Modulation of the complement cascade in cardiovascular disease patients by a bromodomain and extraterminal (BET) protein inhibitor

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Background

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 (DM). BET proteins control recruitment of transcriptional machinery to coordinate gene transcription. BET inhibition by apabetalone has been shown to modulate gene expression of pathways that underlie CVD including reverse cholesterol transport, vascular inflammation, coagulation and complement. Patients with acute coronary syndrome (ACS) have increased post-infarction plasma levels of C5b-9 and levels of C3 and C4 correlate with CVD risk, indicating a role of the complement system in the pathogenesis of CVD.

Purpose

To investigate the effect of BET inhibition on the abundance and function of the complement cascade in clinical samples and cultured cells.

Methods

To assess apabetalone induced changes in levels of circulating complement proteins, plasma from the phase 2b trial ASSURE (n=47) was used to perform a SOMAscan proteomic analysis (measure of ~1300 analytes). SOMAscan™ uses aptamers (short DNA sequences with "protein-like" side chains), each of which is highly specific for its cognate protein. Function of the complement cascade was determined in CH50 and AH50 hemolytic assays (n=11). Primary human hepatocytes (PHH) exposed to apabetalone were surveyed by microarray, mRNA assay and complement protein secretion.

Results

Complement is the top pathway downregulated in CVD patients receiving apabetalone as assessed by the bioinformatics analysis of the plasma proteome (Figure 1). Numerous circulating complement proteins were reduced from 5-50% versus baseline (p-value<0.05) (Table 1). This translated into a reduction in the function of the complement cascade by 26% in both the CH50 and AH50 hemolytic assays after 26 weeks of apabetalone treatment (p<0.01) (Figure 2). This modulation of complement activity did not increase the rate of infections or infestations in the phase 2b trials. Widespread effects of BET inhibition on complement genes and proteins were further confirmed in PHH exposed to apabetalone, where basal and inflammatory stimuli induced mRNA and secretion of complement components C3, C4, C5 and C9 which were decreased by 20-90% (Figure 3).

Conclusion

The role of the complement pathway in the pathogenesis of CVD and ACS represents a novel target for therapeutic intervention. By modulating innate immune pathways such as complement, apabetalone may impact disease and lower the incidence of MACE in patients with high residual CVD risk.

Figure 1. Downregulation of complement cascade in CVD patients treated with apabetalone

Bioinformatics analysis of plasma proteome	
ASSURE phase 2b trial 26 weeks of apabetalone treatment 200mg daily (n=47)	COMPLEMENT CASCADE Rank: 1/332 NES Score: -2.35

