RESVERLOGIX

TSX: RVX

Selective Bromodomain Inhibition with the Bromodomain and Extraterminal Domain (BET) Inhibitor Apabetalone: Discovery to Phase 3 Cardiovascular Outcomes Study

Norman C. W. Wong, MD, FRCPC

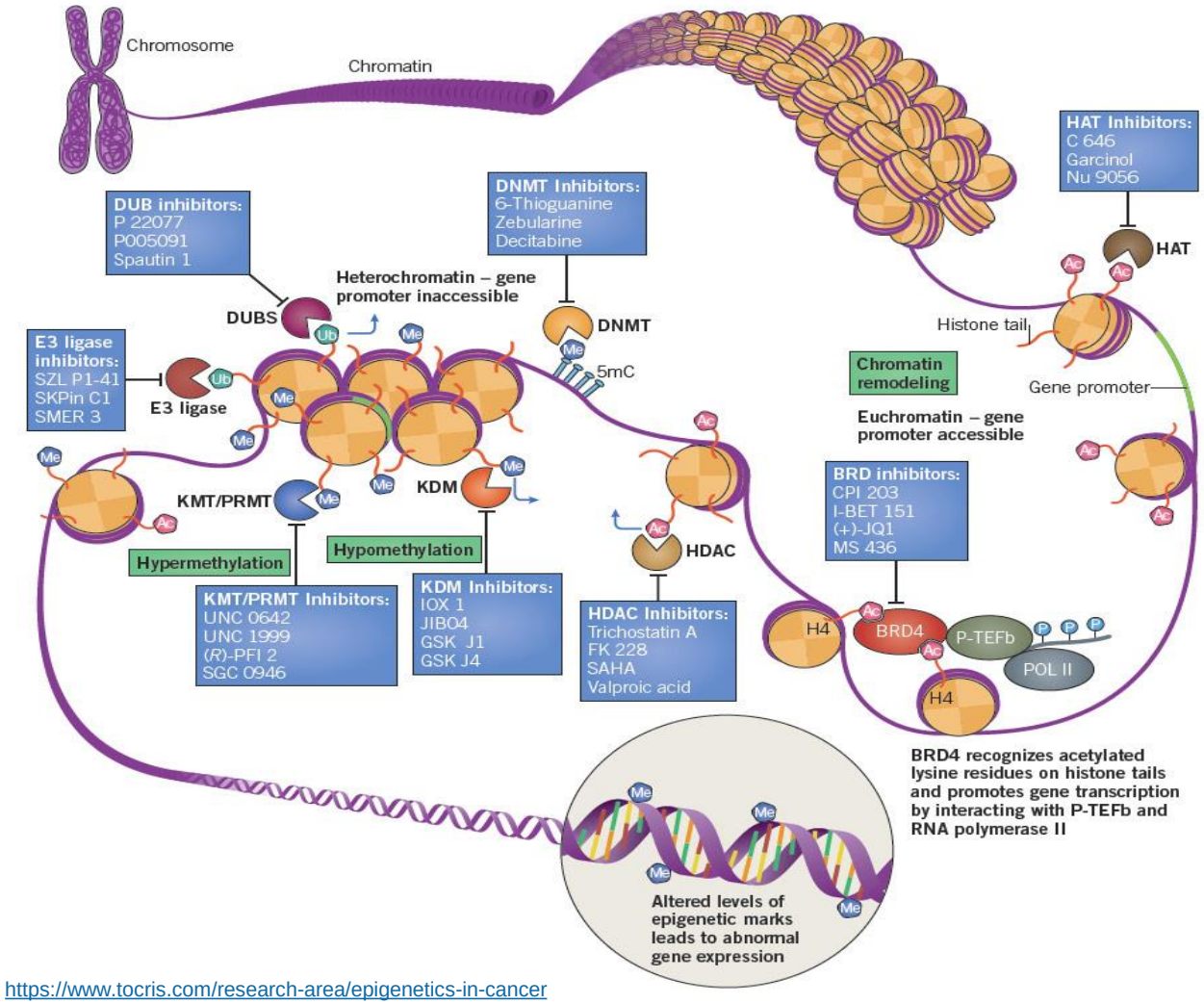
Co-Founder and CSO, Resverlogix Corp.

Outline

1.) Role of epigenetics in disease (cancer and CVD/inflammation)
2.) Activity of Bromodomain and ExtraTerminal Proteins (BET) in epigenetics
3.) Apabetalone (RVX-208), a selective BET inhibitor
    - Unique BD selectivity and mechanism of action
    - Preclinical studies; cells and animals
    - Biomarkers and pathways
    - Clinical data (Phase 1-3 trials)
    - Safety of Apabetalone
4.) Future development of Apabetalone
5.) Summary

Role of Epigenetics in Cancer

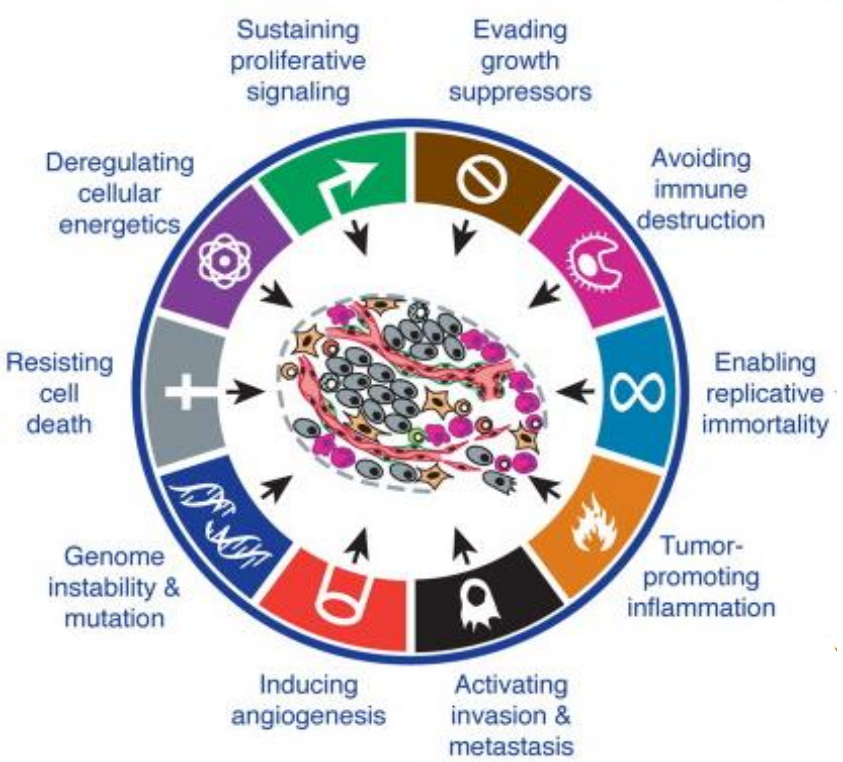
Epigenetics: processes that regulate gene activity which do NOT affect genomic sequence



https://www.tocris.com/research-area/epigenetics-in-cancer

Epigenetic Mechanisms in Cancer: initiation, progression, & metastasis.

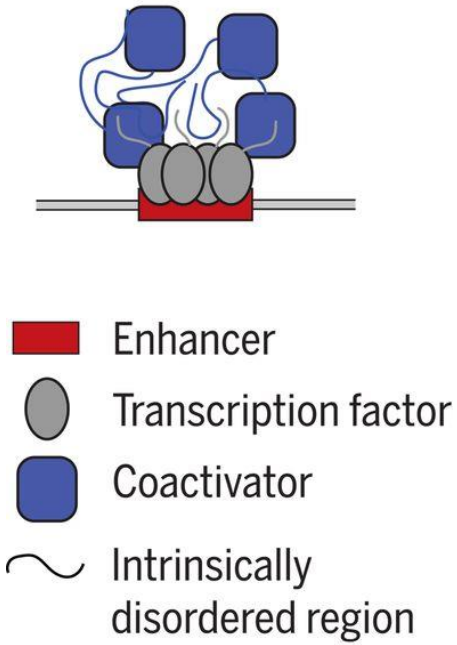
- aberrant DNA methylation
- long non-coding RNAs (lncRNAs)
- histone modifications



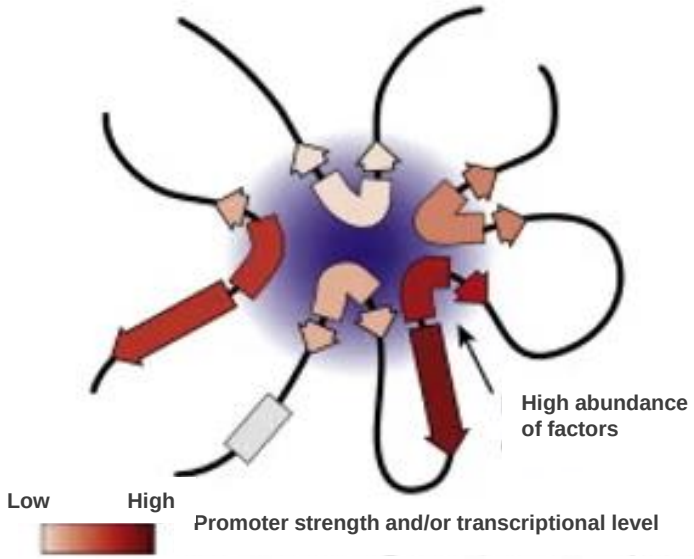
Modified from Hanahan 2011, Cell

Typical-, Super-, and Active-Enhancers in Regulating Gene Activity

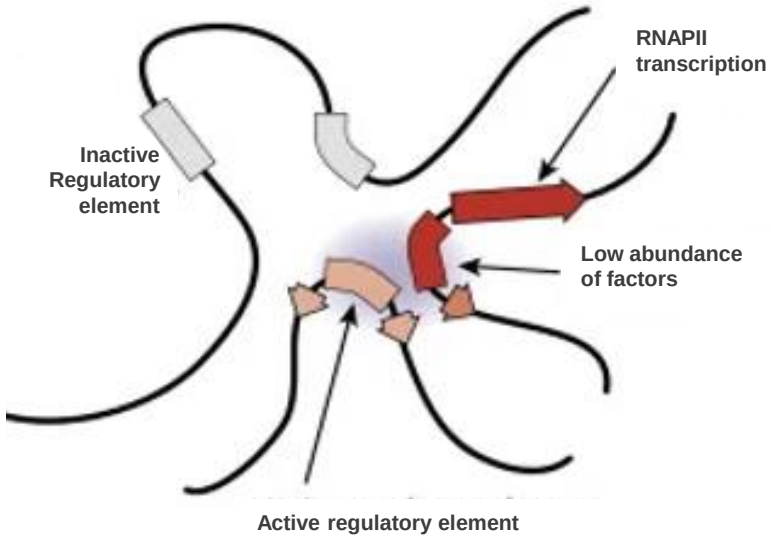
1.) Typical enhancer



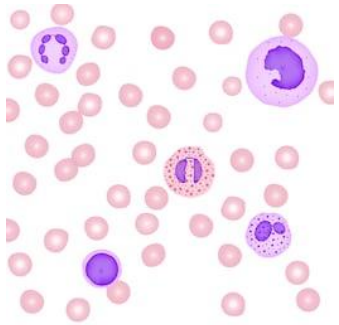
2.) Super-enhancer



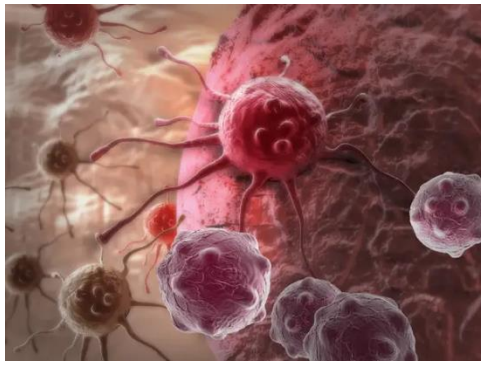
3.) Active enhancer



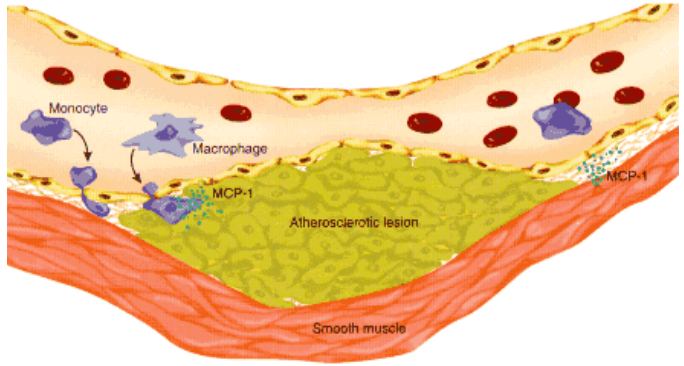
Benjamin R. Sabari et al. Science 2018;361:eaar3958



