Apabetalone (RVX-208), a Selective Bromodomain and Extra-Terminal (BET) Protein Inhibitor, Decreases Abundance and Activity of Complement Proteins in Vitro, in Mice and in Clinical Studies

Sylwia Wasiak[1], Dean Gilham[1], Kamyar Kalantar-Zadeh[2], Christopher Halliday[1], Mike Sweeney[3], Jan Johansson[3], Norman C Wong[1] and Ewelina Kulikowski[1]

[1]Resverlogix Corp, R&D, Calgary, AB, CANADA, [2]University of California Irvine School of Medicine, Irvine, CA, [3]Resverlogix Corp, Clinical Development, San Francisco, CA

INTRODUCTION AND AIMS

RVX-208, an orally active small molecule, reduced the incidence of major acute cardiac events in patients with cardiovascular disease (CVD) and potentially improved eGFR in a subpopulation with chronic kidney disease (CKD) in phase II clinical studies. RVX-208 inhibits the epigenetic readers bromodomain and extraterminal (BET) proteins by competing with acetylated lysines on histone tails that are associated with active transcription. Previously, microarray studies of primary human hepatocytes (PHH) treated with RVX-208 have shown downregulated expression of several complement genes (19 of 26). Since activation of complement in the kidney has been associated with renal disease and reduction in complement activation is beneficial for kidney function, we have examined the effect of RVX-208 on complement expression and activity in vitro, in mice and in clinical samples.

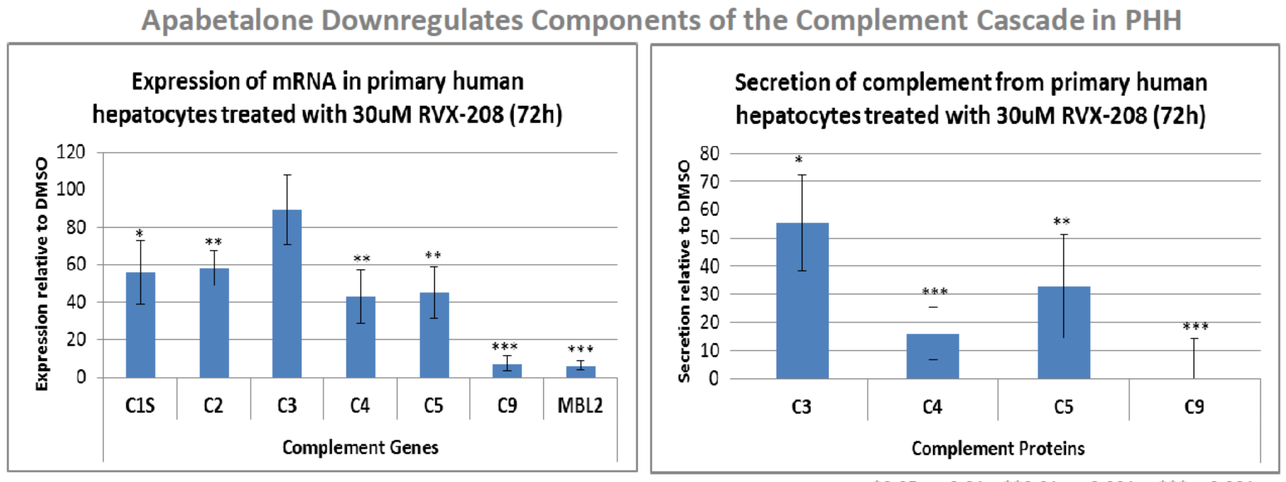
METHODS

Effects of RVX-208 on mRNA expression and secretion of complement proteins was examined in cultured PHH. Expression of complement genes was studied in mice with humanized livers treated with RVX-208 (150 mg/kg, 3 days, b.i.d.). Circulating complement proteins and complement activity were quantified from clinical samples in which patients had been treated with RVX-208 for 6 months, using proteomic approaches and hemolytic assays, respectively.

