Apabetalone (RVX-208) has Anti-atherosclerotic, Anti-Thrombotic and Anti-Inflammatory Effects in Patients with Cardiovascular Disease.

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Abstract

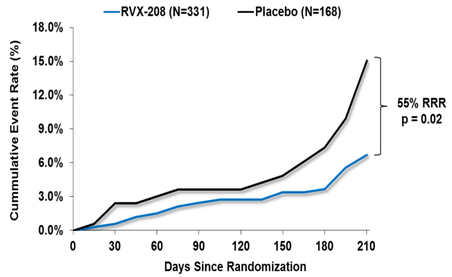
Background: RVX-208 affects epigenetics by inhibiting bromodomain and extraterminal (BET) proteins from binding to their natural ligand, acetyl-lysine marks on histone tails and thereby modulates gene activity. In SUSTAIN and ASSURE phase IIb trials of CVD patients (n=499), giving 200 mg/d of RVX-208 orally lead a 55% relative risk reduction in major adverse cardiovascular events (MACE) vs. placebo. This marked reduction in MACE is unlikely due to RVX-208's modest induction of ApoA-I/HDL, thus prompting studies of RVX-208 for its benefits beyond lipids.

Methods included microarray surveys of human whole blood (WB) or primary hepatocytes (PH) exposed to RVX-208. Cytokines were assayed in U937 macrophage and peripheral blood mononuclear cells (PBMC) exposed to RVX-208. Plasma samples from phase IIb patients were measured using SOMAScan proteomic analysis.

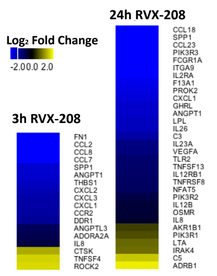
Results of the microarray studies using WB showed a potential anti-atherogenic effect of RVX-208 because it suppressed activity of 37/46 pro-atherogenic while inducing 8/18 anti-atherogenic genes. Additionally, RVX-208 had potential anti-thrombotic properties by affecting 18 genes related to platelet function (e.g. downregulation of CD64 and thrombospondin 1). RVX-208 had anti-inflammatory effects on the expression of >25 cytokines including downregulation of MCP-1, osteopontin and PARC genes. The suppression of these 3 genes by RVX-208 was evident in not only WB but also in the LPS stimulated U937 and/or PBMCs. Whether these in vitro findings extended into patients were examined by SOMAScan showing lower levels of osteopontin and PARC protein in plasma of treated patients. Furthermore, a key marker of inflammation RANTES was markedly lowered in treated patients. Why RVX-208 may affect genes connected to CVD was explored by exposing PH to RVX-208. These studies showed a 25% reduction in levels of mRNA encoding flavin mono-oxygenase-3 (FMO3), an enzyme that produces trimethylamine oxide (TMAO) a metabolite which predicts CVD risk.

Summary, RVX-208 inhibits BET proteins to impact cellular epigenetics that in turn affects expression of genes with known roles in CVD. This activity may underlie RVX-208's anti-atherogenic, -thrombotic and –inflammatory effects in reducing MACE observed in clinical trials.

1. RVX-208 (200 mg/d) added to standard of care leads to a 55% relative risk reduction in MACE (SUSTAIN and ASSURE)



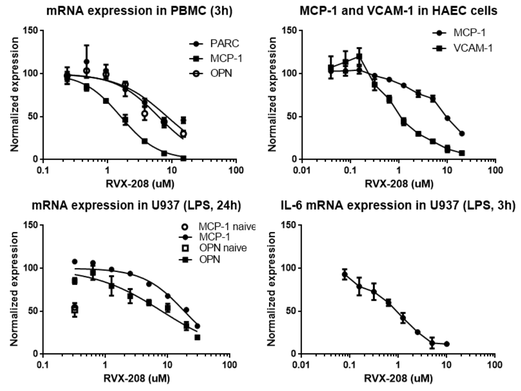
2. RVX-208 reduces pro-atherogenic and pro-inflammatory gene expression in ex vivo treated human whole blood



30uM RVX-208 for 3 and 24 h.

Pro-inflammatory genes	Fold decrease in expression vs CTL
CCL2 (MCP-1)	8-fold
CCL18 (PARC)	7-fold
SPP1 (OPN)	7-fold
CCL23	6-fold
CCL8	6-fold
IL2RA	4-fold
IL23	3-fold
CCR2	2-fold
IL8	1.4-fold

3. RVX-208 in vitro suppresses cytokine mRNA: IL-6, MCP-1, VCAM-1, PARC and osteopontin (OPN)



4. RVX-208 affects genes with roles in platelet activation

Gene Name	Gene Symbol	RVX-208 (20μM, 3H)	p-value	Function
fibronectin 1	FN1	*0.05*	0.0002	Assembled by platelets to stabilize aggregates during vascular injury
PAI-2	SERPINB2	*0.21*	0.007	Monocyte derived PAI-2, pro-thrombotic
thrombospondin 1	THBS1	*0.34*	0.02	Adhesive glycoprotein with a role in platelet activation
CD9 molecule	CD9	*0.46*	0.005	Platelet surface protein for activation, aggregation & release of microparticles
chemokine (C-X-C motif) ligand 13	CXCL13	*2.35*	0.04	Expressed by activated platelets, potential role in plaque stabilization