Normal Cells

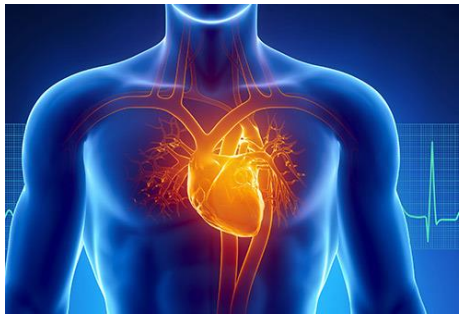


Cancer Cell



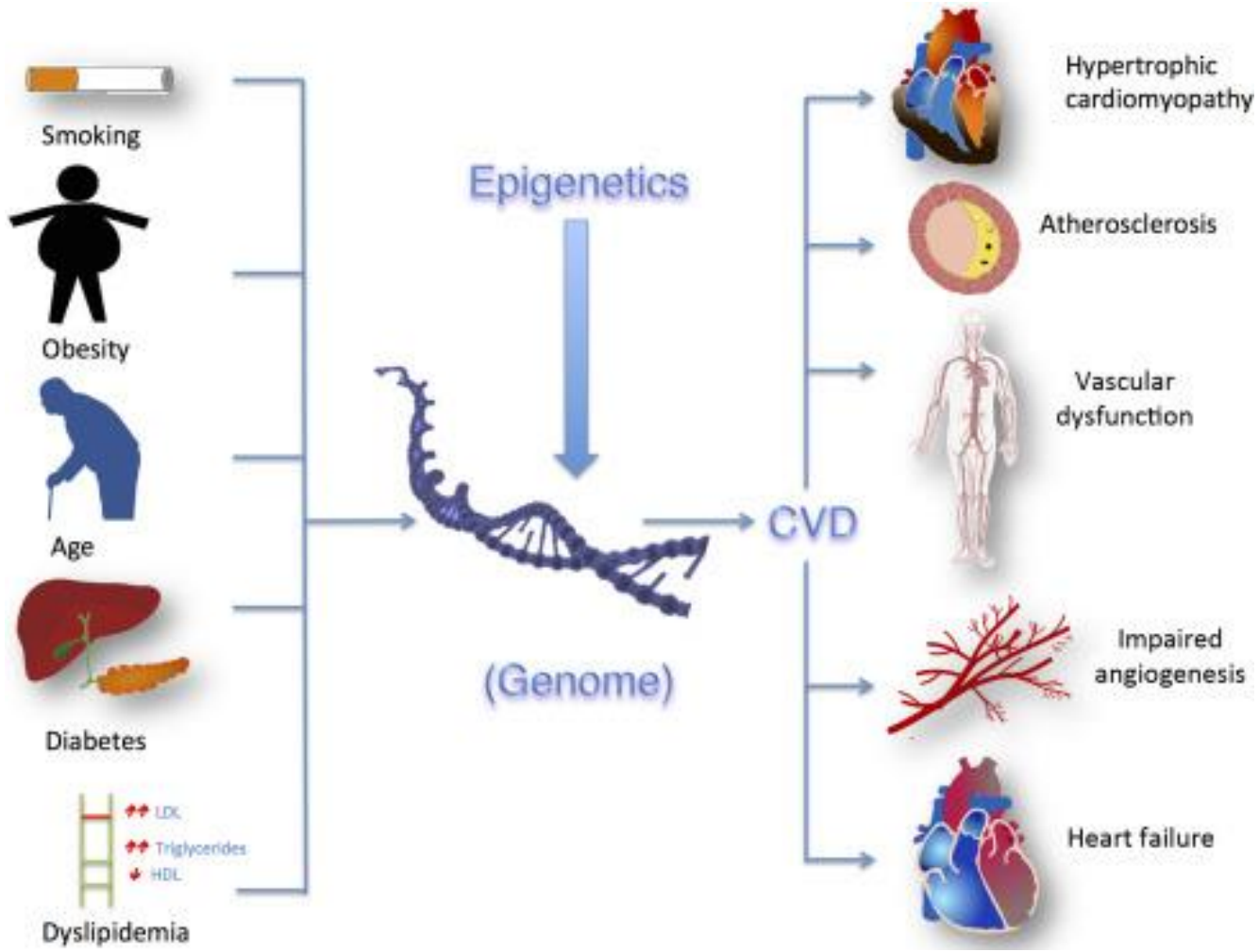
CVD Cells

Commonality amongst diseases is gene activity changes in response to the pathologic process(es).



Role of Epigenetics in Cardiovascular Disease (CVD)

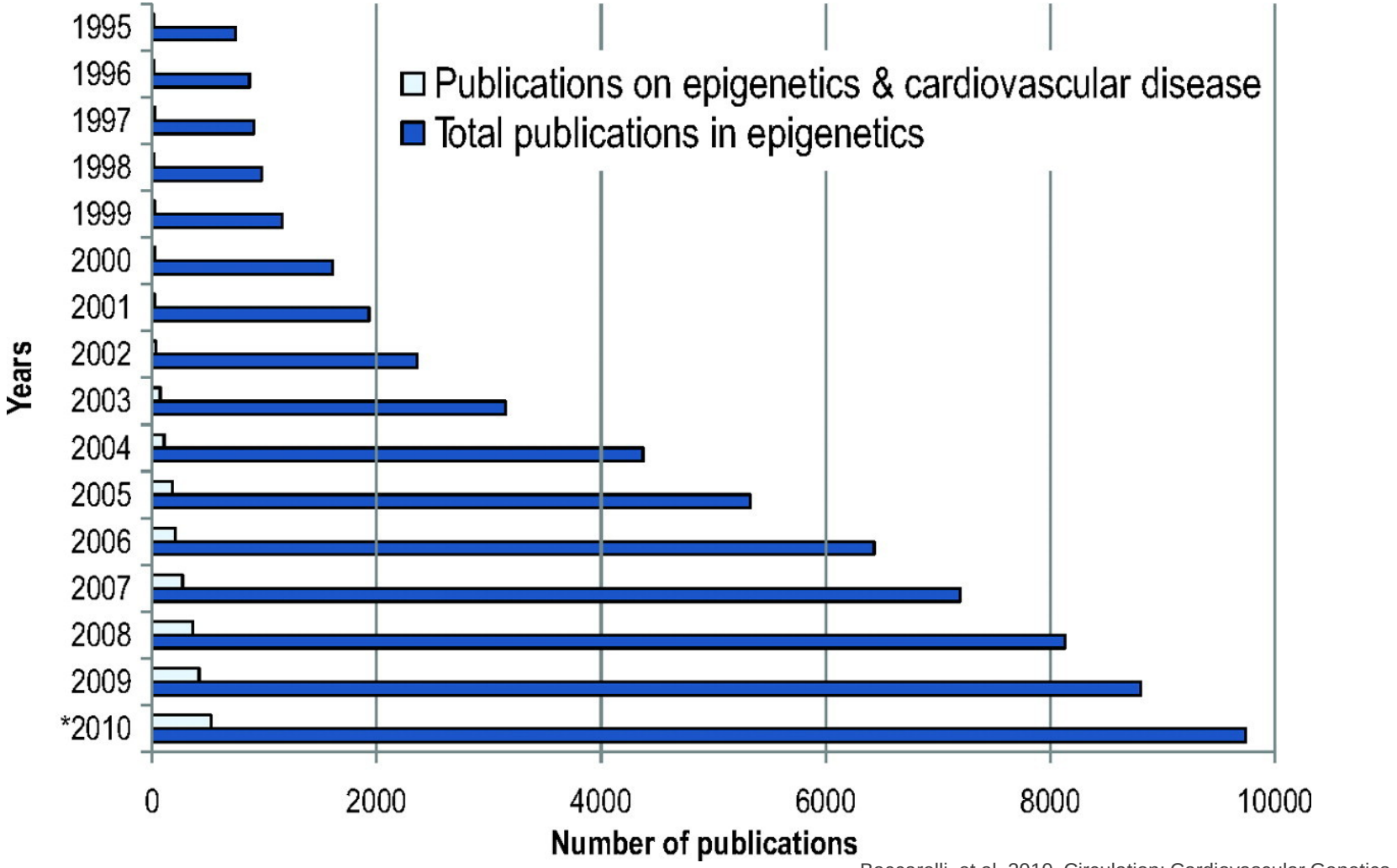
- CVD is multifactorial
- CVD risk factors are associated with epigenetic modifications to the “histone code”



Khalil 2016, Medial Epigenetics

Role of Epigenetics in Cardiovascular Disease (CVD)

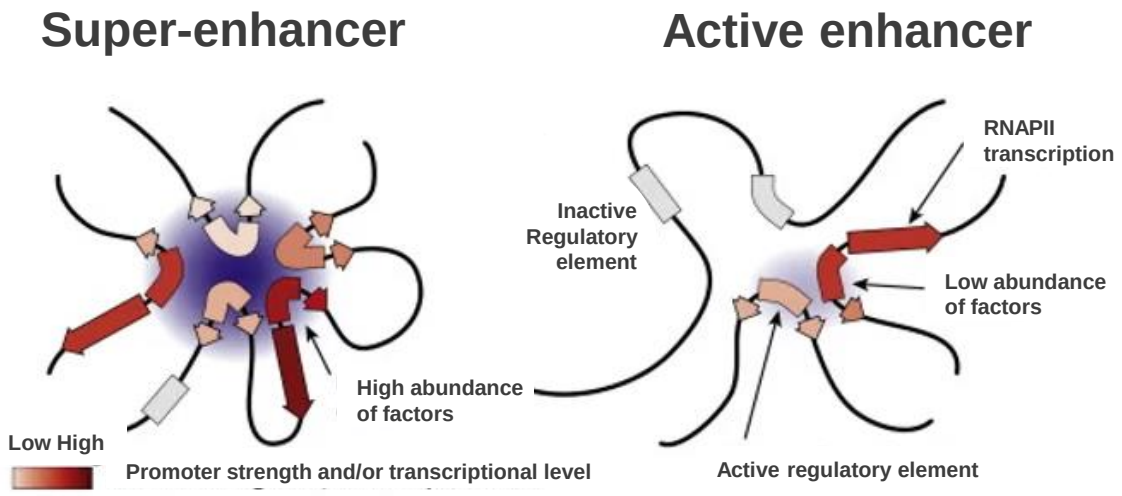
- Increasing evidence for epigenetics in cardiovascular disease (CVD)

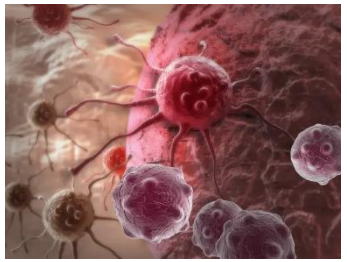


Baccarelli et al. 2010, Circulation: Cardiovascular Genetics

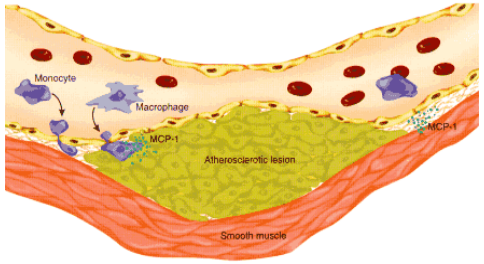
Summary

1.) Human diseases share a common basic mechanism





Cancer Cell



CVD Cells

Outline

1.) ~~Role of epigenetics in disease (cancer and CVD/inflammation)~~
2.) Activity of Bromodomain and ExtraTerminal Proteins (BET) in epigenetics
3.) Apabetalone (RVX-208), a selective BET inhibitor
    - Unique BD selectivity and mechanism of action
    - Preclinical studies; cells and animals
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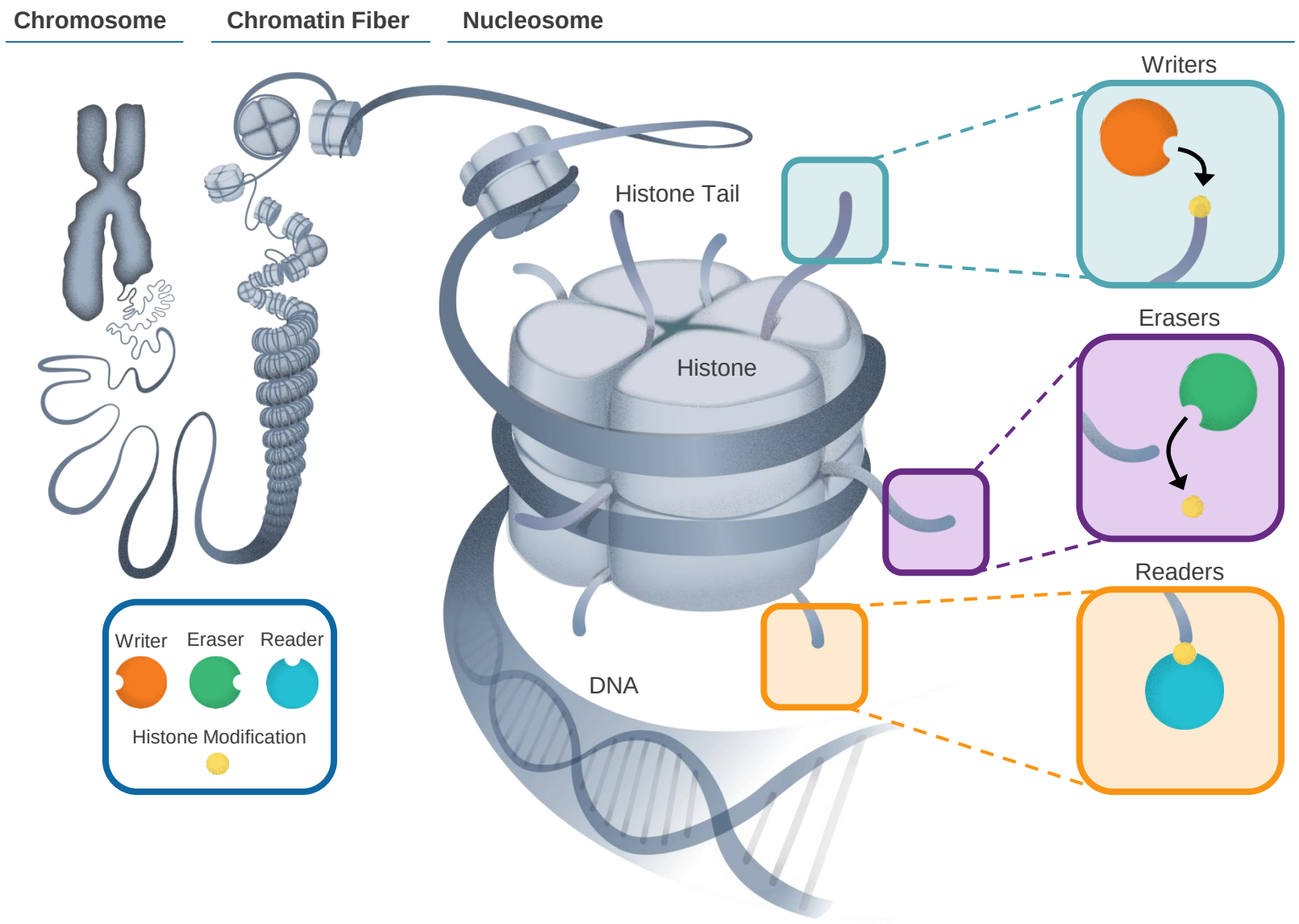
4.) Future development of Apabetalone
5.) Summary

