RVX-208, a selective bromodomain extra-terminal (BET) protein inhibitor, acts on several pathways to benefit cardiovascular risk.

Kulikowski, E.[1], Wasiak, S.[1], Gilham, D.[1], Halliday, C.[1], Lebioda, K.[1], Sweeney, M.[2], Johansson, J.[2], and Wong, N.C.W.[1]

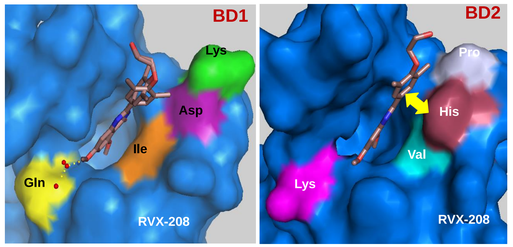
All authors are employees of Resverlogix Corporation [1]Calgary, AB, Canada and [2]San Francisco, CA, USA.

Abstract

The epigenetic modifying compound RVX-208 selectively inhibits the second ligand binding domain in BET proteins to increase ApoA-I/HDL production. In recent trials of CVD patients given standard of care and RVX-208 (200mg/d) there was a 55% relative risk reduction of MACE vs. placebo. This reduction is greater than expected from modest increases in ApoA-I/HDL, thus suggesting benefits of RVX-208 beyond lipid effects. Whether added benefits exist was explored by microarray survey of differential gene expression in primary human hepatocytes (PHH) and whole blood (WB). In PHH, RVX-208 downregulated genes within the complement, fibrin clotting, cholesterol biosynthesis, fatty acid biosynthesis, diabetes and acute phase response (APR) pathways. These data guided more focused analyses on the known HDL proteome encoded by 89 genes of which 30 were affected by RVX-208 and many of these were members of the APR or complement pathways. In support of the microarray data, we used RT-PCR and found RVX-208 lowered mRNA encoding complement 3, 4a/4b and 5 by ≥50% in Huh-7 and HepG2 cells exposed to RVX-208. Consistent with the mRNA data, secretion of these complement proteins were less from the treated cells. Next the cells were exposed to cytokines in order to mimic an inflammatory state thus inducing complement expression. Treatment with RVX-208 blocked this effect by >50%. Furthermore, in Huh-7 cells, RVX-208 repressed both fibrin clotting factors II (prothrombin) and XI (thromboplastin antecedent) consistent with the PHH microarray data thus suggesting an anti-thrombotic effect for RVX-208. To further extend findings in vitro from hepatoma cells, plasma from patients in our clinical trials who received RVX-208 were assayed and found to have decreased levels of both APR and complement proteins. Lastly, microarray studies of WB from healthy donors treated ex-vivo with RVX-208 uncovered differential regulation of anti- and pro-atherogenic gene sets that were activated and suppressed, respectively by RVX-208. In summary, RVX-208, a selective BET inhibitor, affects several pathways involved in CVD including; vascular inflammation, complement, thrombosis, reverse cholesterol transport, and atherogenesis that cumulatively may reduce MACE in high risk CVD patients.

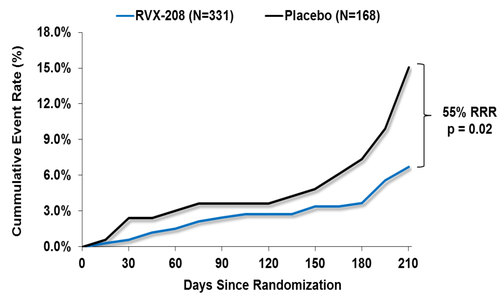
Results

1. RVX-208 selectively binds BD2 of BET proteins.



Each BET protein has dual bromodomains (BD1 & BD2) that bind to acetylated lysines found on histone tails to affect chromatin function. RVX-208 binds selectively to BD2.

2. RVX-208 (200 mg/day) added to standard of care lowers MACE in SUSTAIN and ASSURE human trials.

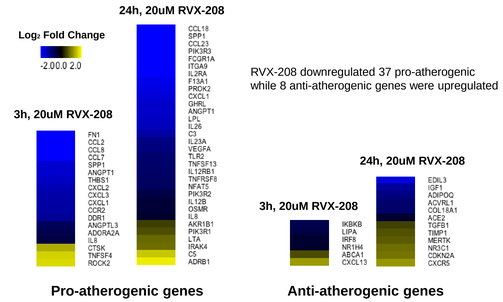


3. RVX-208 increases HDL-c and ApoA-I.

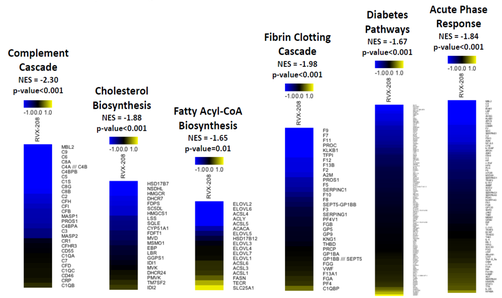
Biomarker	RVX-208 (n=331)		Placebo (n=166)		Difference Between Treatments (%▲)	p-value vs placebo
	Base line	Change from baseline (%▲)	Base line	Change from baseline (%▲)		
HDL-cholesterol (mg/dL)	39.0	+3.0 (+7.69)	38.0	0.0 (0.0)	+3.0 (7.69)	0.0003
ApoA-I (mg/dL)	119.2	+12.3 (+10.3)	118.1	+4.8 (+3.8)	+7.5 (6.5)	0.005

p-value for between group comparison calculated from a 2-sided Van Elteren test. Stratified by study.