Gene Name	Gene Symbol	RVX-208 (20μM , 24H)	p-value	Function
Fc fragment of IgG, high affinity Ia, receptor (CD64)	FCGR1A	*0.21*	0.005	Collagen-induced platelet (Plt) activation, aggregation & thrombus formation
karyopherin alpha 1 (importin alpha 5)	KPNA1	*0.25*	0.02	Translocates p65 & STAT3; promotes ICAM-1 expression & monocyte adhesion
lysophosphatidylcholine acyltransferase 2	LPCAT2	*0.36*	0.02	Role in platelet-activating factor (PAF) biosynthesis
complement component 3	C3	*0.4*	0.005	Mediates complement activation on activated Plts
ST3 beta-galactoside alpha-2,3-sialyltransferase 6	ST3GAL6	*0.48*	0.01	Required for glycosylation & function of selectin ligands (e.g. P-selectin)
vascular endothelial growth factor A	VEGF	*0.5*	0.02	Produced by Plts and endothelial cells; increases vascular permeability.
toll-like receptor 2	TLR2	*0.55*	0.01	Involved in infection and immune cell-mediated activation of platelets
Podoplanin	T1A-2	*0.56*	0.04	Ligand for CLEC-2 which promotes thrombosis
TIMP metalloprotease inhibitor 1	TIMP1	*1.6*	0.003	Reduces phosphatidylserine exposure on activated platelets
platelet activating factor acetylhydrolase 1b	PAFAH1B1	*1.69*	0.03	Phospholipase that degrades Plt-activating factor & oxidated phospholipids
glucocorticoid receptor	NR3C1	*1.83*	0.03	Anti-inflammatory effects on cytokines, adhesion molecules, PAF, etc.
zinc finger CCCH-type containing 12A	ZC3H12A	*1.85*	0.04	Modules immunity & inflammation by degrading specific RNAs, e.g. IL-6, IL12B
enhancer of zeste homolog 2	EZH2	*1.91*	0.04	Required for megakaryocyte maturation
bactericidal/permeability-increasing protein	BPI	*2.71*	0.04	LPS-neutralizing activity; effective against Gram-negative bacteria

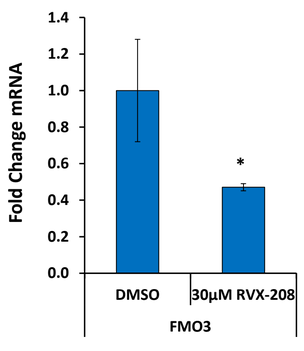
Microarray of human whole blood treated ex-vivo with 30uM RVX-208 for 3 and 24 h. Fold change is relative to vehicle treated samples.

5. RVX-208 affects circulating markers of CVD in ASSERT patients: anti-inflammatory and plaque-stabilizing effects

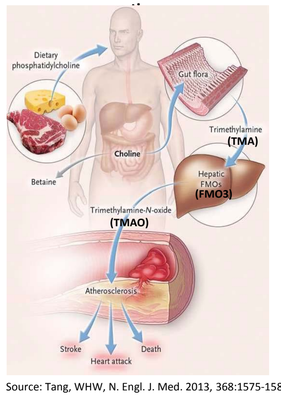
Protein Name	Placebo N=30	RVX-208 200mg daily N=25	Δ treated vs. placebo	p-value vs placebo	Function in CVD/Diabetes
C-reactive protein (CRP)	18.4	-24.3	-42.7	0.01	Risk factor for atherosclerosis, DM, hypertension and CVD
RANTES (CCL5)	21.4	-7.3	-36.7	0.04	Pro-inflammatory, pro-atherosclerotic, pro-thrombotic
sTWEAK (TNFSF12)	13.5	-7.0	-20.5	0.002	Pro-atherogenic in mice, predicts MACE in STEMI patients
Osteopontin (SPP1)	19.7	3.4	-16.4	0.03	Pro-atherogenic, predicts MACE in CAD and type 1 diabetes
PARC (CCL18)	10.4	-3.0	-13.4	0.03	Predicts MACE in CAD and type 1 diabetes
Epiregulin (EREG)	11.5	-1.6	-13.1	0.01	Promotes atherosclerosis and inflammation
TNFSF14	8.4	-2.2	-10.7	0.004	Pro-inflammatory, correlates with plaque number in humans
Pappalysin-1 (PAPPA)	1.9	-11.0	-12.9	0.05	Promotes plaque instability, predicts MACE in ACS
Metalloproteinase inhibitor 2 (TIMP2)	-7.2	6.3	13.5	0.0003	Stabilizes atherosclerotic plaque, predicts risk of death and MI
Metalloproteinase inhibitor 1 (TIMP1)	-0.7	6.6	7.2	0.001	Stabilizes atherosclerotic plaque, correlates with MetS

ASSERT 3 month clinical data : SOMAscan™ assay of 1300+ analytes

6. RVX-208 suppresses expression of the TMAO producing enzyme FMO3 in human primary hepatocytes



TMAO produced by FMO3 in the liver has pro-atherosclerotic and pro-thrombotic



Source: Tang, WHW, N. Engl. J. Med. 2013, 368:1575-1584.

Conclusions

- RVX-208 reduced MACE in phase II trials.
- RVX-208, reduces expression of genes involved atherogenesis, vascular inflammation and platelet function in vitro.
- RVX-208 reduces circulating CVD markers in patients.
- RVX-208 reduces Fmo3 expression which may lower TMAO levels.
- RVX-208 induces transcriptional changes that may impact CVD and lower incidence of MACE observed in the ASSURE and SUSTAIN trials.