Epigenetics Regulate Gene Activity



- Epigenetics refers to **modifications** to chromatin that regulate it's activity
- Transcription is regulated by **addition, removal, or recognition** of these modification
- **Acetylation** is associated with **active transcription** regions of chromatin
- **Bromodomain and Extraterminal Domain (BET)** proteins bind to acetylated histones and recruit additional transcription factors to drive gene expression

BET Proteins Bind to Acetylated Lysines via Dual Bromodomains

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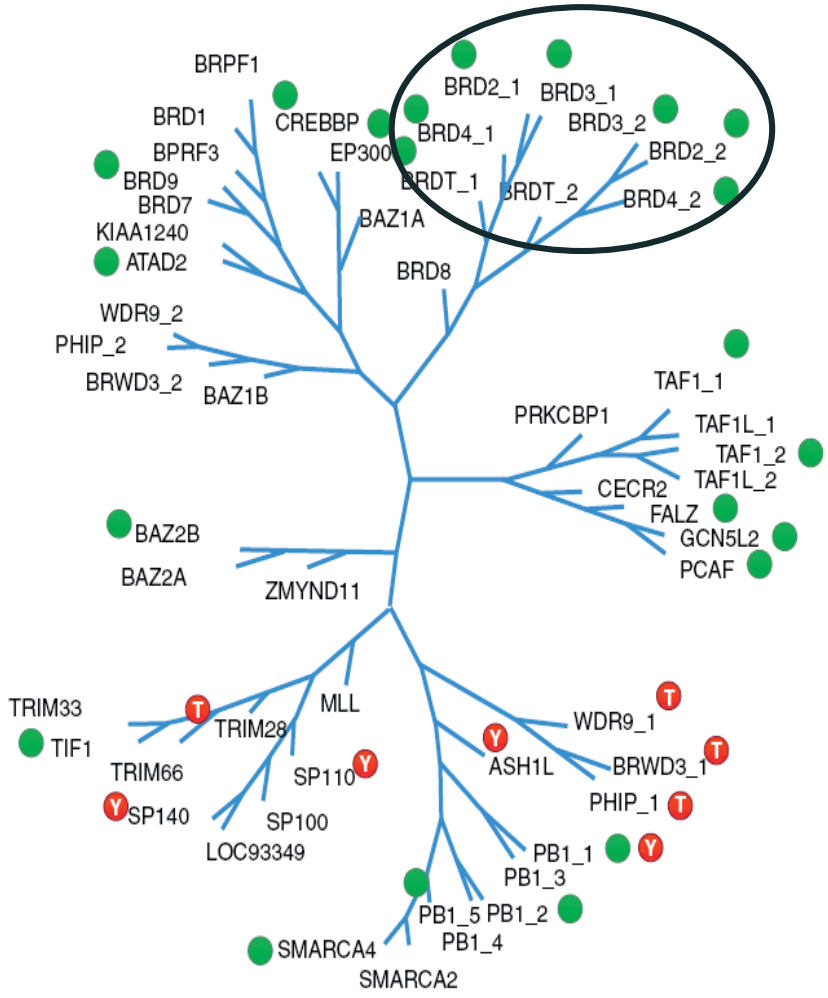
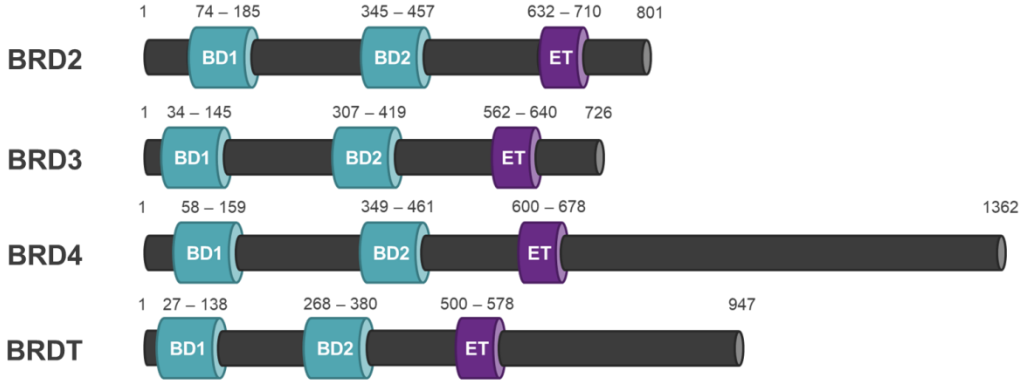
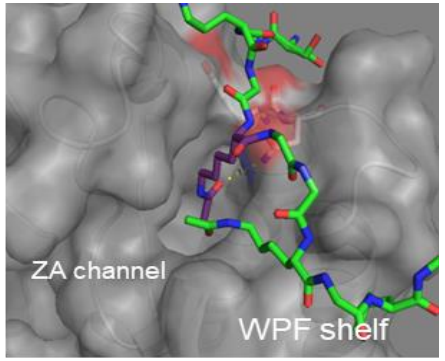
Bromodomain and ExtraTerminal (BET) Protein Superfamily.



Each BET protein has a pair of bromodomains (BD)



Acetylated Lysine bound to a BD



X-ray crystallography Acetylated lysine (color) bound to bromodomain (grey)

BET Proteins Binding to Acetylated Lysines Regulate Gene Expression

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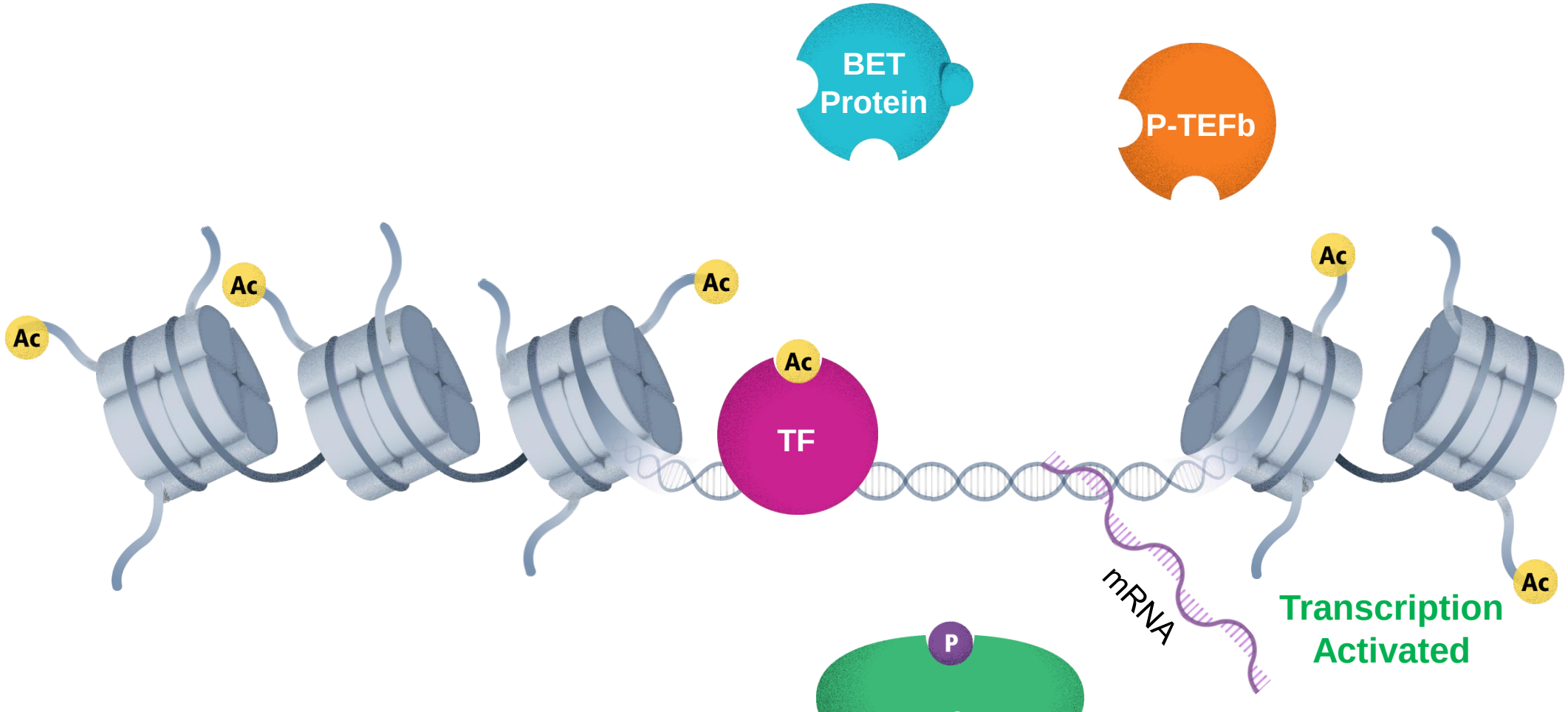
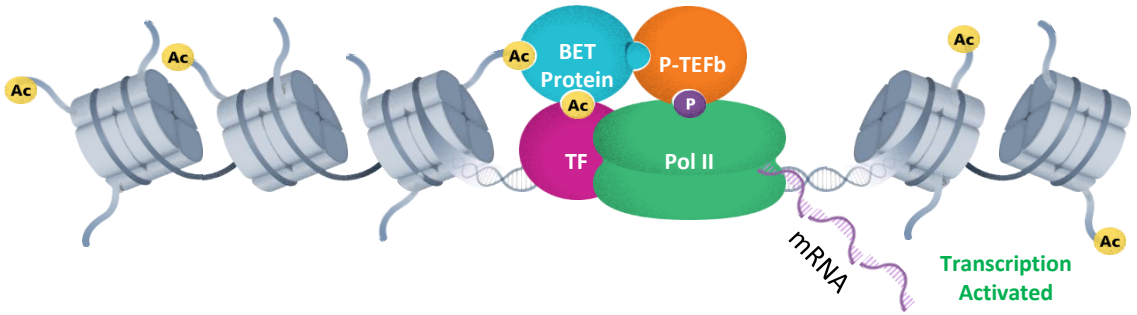
BET Protein Binds Acetylated Histone → Recruits Additional Transcription Factors → Gene Transcription Activated



Summary

1.) Human diseases share a common basic mechanism

2.) BET proteins are important components of the transcriptional machinery in gene regulation.



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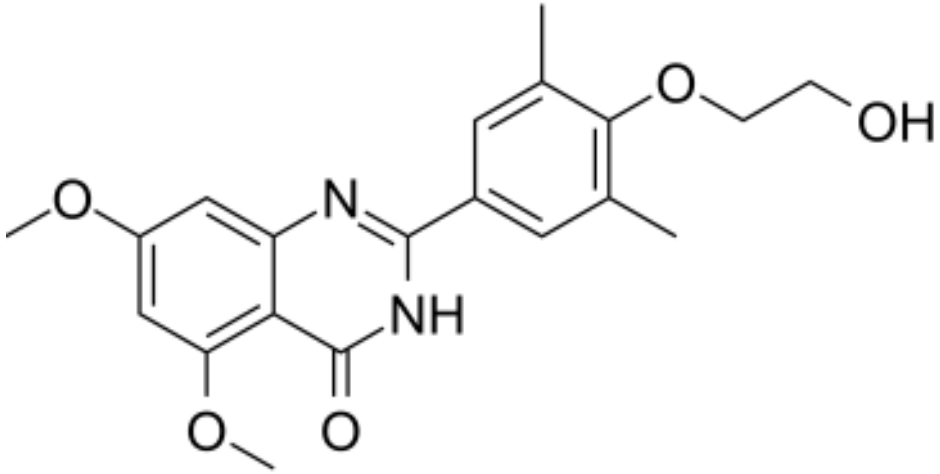
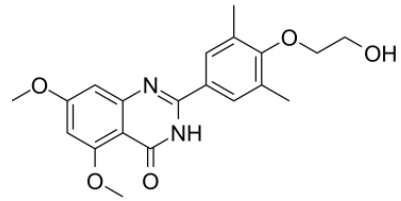


Apabetalone (RVX-208)

Discovery and Characterization of Apabetalone

- **Apabetalone/RVX-208/RVX000222** (2-(4-(2-hydroxyethoxy)-3,5-dimethylphenyl)-5,7-dimethoxyquinazolin-4(3H)-one)



- Discovered and synthesized in 2006 at Resverlogix as part of the medicinal chemistry program aimed at discovering compounds to upregulate Apolipoprotein A-I, the building block of HDL
- Selected using a cell based screen for Apolipoprotein A-I induction in liver cells, *in vitro*
- Tested in numerous cell and animal models of cholesterol metabolism at Resverlogix including a variety of cell and tissue types (i.e. liver/intestine) and models of cardiovascular disease and obesity
- Apabetalone was identified as a BET inhibitor in 2013 (McLure; Picaud) using binding assays, x-ray crystallography, chromatin displacement. Apolipoprotein A-I expression is regulated by BET proteins.

Binding of Apabetalone to the BD is unique vs other BET inhibitors.