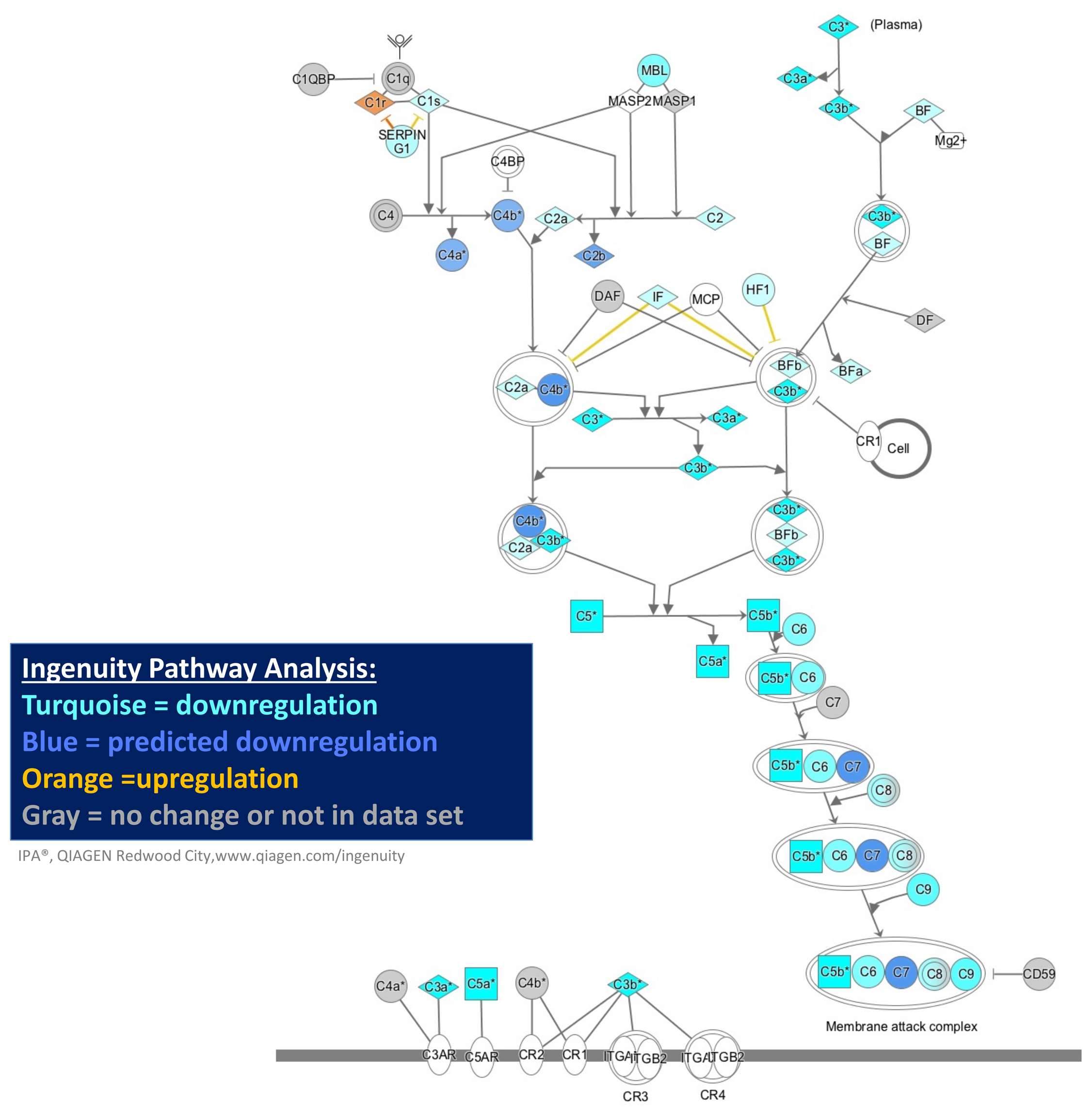