RESULTS

RVX-208 downregulated basal mRNA expression and protein secretion of complement C3, C4, C5 and C9 in cultured PHH by 10%-100%. In a cytokine induced inflammatory state, the observed reductions in mRNA expression and protein secretion was further confirmed. Chimeric mice with humanized livers treated with RVX-208 decreased expression of C4, C9 and MBL2 mRNA by 36%, 46% and 61%, respectively. Proteomic analysis of clinical samples from the ASSURE and SUSTAIN trials in patients with established CVD showed a significant decrease in circulating complement proteins following treatment with RVX-208 (between -5% and -20%, vs. baseline). To establish if the observed decrease in protein abundance affected -activity, CH50 and AH50 hemolytic assays were performed on 11 plasma samples at baseline and after 26 weeks of RVX-208 treatment. A significant decrease in complement activity of ~26% ($p<0.01$) was observed in both assays. No increase in infections have been reported in patients receiving RVX-208 in any trial.

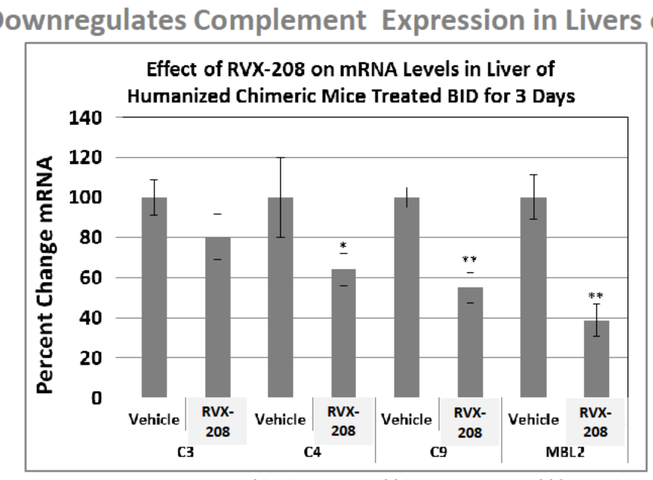
Apabetalone Downregulates Components of the Complement Cascade in PHH



*0.05>p>0.01 **0.01>p>0.001 ***p<0.001

RVX-208 treatment for 72h reduces mRNA expression and protein secretion of components of the complement cascade at steady state.

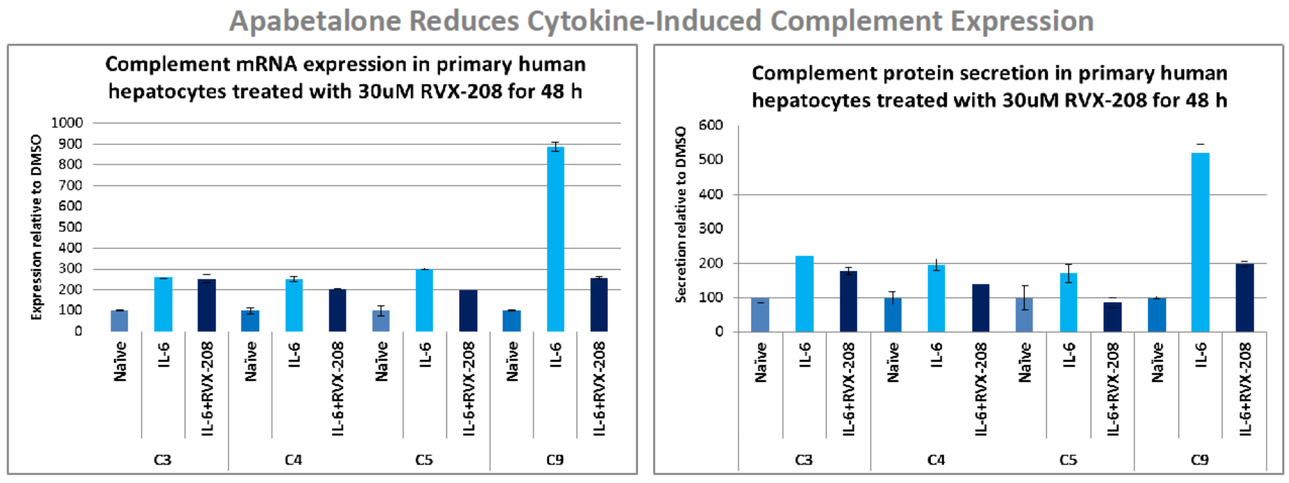
Apabetalone Downregulates Complement Expression in Livers of Chimeric Mice



*0.05>p>0.01 **0.01>p>0.001 ***p<0.001

Mouse model where mouse hepatocytes are replaced with human hepatocytes. Mice were treated with RVX-208, and expression of components of the complement cascade were measured by real-time PCR with human specific probes.