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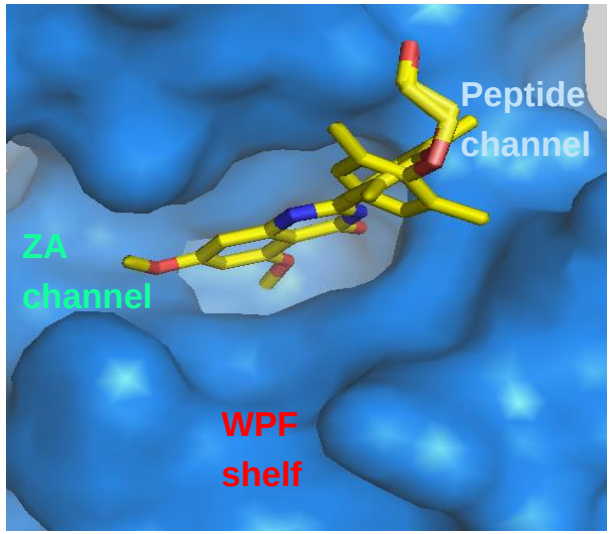
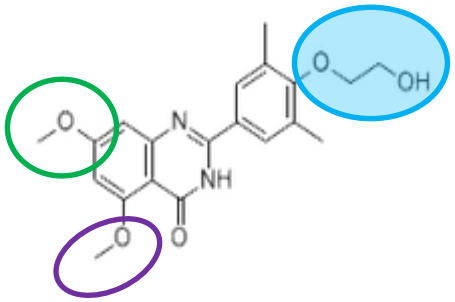
Apabetalone (RVX-208)



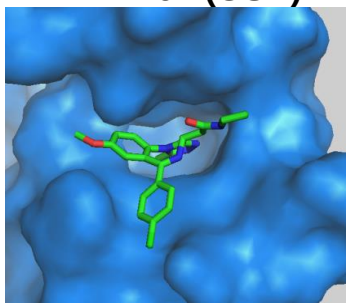


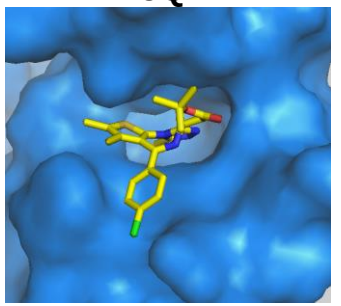
Purple – interaction with Asn140 deep in the pocket

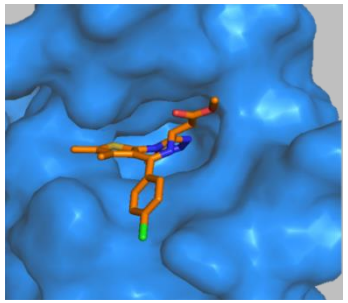
Green – interactions in the ZA channel

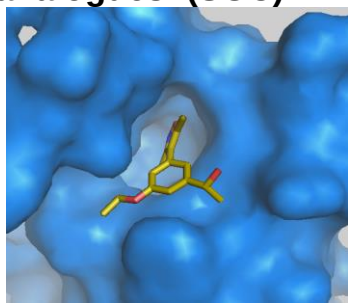
Red – interaction with the WPF shelf (absent in RVX-208)

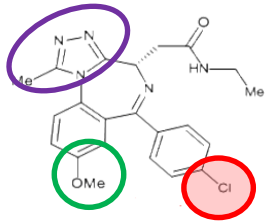
Blue – interaction in the "peptide" channel (unique to RVX-208)

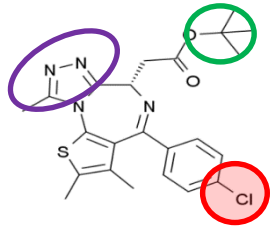

I-BET762 (GSK)

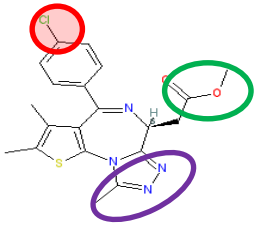

JQ1

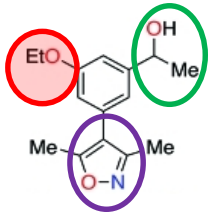

MS417

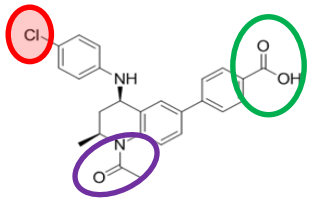

3,5-dimethylisoxazole analogues (SGC)

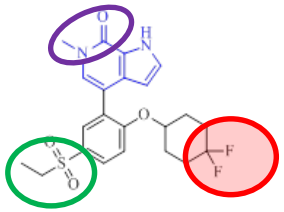


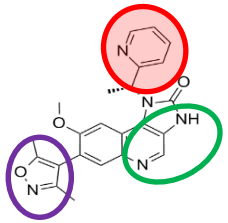


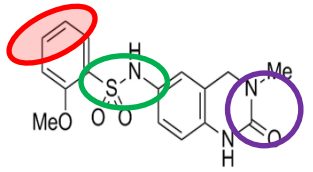


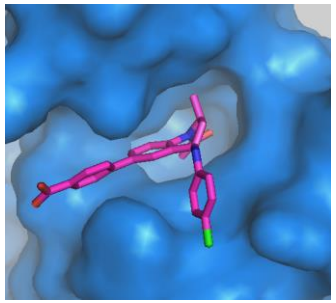


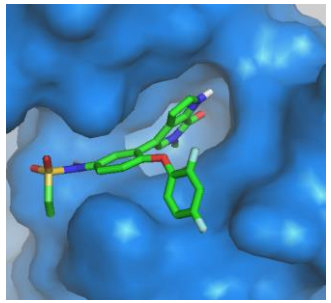


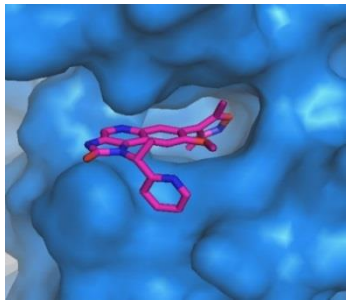


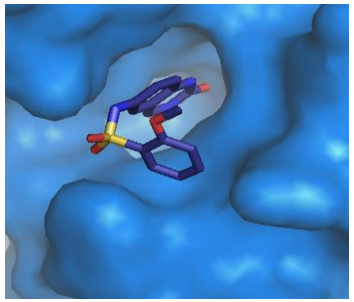





I-BET726 (GSK)

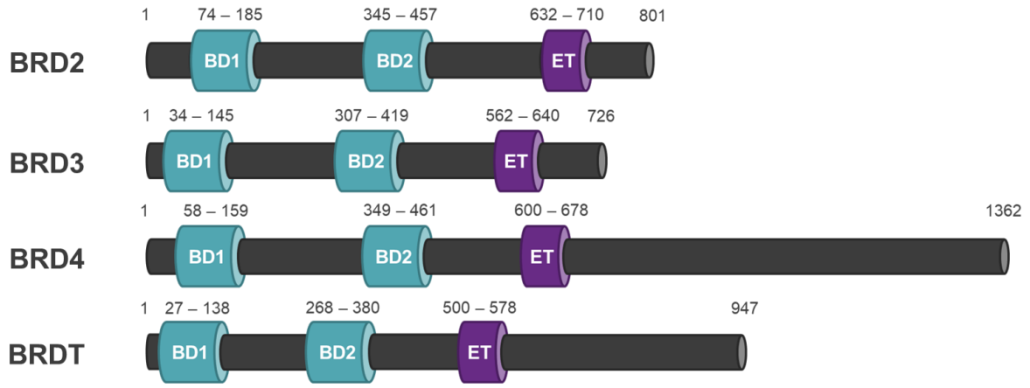

Abbvie


I-BET151 (GSK)

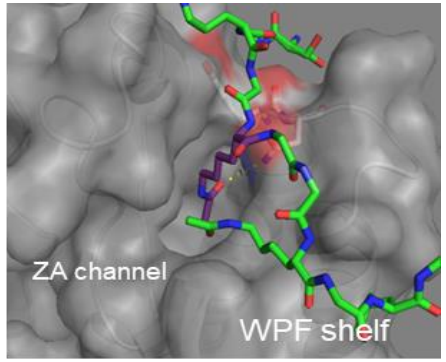

PFI-1(Pfizer)

Apabetalone Inhibits BET Protein Binding to Acetylated Lysines

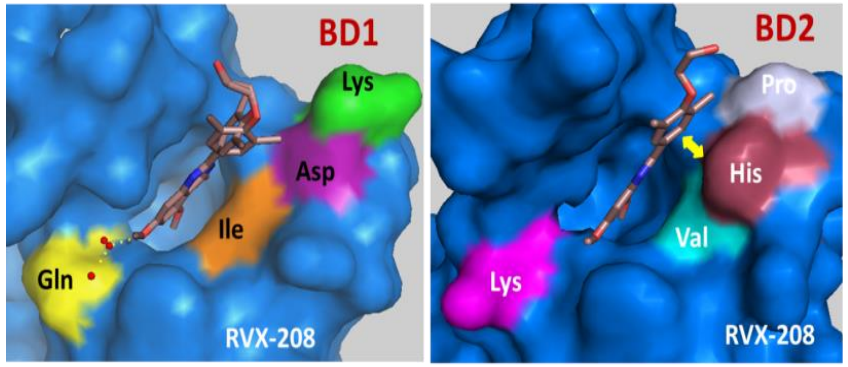
Each BET protein contains two bromodomains (BD)



Apabetalone has selectivity for bromodomain 2 (BD2) within BET proteins



X-ray crystallography Acetylated lysine (color) bound to bromodomain (grey)



X-ray crystallography Apabetalone bound to bromodomain

Apabetalone has selectivity for bromodomain 2 (BD2) within BET proteins

- Using biochemical assays including; alphascreen (competition assay), Bromoscan (direct binding assay), and isothermal calorimetry (direct binding), apabetalone has shown BD2 selectivity with approximately 27-fold and binding to BD2 in the 100-200 nanomolar range

AlphaScreen Selectivity Panel (IC50, uM)

	BRD2(1)	BRD2(2)	BD1/BD2	BRD3(1)	BRD3(2)	BD1/BD2	BRD4(1)	BRD4(2)	BD1/BD2
RVX-208	6.17	0.22	27	4.88	0.17	29	3.22	0.12	27
JQ1	0.08	0.05	1.6	0.04	0.03	1.3	0.06	0.04	1.7

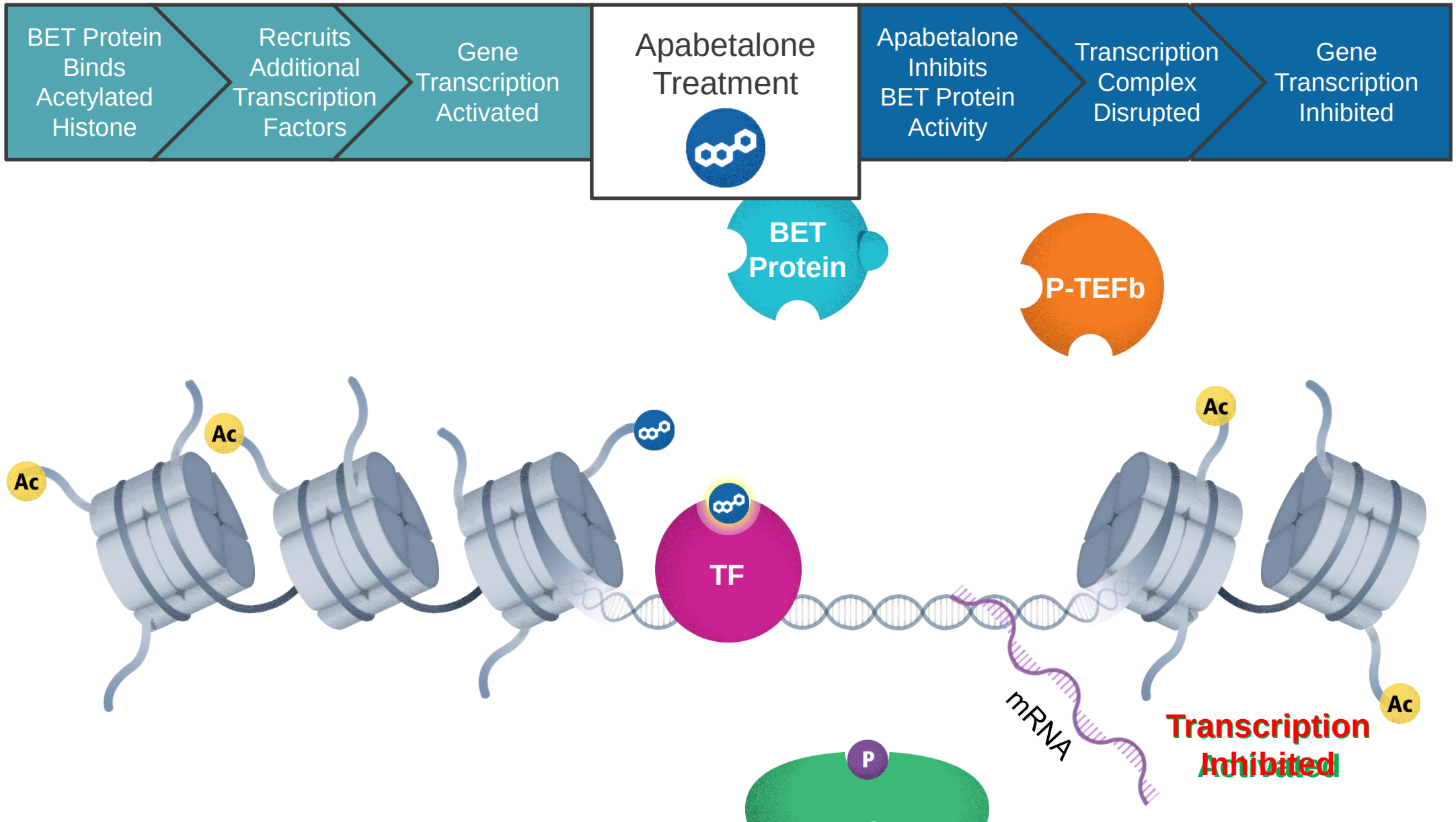
- Using live in cell assays such as NANOBRET, apabetalone has been shown to displace full length Brd4 or Brd4-BD2 at an IC50 of 1um

Table 3. Binding of RVX-208 to individual bromodomains.

