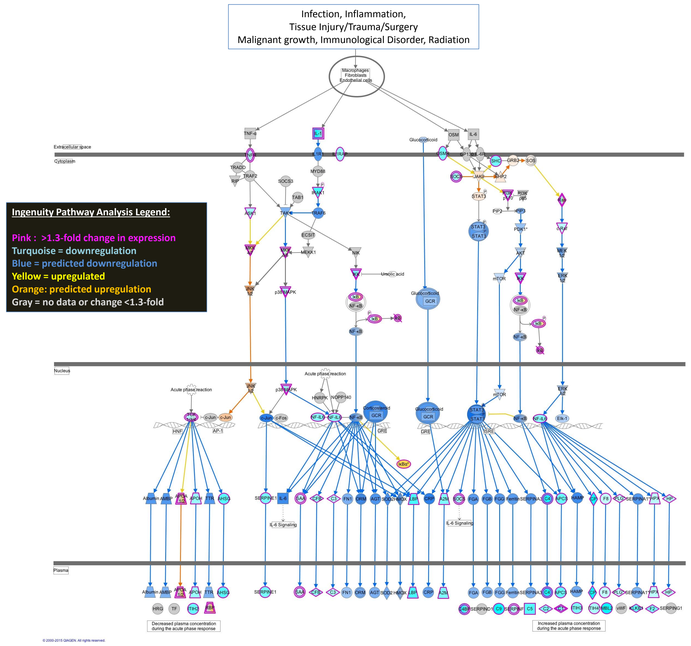
BET inhibition by apabetalone decreases basal and inflammatory transcription of APR markers which correlate with CVD. Clinical trials demonstrate that apabetalone reduces circulating levels of APR proteins, which may partly contribute to the reduction in MACE in patients with high residual CVD risk.

1. 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

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

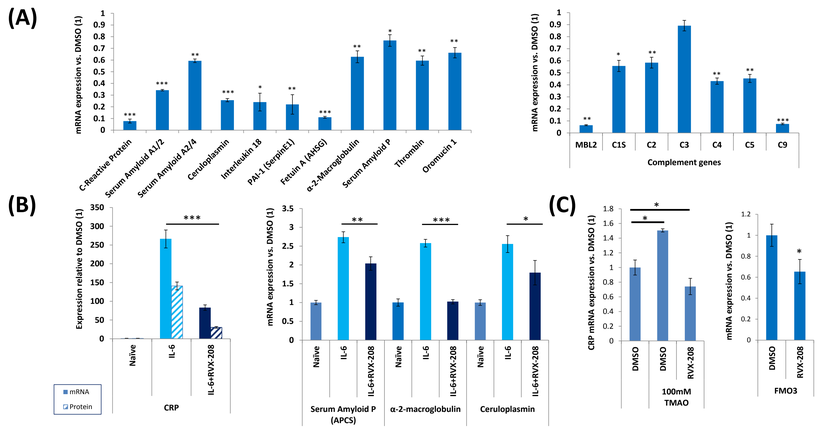


2. Apabetalone reduces expression of APR genes that correlate with CVD risk

Gene Expression Microarray Data from Primary Human Hepatocytes (relative to 1)			
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 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	**0.50**	**0.75**
Amyloid P component, serum	APCS	**0.50**	**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**	**0.60**
Tumor necrosis factor receptor superfamily 1B	TNFRSF1B	**0.66**	**0.78**
Complement component 3	C3	**0.70**	1.08
Serum amyloid A2/A4	SAA2/SAA4	**0.73**	**0.57**
Haptoglobin	HP	**0.73**	**0.76**
Tumor necrosis factor receptor superfamily 1A	TNFRSF1A	0.74	**0.74**
Serum amyloid A1/A2	SAA1/SAA2	0.80	**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, pentraxin-related	CRP	1.07	**0.54**
Transthyretin	TTR	1.26	**1.40**
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.

3. Apabetalone downregulates APR expression in primary human hepatocytes at steady state (A) and in inflammatory conditions (B and C)



4. Downregulation of the APR pathway in CVD patients treated with apabetalone

Bioinformatics (IPA) Analysis of the Plasma Proteome (SOMAscan™)	
Trial	**Acute Phase Response pathway**
ASSERT phase 2b trial 12 weeks of apabetalone treatment 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 200mg daily (n=47) vs. placebo (n=47)	Rank: 1st out of 351 pathways IPA z-score: -2.0 (predicated downregulation)

5. Apabetalone treatment reduces levels of circulating APR proteins in CVD patients

SOMAscan™ Proteomics Data: % Change in Serum Protein Abundance				
	Protein Name	**Gene Symbol**	**Apabetalone vs. placebo**	**p-value vs. placebo**
ASSERT	C-reactive protein	CRP	-42.7	0.01
	D-dimer	FGA GGB FGG	-19.0	0.02
	Fibrinogen	FGA FGB FGG	-19.0	0.03
	Fibrinogen gamma chain	FGG	-16.9	0.01
	Interleukin-1 receptor antagonist protein	IL1RN	-13.8	0.003
	Complement C3	C3	-13.3	0.05
	Complement C4	C4	-10.1	0.04
ASSURE	C-reactive protein	CRP	-21.3	0.02
	Osteoprotegerin	TNFRSF11B	-14.0	0.003
	Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B	-11.2	0.01
	Plasma kallikrein	KLKB1	-11.1	0.001
	Complement C2	C2	-10.9	0.0002
	Complement C5	C5	-10.8	0.0001
	Serum amyloid P-component	APCS	-10.8	0.001

Summary

- **Acute phase response is amongst the top downregulated pathways by apabetalone in primary human hepatocytes and in plasma from treated patients.**
- **Apabetalone reduces expression of APR genes linked to CVD risk and MACE, including CRP, in resting and cytokine-treated primary human hepatocytes.**
- **In CVD patients from two clinical trials, apabetalone reduces levels of circulating APR proteins that correlate with CVD risk, including CRP, fibrinogen, complement proteins and serum amyloid P.**
- **Apabetalone-mediated downregulation of the APR pathway in CVD patients may contribute to reductions in MACE observed in clinical trials.**