4. RVX-208 beneficially affects expression of pro- and anti-atherogenic genes in human whole blood.



5A. RVX-208 suppresses pathways in primary human hepatocytes with known roles in MACE.



Normalized enrichment scores (NES) indicate downregulation of gene expression

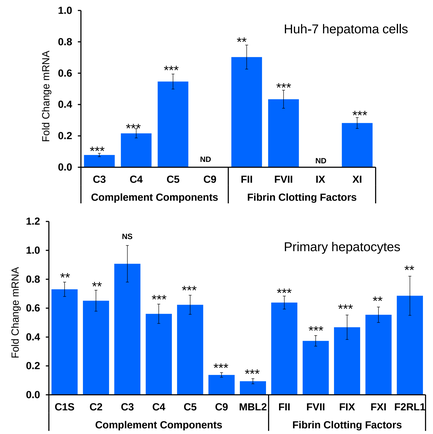
5B. RVX-208 suppresses expression of acute phase response genes associated with HDL.

Gene Name	Fold change	Functional category
complement component 9	0.24	hemostasis
ceruloplasmin (ferroxidase)	0.27	metal binding
alpha-2-HS-glycoprotein, fetuin A	0.31	inflammation
inter-alpha-trypsin inhibitor heavy chain 2	0.51	proteolysis/inhibition/inflammation
lipopolysaccharide binding protein	0.60	immune response
alpha-2-macroglobulin	0.63	hemostasis
serum amyloid A1/A2/A4	0.69	lipid metabolism and transport
amyloid P component, serum	0.72	inflammation
complement component 1, s subcomponent	0.74	immune response
coagulation factor II (thrombin)	0.76	hemostasis
apolipoprotein H	0.78	hemostasis
haptoglobin	0.82	inflammation
complement component 2	0.85	immune response
serpin peptidase inhibitor, clade A, member 4	1.36	proteolysis/inhibition
histidine-rich glycoprotein	1.93	metal binding/hemostasis
apolipoprotein A-I	2.17	lipid metabolism and transport

BLUE: REPRESSION; YELLOW: INDUCTION; p<0.05

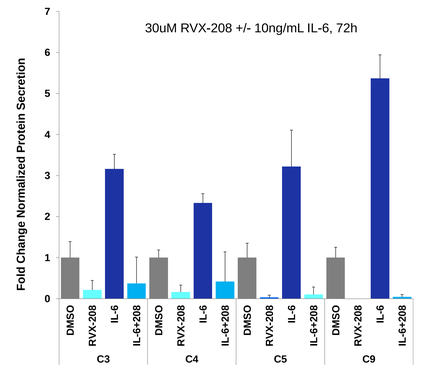
Gene expression changes = fold change relative to DMSO from microarray analysis of primary human hepatocytes exposed to 30uM RVX-208, 48h

5C. RVX-208 (30 uM for 72 hrs) reduces mRNA in complement and coagulation pathways.



ND: not detected; NS: not significant

5D. RVX-208 reduces basal and inflammatory secretion of complement from PHH.



6. RVX-208 reduces circulating complement C3 & acute phase response proteins in human samples

Analyte	N	Baseline	Units	Change from baseline	% Change from baseline	P-value vs baseline
Complement C3	20	1.10	mg/mL	-0.10	**-9.28**	0.002
Serum Amyloid P-Component	20	16.2	ug/mL	-2.64	**-15.0**	0.001
Ceruloplasmin	17	186	ug/mL	-19.9	**-7.35**	0.02

Note: Potential changes in complement and fibrin clotting pathways had no impact on rates of infection or bleeding in study patients.

Summary

RVX-208, an orally active small molecule, selectively inhibits BD2 in BET proteins leading to modest rises in ApoA-I and HDL.

RVX-208 induced lipid changes cannot account for the marked MACE reductions observed in SUSTAIN and ASSURE.

RVX-208 ex-vivo treatment of WB and HPP downregulate genes and pathways with known roles in CVD risk, including inflammatory mediators i.e. complement system and acute phase response.

RVX-208 also downregulates production of several complement proteins in vitro and circulating components of the complement system and APR in patients given RVX-208.

Conclusions

RVX-208 appears to have beneficial effects on CVD risk that go beyond raising ApoA-I/HDL that affect pathways including vascular inflammation, complement, thrombosis, reverse cholesterol transport, and atherogenesis.

These activities may provide biologic plausibility for the MACE reduction in SUSTAIN and ASSURE..