Bromodomain	Thermal denaturation ΔT_m (°C)		TR-FRET IC50 (μM)	
	RVX-208	JQ-1	RVX-208	JQ-1
BRD2[BD1]	1.9	6.7	2.6	0.09
BRD2[BD2]	5.1	7.7	0.09	0.01
BRD3[BD1]	2.6	9.6	3.1	0.04
BRD3[BD2]	6.8	9.0	0.28	0.03
BRD4[BD1]	3.9	10.3	1.8	0.12
BRD4[BD2]	7.7	5.5	0.04	0.01

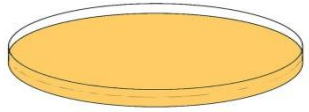
McLure et al. 2013, PLOSOne

Apabetalone BET Inhibition Reduces Gene Expression

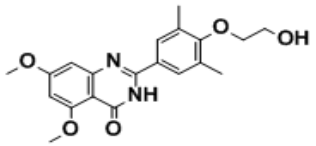


Mechanism of Action and Effect of Apabetalone

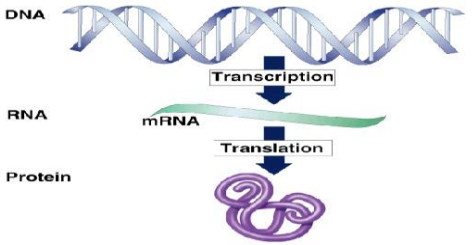
Studies in cells, animals and humans



Primary cells and cell lines
liver, endothelial, immune, kidney, pancreatic, intestinal

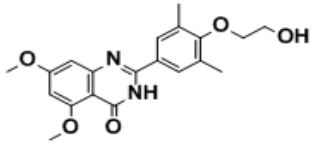
\+ 

apabetalone



microarray analysis
mRNA expression & protein analysis
cytokine secretion
CHIP-qPCR & ChIP-seq
adhesion & calcification assays

Mouse
Hyperlipidemic apoE -/-
Carotid ligation apoE -/-
LPS endotoxemia model
Chimeric mice w. humanized livers
Diet induced obesity mouse model

\+ 

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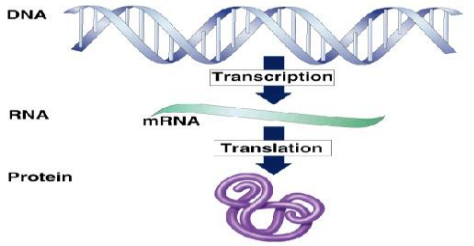
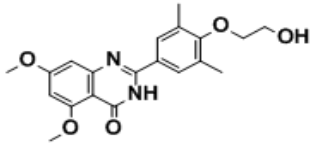
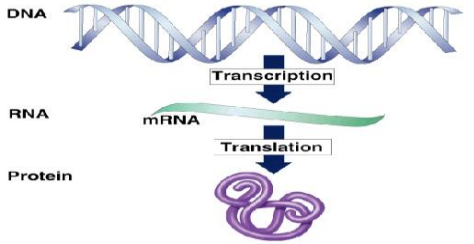


serum lipid & cytokine profiles
aortic lesion measurements
mRNA expression & protein analysis in tissue

CVD patients
Phase 2 ASSERT: 12w dose finding, safety, efficacy
Phase 2b SUSTAIN: 24w single dose, safety, efficacy
Phase 2b ASSURE: 26w IVUS study
Phase 1 PK study: PK in patients w. severe renal impairment
Phase 3 BETonMACE: secondary prevention of MACE in patients w. CVD/diabetes and low HDL

\+ 

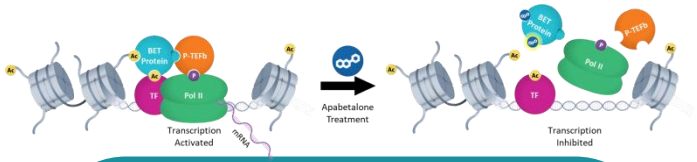
apabetalone



MACE (major adverse cardiovascular events)
intravascular ultrasound (IVUS)
serum lipid, ALP, cytokine profiles
plasma proteome and biomarkers of interest

BET Proteins Affect Expression of Mediators of CVD;

evidence from cell/animal and human studies



Many individual mediators, classified as associative or causative risk factors for poor CVD or CKD outcomes, are regulated by epigenetic processes, and sensitive to BET inhibition

Fibrosis

Genes	BET	Cell type	Disease model	Reference
IL6, ACTA2, PAI1, COL1A1, FN1	BRD2 BRD4	Human primary lung fibroblasts	Bleomycin-induced pulmonary fibrosis	Tang 2013 AJP
COL1A1, ACTA2, COL1A2, DES, PDGFRB, CCND1, FBN1, FN1, TIMP1, TGFB1	BRD4	Human activate hepatic stellate cells (LX-2 cell line) Primary mouse HSCs	Carbon tetrachloride mouse model of liver injury	Ding 2015 PNAS
TS2, HNF4A, JUNB, FOXP1, CDH2	BRD4	Human lung cancer A549 cell line		Chang 2016 NAR
ACTA2, COL1A1, P53, FBN1, SMAD7, CCL2, c-MYC	BRD4	Renal interstitial fibroblast cells (NRK-49F)	Unilateral ureteral obstruction	Xiong 2016 Oncotarget
MCP1, OPN	BRD2 BRD3 BRD4	U937 macrophages	Inflammation (LPS stimulation)	**Gilham 2017 AHA** **Kulikowski 2017 ERA-EDTA**
F9, F7, F11, PROC, KLKB1, TFPI, F12, F13B, F2, A2M, PROS1, F5	BRD2 BRD3 BRD4	Human primary hepatocytes and whole blood	Atherosclerosis	**Gilham 2016 Atherosclerosis**
Thrombin, F9, F10, F11, F12, KLKB1	BRD2 BRD3 BRD4	Human primary hepatocytes, Huh-7 cells, chimeric mice w. humanized livers,		**Wasiak 2017 JCTR**
F2, F10	BRD2 BRD3 BRD4	Serum	CVD patients (ASSURE & ASSERT phase 2 clinical trials)	**Wasiak 2017 JCTR**
FN1, MMP3, MMP10	BRD2 BRD3 BRD4	Serum	Stage 4/5 CKD patients	**Wasiak 2018 KIR**

Complement Cascade

Genes	BET	Cell type	Disease model	Reference
MBL2, C9, C6, C8A, C4A-B, C4BPB, C5, C1S, C8G, C2, CFH, C3	BRD2 BRD3 BRD4	Human primary hepatocytes and whole blood	Atherosclerosis	**Gilham 2016 Atherosclerosis** **Wasiak 2016 DIP**
MBL2, C1S, C2, C3, C4, C9, C6, C8A, SAP, CRP, C5a, C3b, C5b-C6, COLEC11	BRD2 BRD3 BRD4	Human primary hepatocytes, Huh-7 cells, chimeric mice w. humanized livers	Basal, IL6 and IFN stimulation	**Wasiak 2017 JCTR**
CRP, C3, VTN, C4A C4B, F10, C7, C6, CFI, F2, C9	BRD2 BRD3 BRD4	Serum	CVD patients (ASSERT phase 2 clinical trial)	**Wasiak 2017 JCTR**
CRP, C5, C6, COLEC11, C8, SerpinG1, C2, APCS, COLEC12, F10, CD55, CFB, FCN1, C1R	BRD2 BRD3 BRD4	Serum	CVD patients (ASSURE phase 2 clinical trials)	**Wasiak 2017 JCTR** **Tsujikawa 2019 Clinical Epigenetics**
C3b, C3d, C5	BRD2 BRD3 BRD4	Serum	Stage 4/5 CKD patients	**Wasiak 2018 KIR**

Acute Phase Response

Genes	BET	Cell type	Disease model	Reference
MBL2, C9, CP, IRAK1, LBP, C5, AHSG, KLKB1, APCS, ITIH2, C1S OSMR, F2, SHC, SERPINE1, C2, IL1RN	BRD2 BRD3 BRD4	Human primary hepatocytes Whole blood	Atherosclerosis	**Gilham 2016 Atherosclerosis** **Wasiak 2016 DIP** **Wasiak 2019 PLOS****
APCS, C3, CP, C5, CRP	BRD2 BRD3 BRD4	Serum	CVD patients (ASSURE phase 2 clinical trials)	**Gilham 2016 Atherosclerosis** **Wasiak 2017 JCTR** **Tsujikawa 2019 Clinical Epigenetics**
APCS	BRD2 BRD3 BRD4	Mouse liver	LPS mouse model of systemic inflammation	**Wasiak 2019 PLOS****
IL1RN, FGG, CRP, ITIH4, FGA FGB FGG, MAPK9	BRD2 BRD3 BRD4	Serum	CVD patients (ASSERT phase 2 clinical trials)	**Wasiak 2019 PLOS****

**Submitted

Vascular Calcification

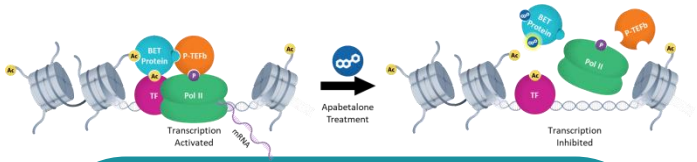
Genes	BET	Cell type	Disease model	Reference
ALPL, OPG, RANKL, MCP1, IL8, BMP2, OPN	BRD2 BRD3 BRD4	Primary human hepatocytes	Basal conditions	**Gilham 2019 Atherosclerosis** **Gilham 2017 AHA** **Kulikowski 2017 ERA-EDTA**
ALPL, RUNX2, WNT5A, MGP, LEP, POSTN, NFATC1, ACVR2A, ROR2	BRD2 BRD3 BRD4	Human vascular smooth muscle cells	Osteogenic and calcifying conditions	**Gilham 2019 Atherosclerosis**
MCP1, OPN	BRD2 BRD3 BRD4	U937 macrophages	Inflammation (LPS stimulation)	**Gilham 2017 AHA** **Kulikowski 2017 ERA-EDTA**
ALP, OPG, OPN	BRD2 BRD3 BRD4	Serum	CVD patients (ASSERT/ASSURE/SUSTAIN phase 2 clinical trials)	**Haarhaus 2019 Atherosclerosis** **Gilham 2017 AHA** **Kulikowski 2017 ERA-EDTA**
ALP	BRD2 BRD3 BRD4	Serum	CVD patients w. eGFR < 60 mL/ min/ 1.73 m^2 (ASSURE/SUSTAIN phase 2 clinical trials)	**Kulikowski 2018 KBPR**
OPN	BRD2 BRD3 BRD4	Serum	Stage 4/5 CKD patients	**Wasiak 2018 KIR**
NFATc1, RUNX2	BRD2 BRD3 BRD4	Osteoclasts, osteoblasts	Post-ovariectomy osteoporosis	Baud'huin 2017 Bone

Inflammation

Genes	BET	Cell type	Disease model	Reference
VCAM1, SELE, CCL2, CSF2, LTB, TNFAIP3, IRAK2, CSF2RB, CXCR7, CXCL1, ICOSLG	BRD4	Human umbilical vein endothelial cells	Atherosclerosis (hypercholesterolemic mice)	Brown 2014 Mol Cell
VCAM1, SELE, CCL2, IL8	BRD4	Human umbilical vein endothelial cells	TNFα stimulation	**Tsujikawa 2019 Clinical Epigenetics**
TNFA, IL1B, IL6, CCL2 (MCP-1), IL10	BRD2 BRD3 BRD4	Murine bone marrow-derived macrophages	Endotoxemic (LPS-induced "cytokine storm")	Belkina 2013 J Immunol
CCL2, CCL5 (RANTES), IL6, CSF2, CCL20, LTB, ICOSLG, IL8	BRD4	Human tubular epithelial cells (HK2 cell line)	Unilateral ureteral obstruction Systemic infusion of ANGII Nephrotoxic serum nephritis	Suarez-Alvarez 2017 JASN
IL6, TIMP1, NOX4, COL8A1, CCL21A, CTGF	BRD4	Neonatal rat ventricular cardiomyocytes (NRVM)	Transverse aortic constriction (TAC) (cardiac hypertrophy)	Anand 2013 Cell
IL6	BRD2 BRD3 BRD4	U937 macrophage		**McLure 2013 PlosONE**
Haptoglobin, VCAM1, IL18, SAP, MIP1α, MCP1, IL6	BRD2 BRD3 BRD4	Mouse serum, HAEC, U937 cells	Hyperlipidemic apoE⁻/⁻ mice Carotid artery ligation	**Jahagirdar 2014 Atherosclerosis**
FN1, CCL2, CCL8, CCL7, CCL18, SPP1, CCL23, PIK3R3, FCGR1A, ITGA9, IL2RA, F13A1, PROK2, CXCL1	BRD2 BRD3 BRD4	Human primary hepatocytes and whole blood	Atherosclerosis	**Gilham 2016 Atherosclerosis** **Wasiak 2016 DIP**
ANGPT2, APCS, APP, C5, CCL1, CCL5, CD38, CCL18, CRP, IL18, ICAM1, IL12B, TLR4, VCAM1, IL5, TNFRSF11B, CX3CL1, CXCL2, CXCL3, CXCL13, MMP3	BRD2 BRD3 BRD4	Serum	CVD patients (ASSURE phase 2 clinical trials)	**Gilham 2016 Atherosclerosis** **Tsujikawa 2019 Clinical Epigenetics**
IL6, IL17A, IL15RA, IL12, IL1A	BRD2 BRD3 BRD4	Serum	Stage 4/5 CKD patients	**Wasiak 2018 KIR**
COX2, CSF-2, IL1B, IL6, OPG, MYD88, CD44, CXCL10, CXCL3, IL15, IL18, LTB, TGFB3, TNF, IRF1, RELB, TLR2, IL1R, TRADD, C1S, CFB, IFIT2, TNFAIP3, CCR1, CCR2, TLR4, VLA4	BRD2 BRD3 BRD4	Human umbilical vein endothelial cells, THP1 monocytes	TNFα, IL1B, LPS stimulation	**Tsujikawa 2019 Clinical Epigenetics**
IL8, MCP1, CCL5, IL6	BRD2 BRD3 BRD4	Pulmonary microvascular endothelial cells Whole lung lysates	TNFα stimulation PAH rat models	**Van der Feen 2019 AJRCCM**
GM-CSF, CX3CL1, MCP3, IP10, IL6, VCAM1, SELE,	BRD2 BRD3 BRD4	Primary human brain microvascular endothelial cells, brain endothelial hCMEC/D3 cell	TNFα, IL1B, LPS stimulation	**Wasiak 2019 CTAD**
SELE, ICAM, CCR2, CD68	BRD2 BRD3 BRD4	Mouse brain	LPS mouse model of systemic inflammation	**Wasiak 2019 CTAD**
IL2, IFN, TNF, Tbet	BRD2 BRD3 BRD4	Activated T cells	T2DM CVD patients	**Wong 2019 EASD**
IL1, IL8, TNF, MYD88, RELA, TLR4, CCL2, CCL7, CCL8, CXCL9, CXCL10, CCR1, TLR1, TLR8, LY96, FPR2, TICAM2, MSR1	BRD2 BRD3 BRD4	Human monocytes baseline & IFN stimulated	T2DM CVD patients	**Wasiak 2020 ACC****