Apabetalone Reduces Cytokine-Induced Complement Expression



Inflammatory expression of complement genes was induced by 24h pre-treatment with IL-6 (10ng/mL) in PHH, subsequent co-administration of RVX-208 for 48h reduced inflammatory expression of complement.

Apabetalone Downregulates Expression of Complement in PHH Microarrays

Gene Set	Donor 1 -FBS	Donor 2 +FBS
REACTOME_COMPLEMENT_CASCADE	-2.33	-2.07
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	-2.38	-2.05

GSEA canonical pathway analysis shows downregulation of complement pathways

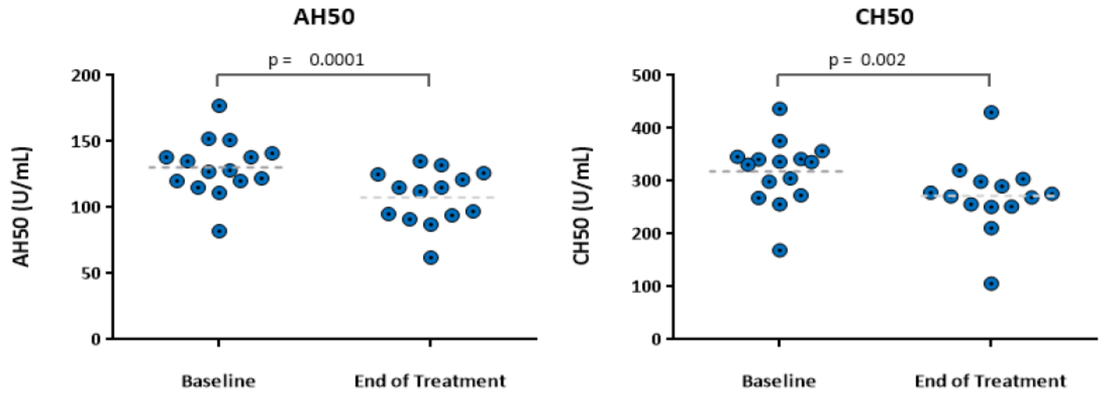
Apabetalone Reduces Levels of Circulating Complement Proteins in Clinical Plasma Samples

Percent Change from baseline

Protein	Gene Symbol	Placebo (n=47)	Apabetalone, 200mg daily (n=47)	Treatment Difference	p-value vs placebo
C5a anaphylatoxin	C5	22.7	-28.7	-51.4	0.0001
C-reactive protein	CRP	-22.3	-43.6	-21.3	0.02
Complement component C6	C6	0.9	-15.3	-16.1	0.002
Complement component C8	C8A C8B C8G	1.9	-10.1	-12.0	0.01
Complement C2	C2	4.2	-6.7	-10.9	0.0002
Complement C5	C5	-0.9	-11.7	-10.8	0.0001
Serum amyloid P-component	APCS	-2.1	-12.9	-10.8	0.001
Complement C5b-C6 complex	C5 C6	-1.6	-12.0	-10.4	0.002
Complement decay-accelerating factor	CD55	2.0	-6.3	-8.3	0.02
Complement C1r subcomponent	C1R	-14.9	14.8	29.7	0.01
Complement factor B	CFB	-1.1	-6.8	-5.7	0.05
Complement C1s subcomponent	C1S	-1.2	-6.1	-4.9	0.08
Complement component C7	C7	4.3	0.5	-3.8	0.06
Complement factor H	CFH	-2.0	-5.6	-3.6	0.05

The SOMAscan™ is a multiplexed, sensitive, and reproducible assay to measure 1,310 protein analytes in clinical samples. P-value vs placebo calculated using Mann-Witney test

Apabetalone Reduces the Activity of the Complement Cascade in Clinical Plasma Samples



Select CVD patients (n=11) treated with Apabetalone over 6 months demonstrated reduction in the activity of the complement cascade (classical and alternative) by 26% from baseline. p-value calculated using paired t-test.

CONCLUSIONS

RVX-208 decreases complement component expression and cascade activity, which may result in decreased pathologic activation of complement in CKD. The potential of RVX-208 for the treatment of high-risk CVD patients with type II diabetes and CKD is currently being explored in the phase III BETonMACE clinical study.

53 ERA
71--SP
Renal pathology. Experimental and clinical.
Norman Wong
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS VIENNA MAY 21-24, 2016 AUSTRIA
PosterSessionOnline.com