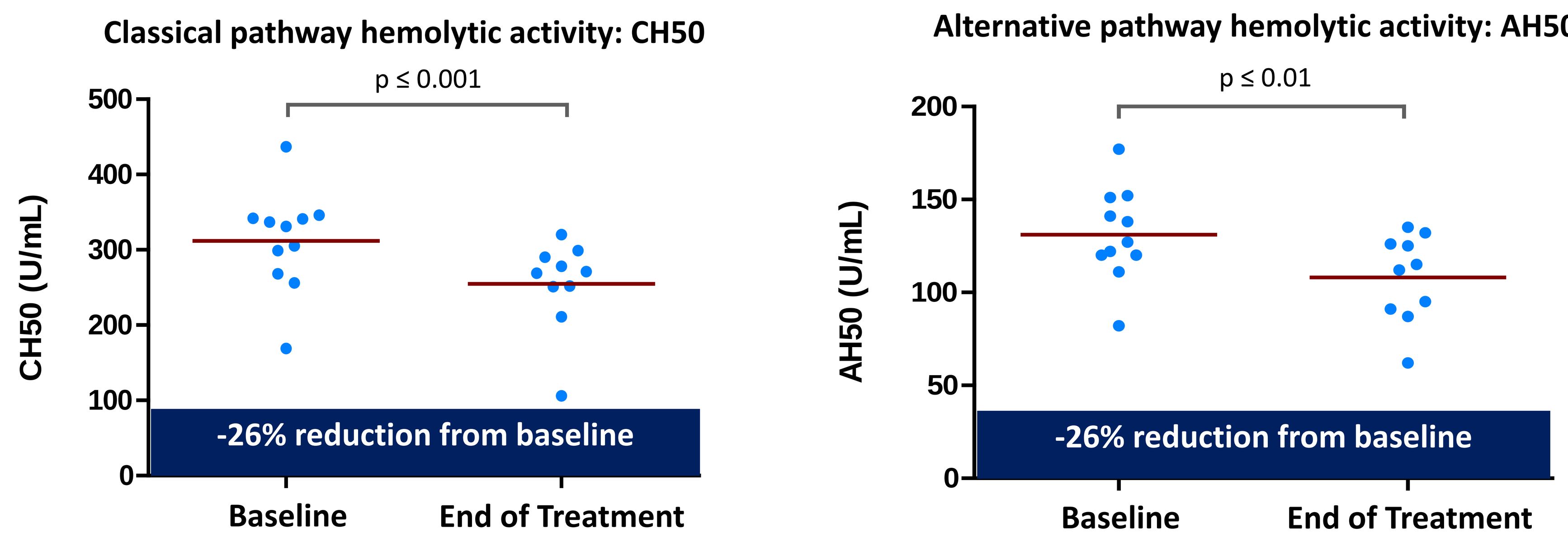