BET Proteins Affect Expression of Mediators of CVD;

evidence from cell/animal and human studies



Many individual mediators, classified as associative or causative risk factors for poor CVD or CKD outcomes, are regulated by epigenetic processes, and sensitive to BET inhibition

Fibrosis

Complement Cascade

Fibrosis

Complement Cascade

Acute Phase Response

Inflammation

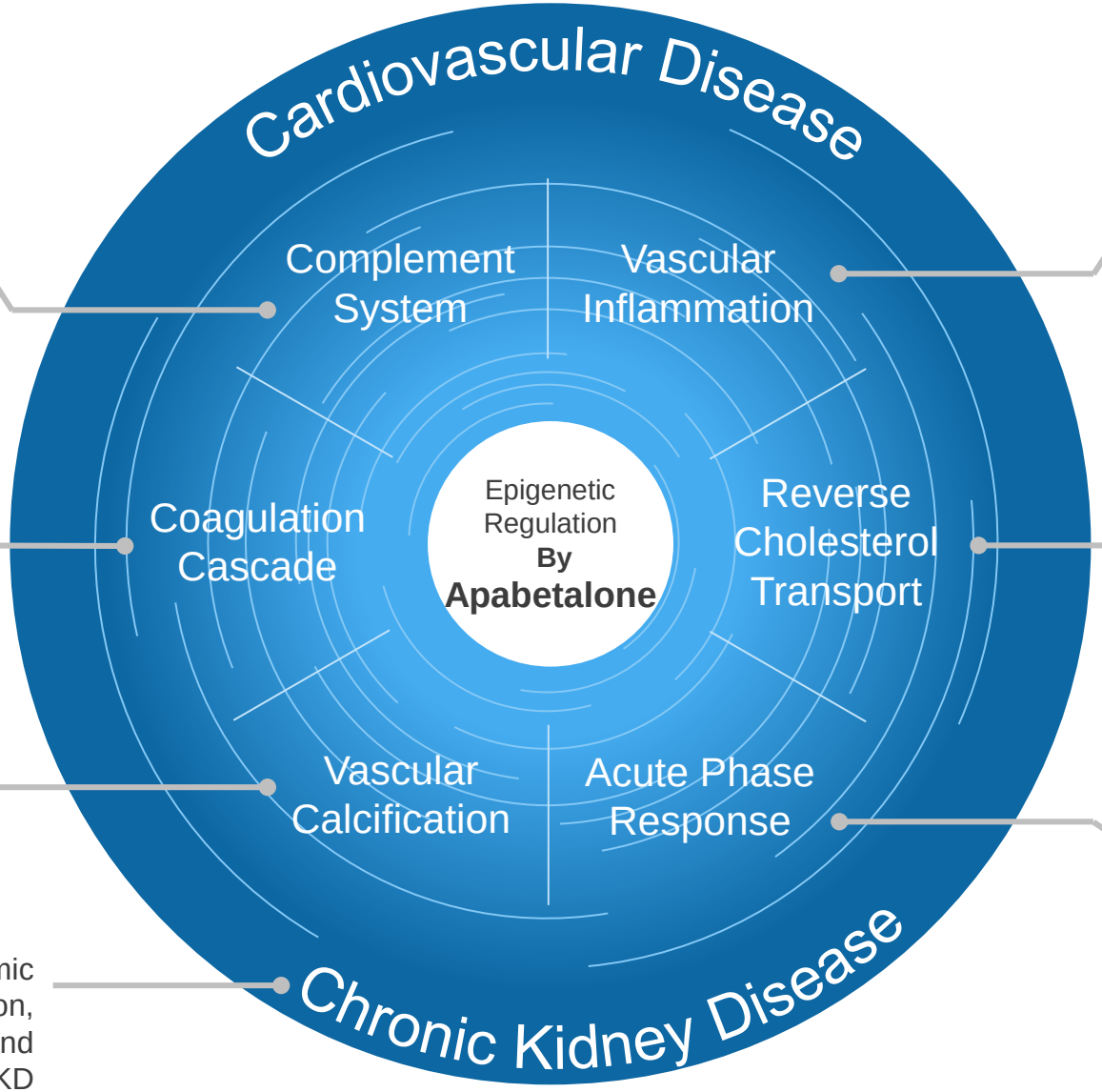
Acute Phase Response

Vascular Calcification

Vascular Calcification

Inflammation

Apabetalone Affects Pathways Leading to Vascular Disease



Apabetalone reduces the expression of multiple components of the complement cascade

Gilham et al. 2016
Wasiak et al. 2016
Wasiak et al. 2017

Apabetalone reduces the expression of several factors within the coagulation system

Gilham et al. 2016
Wasiak et al. 2017

Levels of ALP, osteopontin and other drivers of vascular calcification and fibrosis are lowered by apabetalone

Gilham et al. 2019

Apabetalone reduces markers of systemic inflammation, endothelial dysfunction, vascular calcification, fibrosis, and atherosclerosis in patients with CKD

Wasiak et al. 2018

Treatment with apabetalone reduces mediators that drive endothelial activation, monocyte recruitment and plaque destabilization

Tsujikawa et al. 2019
Wasiak et al. 2020 (submitted)

Apabetalone contributes to remodeling of the HDL proteome and lipidome, including increased ApoA-1 and HDL particle size

Jahagirdar et al. 2014
Gilham et al. 2016
Nicholls et al. 2017

Apabetalone reduces markers of systemic inflammation including acute phase reactants

Wasiak et al. 2020 (submitted)

Reduced Incidence of MACE & CHF

Nicholls et al. 2017 & Ray et al. 2020

Apabetalone Preclinical Work

Reverse Cholesterol Transport

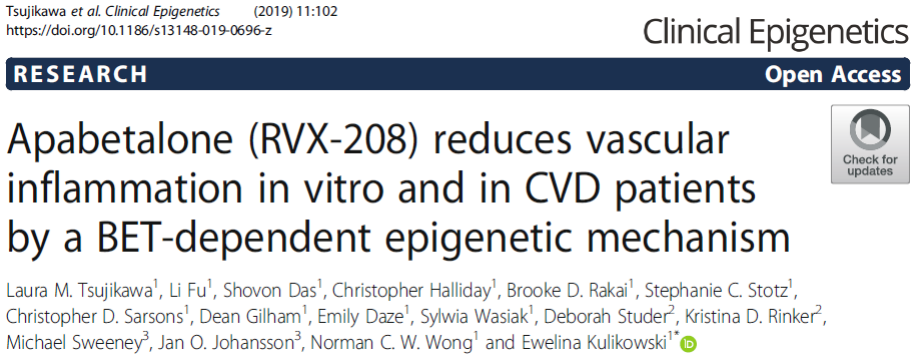
Complement and Coagulation

Acute Phase Response

Vascular Calcification and Fibrosis

Vascular Inflammation

Apabetalone prevents inflammatory (TNFα, LPS, or IL-1β) induction of genes that drive endothelial activation, monocyte recruitment, adhesion, and plaque destabilization. Apabetalone reduces BRD4 abundance on the promoters and enhancers of inflammation and adhesion genes. Pre-treatment of endothelial cells with apabetalone reduces monocyte adhesion to the endothelial monolayer. CVD patients treated with apabetalone have lower VI mediators in their plasma versus placebo.



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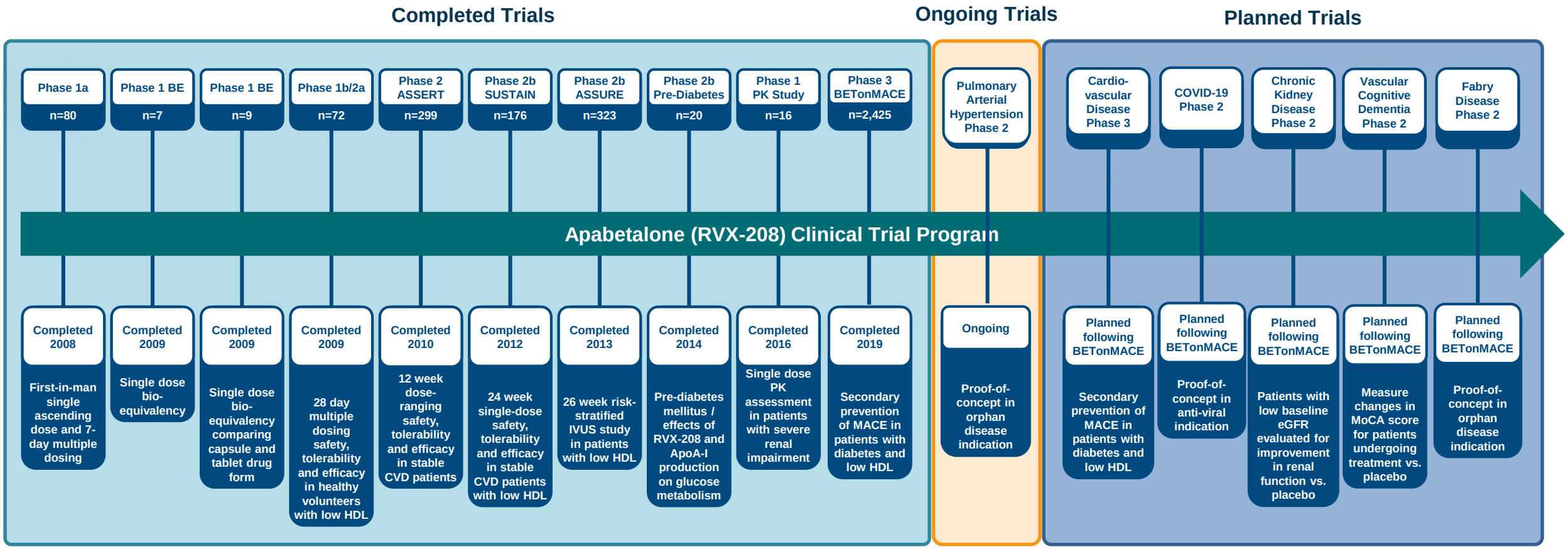
Clinical Epigenetics

RESEARCH Open Access

Apabetalone (RVX-208) reduces vascular inflammation in vitro and in CVD patients by a BET-dependent epigenetic mechanism

Laura M. Tsujikawa[1], Li Fu[1], Shovon Das[1], Christopher Halliday[1], Brooke D. Rakai[1], Stephanie C. Stotz[1], Christopher D. Sarsons[1], Dean Gilham[1], Emily Daze[1], Sylwia Wasiak[1], Deborah Studer[2], Kristina D. Rinker[2], Michael Sweeney[3], Jan O. Johansson[3], Norman C. W. Wong[1] and Ewelina Kulikowski[1*]

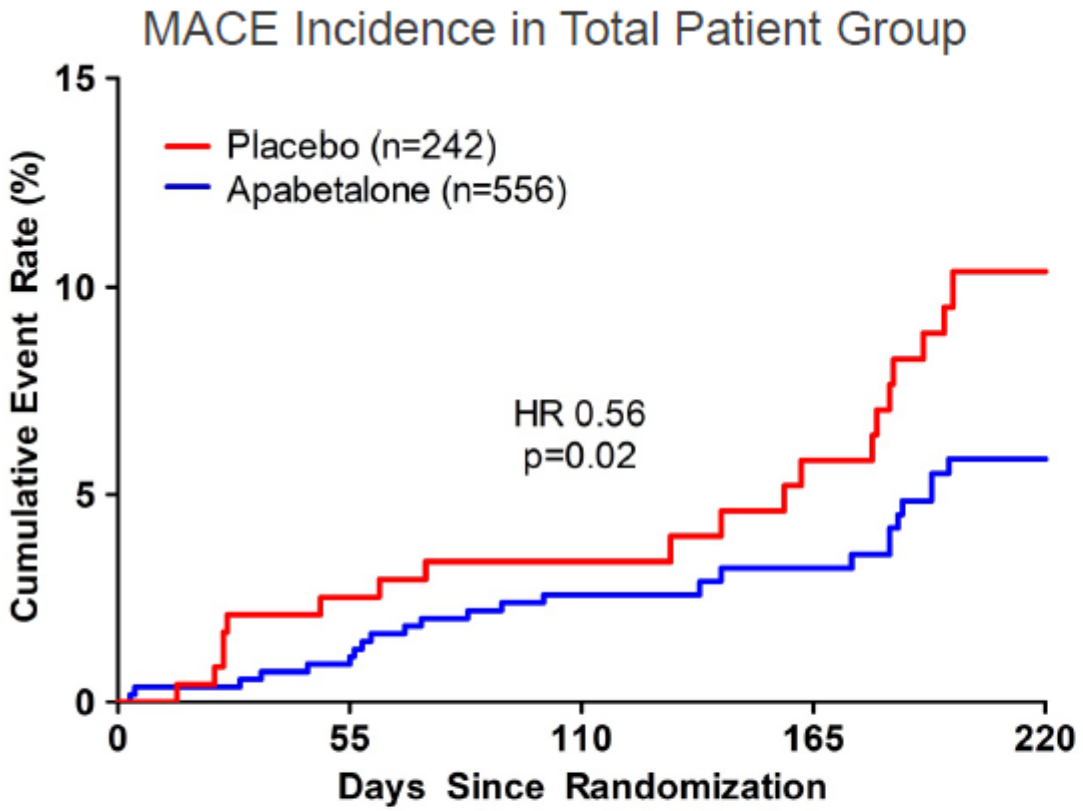
Clinical Program: Apabetalone for treating CVD

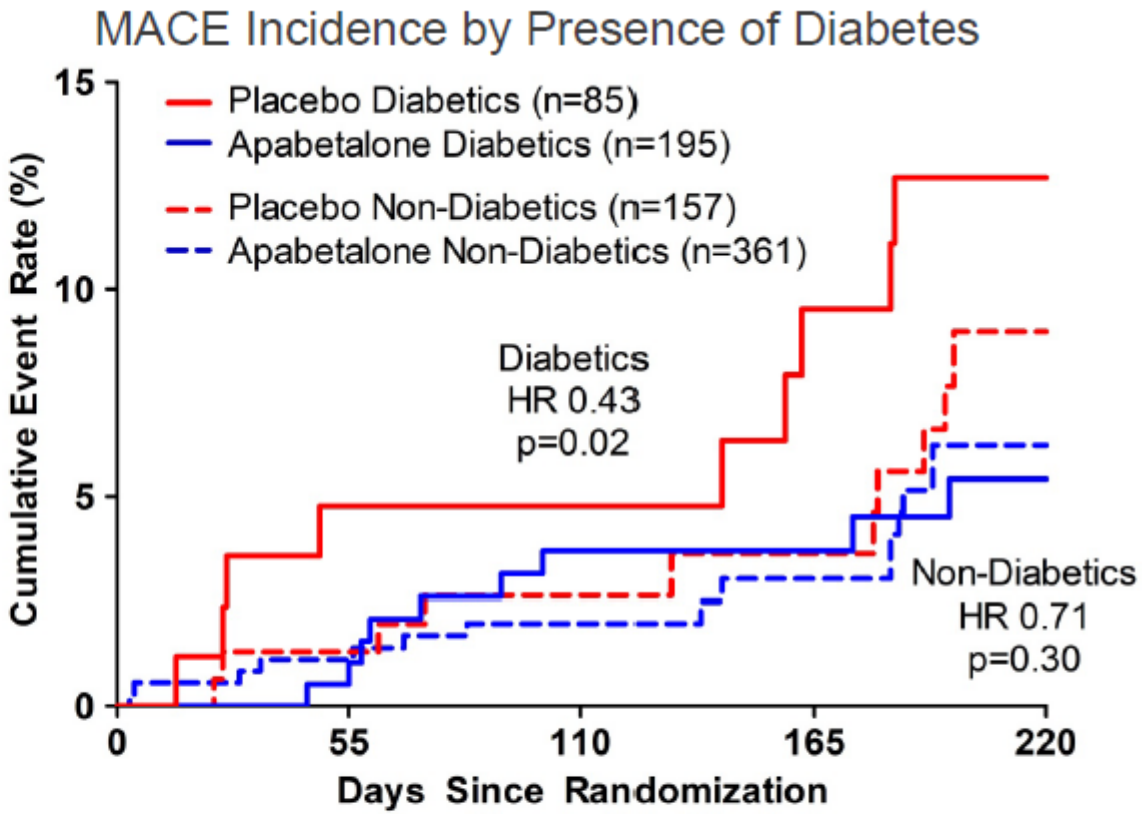


Summary of phase 2 clinical studies data

Trial Name	Study Number	Dose	Duration	Patient criteria	Patient number		MACE Endpoints	
					Apabetalone	Placebo	Apabetalone	Placebo
ASSERT	RVX222-CS-005	Multiple doses: 100mg, 200mg, 300mg daily	12 week	Stable coronary artery disease on standard of care therapy	76 on 100 mg 75 on 200 mg 74 on 300 mg	74	2.2%	2.7%
SUSTAIN	RVX222-CS-008	200 mg daily	24 week	Statin-treated patients with stable coronary artery disease and/or dyslipidemia, with low baseline HDL-C concentrations	88	88	0.4%	7.5%
ASSURE	RVX222-CS007	200 mg daily	26 week	Statin-treated patients with stable coronary artery disease and/or dyslipidemia, with low baseline HDL-C concentrations	243	80	7.5%	14.3%
Combined Pooled Analysis					556	242	5.9%	10.4%
				Subgroup: + T2DM			5.4%	12.7%
				Subgroup: + low HDL-C			5.5%	12.8%
				Subgroup: + high hsCRP			5.4%	14.2%

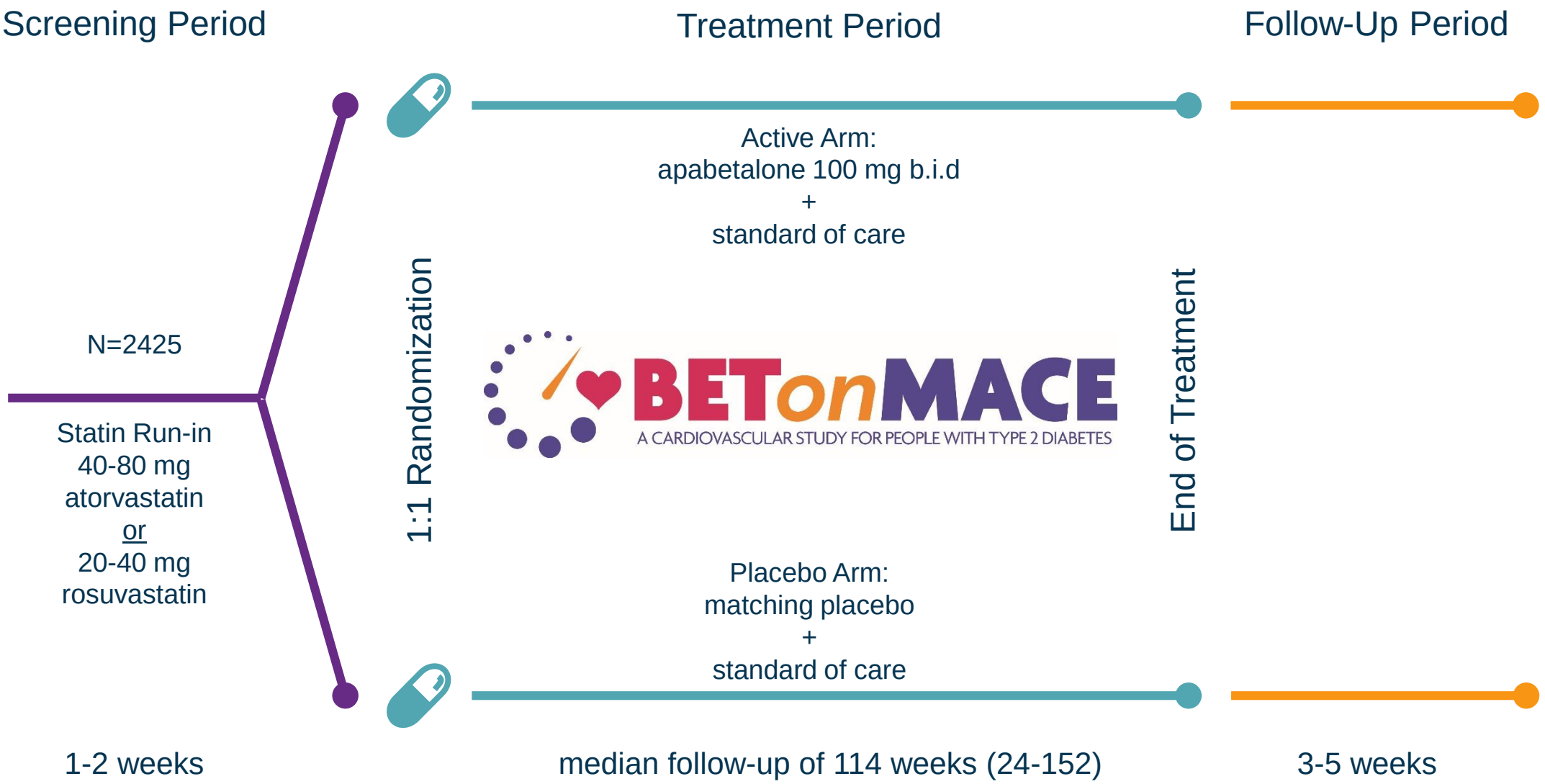
Apabetalone; Phase 2 results - Pooled Analysis: Potential CV Benefit





- MACE (major adverse cardiovascular events) including death, myocardial infarction, coronary revascularization, and hospitalization for cardiovascular causes.
- Other characteristics associated with greater effect of apabetalone in pooled Phase 2 were low HDL-C and high hsCRP
- **Data shown are aggregate from the following trials: ASSERT;ASSURE;SUSTAIN. *Nicholls Am J Cardiovasc Drugs 2018***

Design of Phase 3 Clinical Trial: BETonMACE



Phase 3 Clinical Trial: BETonMACE Objective and Endpoints

Primary Objective

- To evaluate if treatment with apabetalone as compared to placebo increases **time to the first occurrence of Primary MACE.**

Key Inclusion Criteria

- Type 2 Diabetes Mellitus
  - HbA1c >6.5% or history of diabetes medications
- Acute coronary syndrome 7-90 days prior to the screening visit
  - Unstable angina (Limited to 25% of total participants) or acute myocardial infarction
- Low HDL cholesterol
  - <40 mg/dL (1.04 mmol/L) for males; <45 mg/dL (1.17 mmol/L) for females at the screening visit

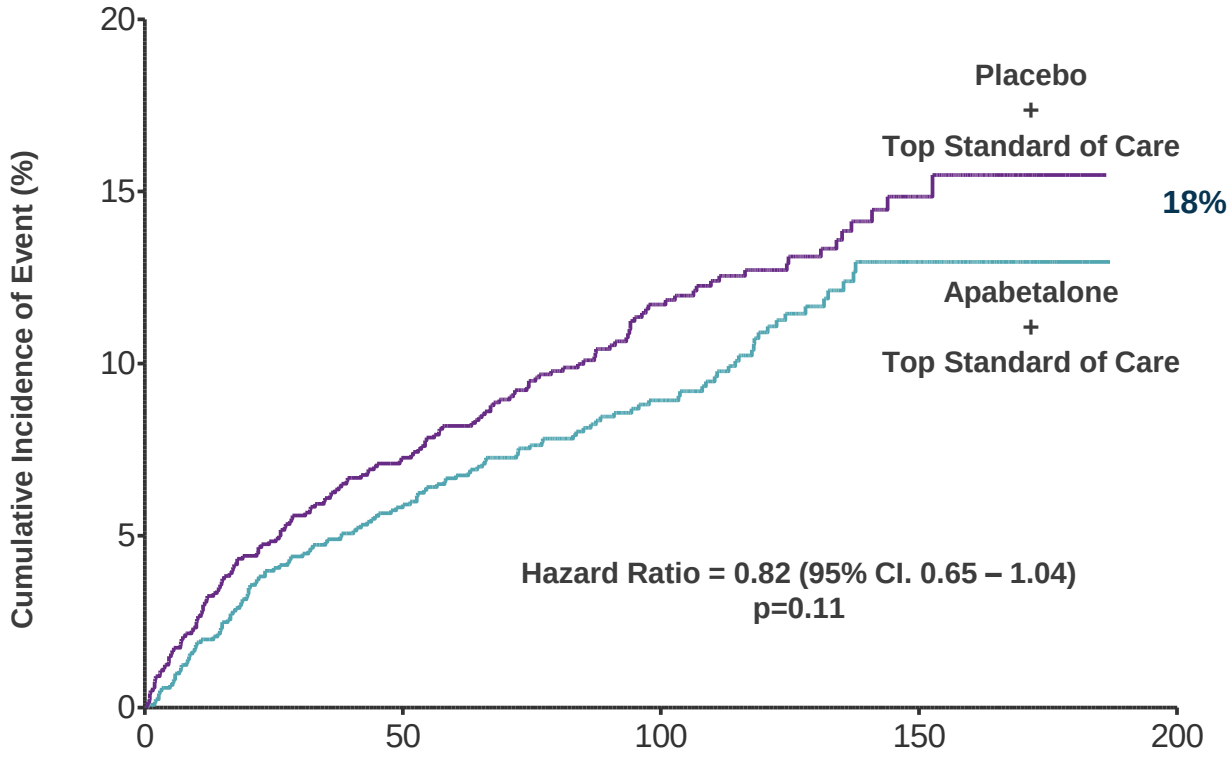
Primary Endpoint

- **Time to first occurrence of adjudication-confirmed primary MACE (CV death, non-fatal MI and stroke)**

Key Secondary and Exploratory Endpoints

- Change in kidney function in chronic kidney disease sub-population
  - Baseline estimated glomerular filtration rate (eGFR) <60 mL/min/1.7m^2
- Change in Montreal Cognitive Assessment (MoCA)
  - Evaluated in at-risk sub-population (>70 years old at randomization)