Table 1. Apabetalone treatment reduces levels of multiple circulating complement factors, activators and inhibitors in CVD patients

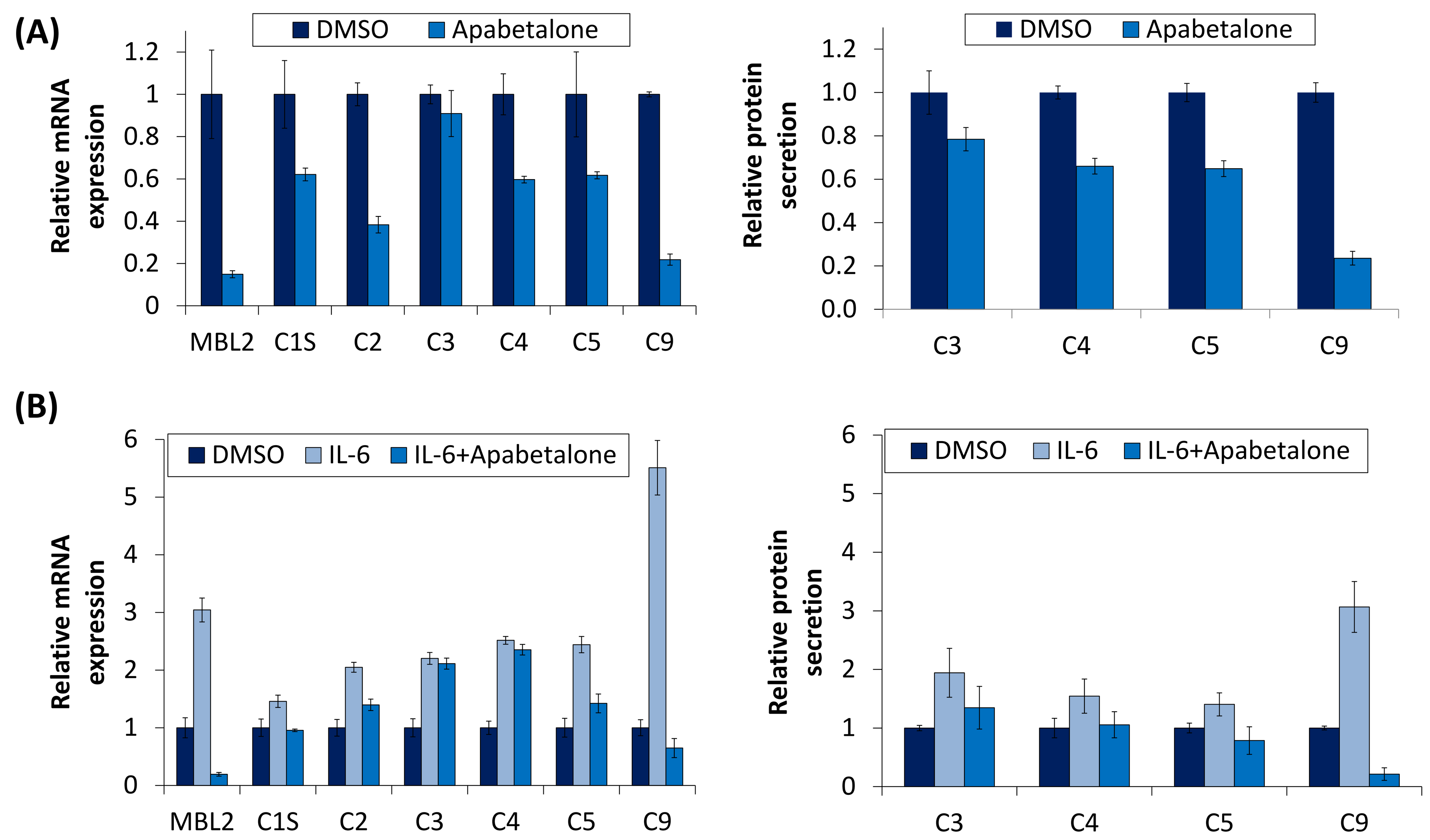
ASSURE: 26-Week Phase 2b Trial, Apabetalone 200mg daily, n=47 Placebo, n=47		SOMAscan™ Proteomic Data % Change in Protein Abundance			
Protein Name	**Gene Symbol**	**Apabetalone vs. Baseline**	**p-value**	**Apabetalone vs. Placebo**	**p-value**
Complement C3b	C3	-52.7	0.001	-13.4	0.4
C-reactive protein	CRP	-43.6	0.0001	-21.3	0.02
C5a anaphylatoxin	C5	-28.7	0.0002	-51.4	0.0001
Complement component C9	C9	-18.3	0.0001	-10.4	0.4
Mannose-binding protein C	MBL2	-14.6	0.0002	-6.3	0.2
Complement component C6	C6	-14.3	0.0001	-16.2	0.002
Serum amyloid P-component	APCS	-12.9	0.0001	-10.8	0.001
Complement C5b-C6 complex	C5 C6	-12.0	0.0001	-10.4	0.002
Complement C5	C5	-11.7	0.0001	-10.8	0.0001
Complement component C8	C8	-10.1	0.004	-12.0	0.009
Plasma protease C1 inhibitor	SERPING1	-8.5	0.01	-11.9	0.007
Complement factor B	CFB	-6.8	0.001	-5.7	0.05
Complement C2	C2	-6.7	0.001	-10.9	0.0002
Complement factor I	CFI	-6.4	0.01	-4.1	0.1
Complement C1s	C1S	-6.1	0.02	-4.1	0.08
Complement factor H	CFH	-5.6	0.0001	-3.6	0.05

Figure 2. Apabetalone reduces activity of the overall complement cascade in plasma from CVD patients



A reduction in complement cascade activity potentially decreases chronic pro-inflammatory signaling which contributes to disease progression in CVD patients.

Figure 3. Basal (A) and cytokine-induced (B) expression of complement components are decreased by apabetalone at the mRNA and secreted protein level in primary human hepatocytes (PHH)



Summary

- Apabetalone reduces basal and cytokine-induced expression of complement factors in hepatocytes.
- Complement is the top pathway downregulated by apabetalone in plasma from CVD patients.
- Apabetalone reduces levels of complement proteins linked to CVD and major adverse cardiac events (MACE).
- Apabetalone reduces overall complement activity in plasma from CVD patients.
- This reduction in complement activity does not impact rate of infections or infestations in patients.