Phase 3 Clinical Trial: BETonMACE - Primary Endpoint of Narrow MACE



No. at Risk			Weeks		No. of Events
Placebo	1,206	1,105	701	169	149
Apabetalone	1,212	1,119	724	183	125

- The effect of the co-administration of apabetalone and top standard of care **– quantified by MACE defined as CV death, non-fatal MI and stroke –** illustrated a reduction of events compared to placebo and top standard of care
  - HR = 0.82 (95% CI, 0.65–1.04; p=0.11)
- Apabetalone was well tolerated with similar rates of adverse events compared to placebo in over 4,200 patient years

Top standard of care includes: high intensity statins, ACE inhibitors/angiotensin II blockers, beta blockers, antiplatelet agents

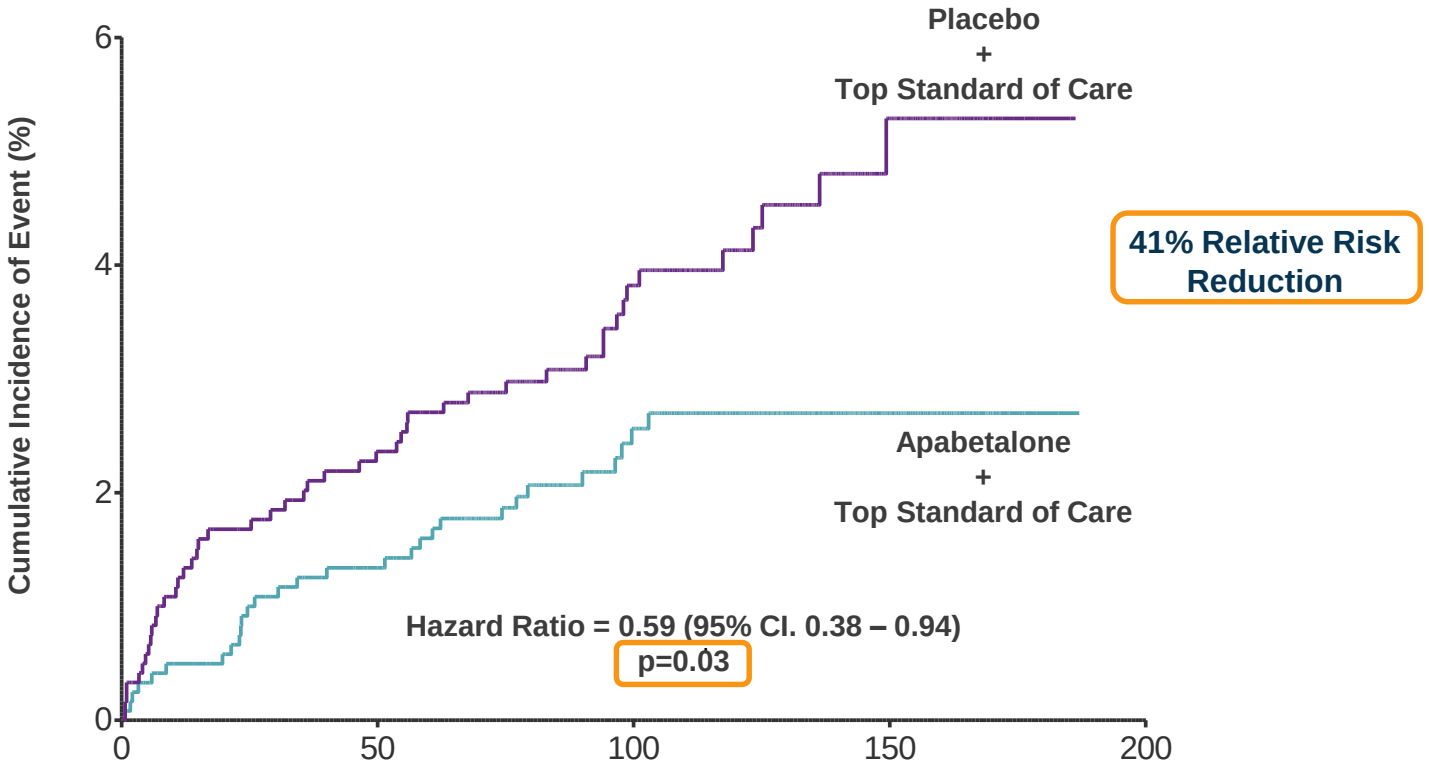
Apabetalone treatment led to a favorable 18% hazard reduction of MACE compared to placebo

Phase 3 Clinical Trial: Top Line Data Cardiovascular End-Points

	Apabetalone	Placebo	Hazard Ratio (95% CI)
Primary End Point	no. of events (% of patients)		
First occurrence of primary end point	125 (10.3%)	149 (12.4%)	0.82 [0.65, 1.04] p=0.11
Key Secondary End Points			
First occurrence of primary end point or hospitalization for unstable angina or urgent or emergency revascularization procedure	144 (11.9%)	166 (13.8%)	0.85 [0.68, 1.06]
First and recurrent primary end point events	171	203	0.79 [0.60, 1.06]
Cardiovascular death or non-fatal myocardial infarction	112 (9.2%)	139 (11.5%)	0.79 [0.61, 1.01]
Coronary heart disease death or non-fatal myocardial infarction	110 (9.1%)	136 (11.3%)	0.79 [0.61, 1.02]
Non-fatal myocardial infarction	77 (6.4%)	94 (7.8%)	0.80 [0.59, 1.08]
Cardiovascular death	45 (3.7%)	55 (4.6%)	0.81 [0.54, 1.19]
Stroke	17 (1.4%)	17 (1.4%)	1.01 [0.52, 1.98]
All cause mortality	61 (5.0%)	69 (5.7%)	0.88 [0.62, 1.24]
First hospitalization for congestive heart failure	29 (2.4%)	48 (4.0%)	0.59 [0.38, 0.94]
Other Pre-specified End Points			
First and recurrent hospitalization for congestive heart failure	35	70	0.47 [0.27, 0.83]
First occurrence of primary end point, excluding undetermined death	113 (9.3%)	140 (11.6%)	0.79 [0.62, 1.01]

Apabetalone had favorable trend on all cardiac endpoints that reached nominal statistical significance for congestive heart failure with a neutral effect on strokes

Phase 3 Clinical Trial: BETonMACE – Key Secondary Endpoint of Hospitalization for CHF



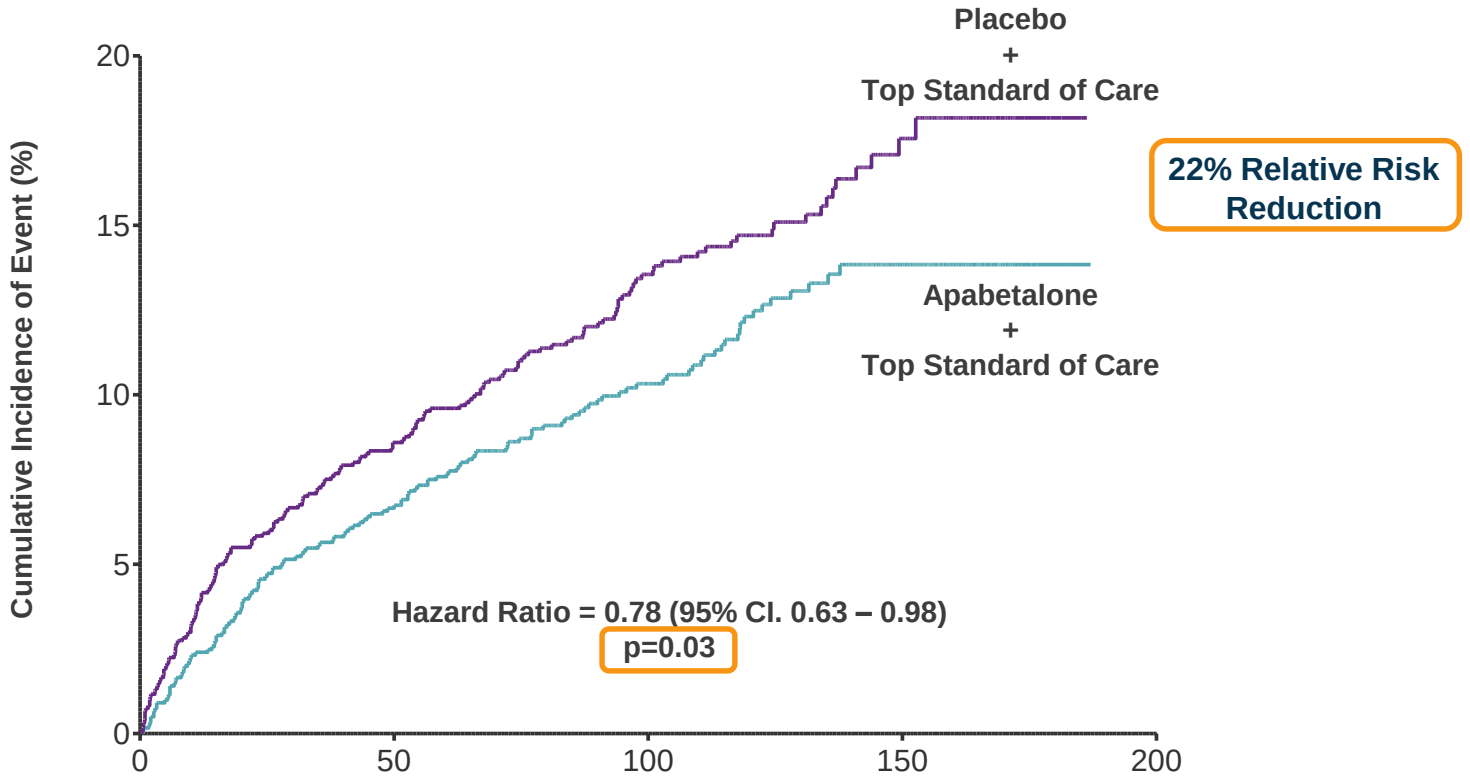
No. at Risk					No. of Events
Placebo	1,206	1,139	736	189	48
Apabetalone	1,212	1,152	749	198	29

- The effect of the co-administration of apabetalone and top standard of care **– quantified by hospitalization for congestive heart failure (CHF) –** illustrated a significant reduction of events compared to placebo and top standard of care
  - HR = 0.59 (95% CI, 0.38–0.94; p=0.03)
- Apabetalone was well tolerated with similar rates of adverse events compared to placebo in over 4,200 patient years

Top standard of care includes: high intensity statins, ACE inhibitors/angiotensin II blockers, beta blockers, antiplatelet agents

Apabetalone treatment led to a significant 41% RRR of hospitalization for CHF compared to placebo

Phase 3 Clinical Trial: BETonMACE – Composite of Narrow MACE and Hospitalization for CHF



No. at Risk					No. of Events
Placebo	1,206	1,091	688	168	173
Apabetalone	1,212	1,109	710	181	139

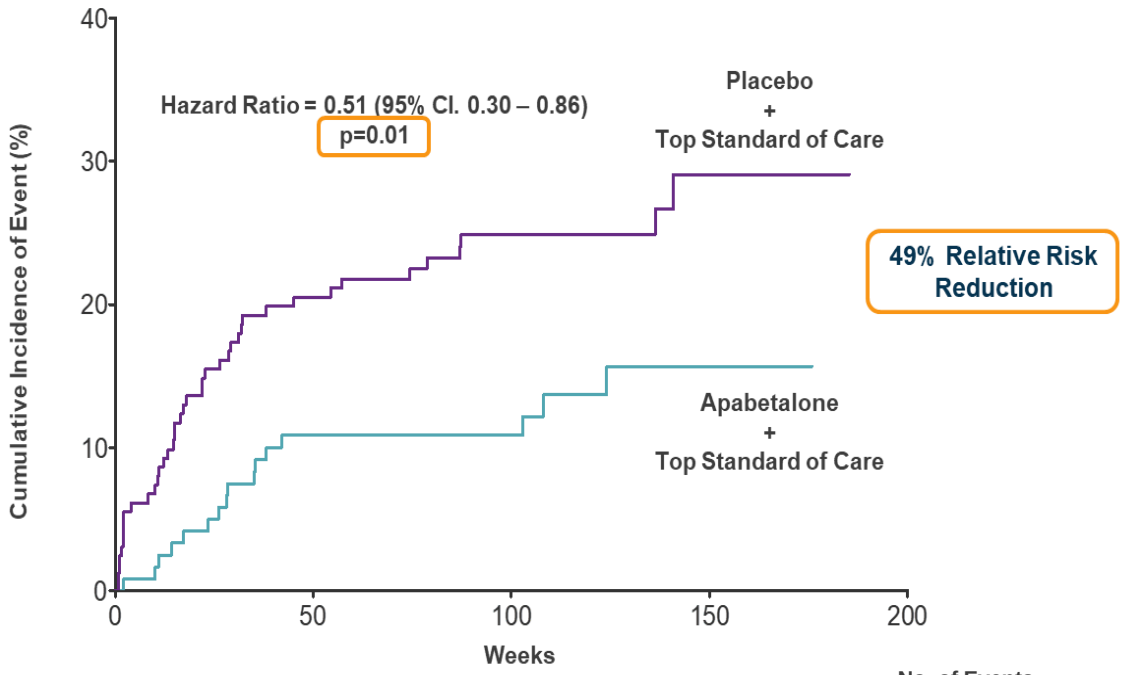
- The effect of the co-administration of apabetalone and top standard of care **– quantified by MACE defined as CV death, non-fatal MI, stroke and hospitalization for congestive heart failure (CHF)** – illustrated a significant reduction of events compared to placebo and top standard of care
  - HR = 0.78 (95% CI, 0.63–0.98; p=0.03)
- Apabetalone was well tolerated with similar rates of adverse events compared to placebo in over 4,200 patient years

Top standard of care includes: high intensity statins, ACE inhibitors/angiotensin II blockers, beta blockers, antiplatelet agents

Apabetalone treatment led to a significant 22% RRR of MACE and hospitalization for CHF compared to placebo

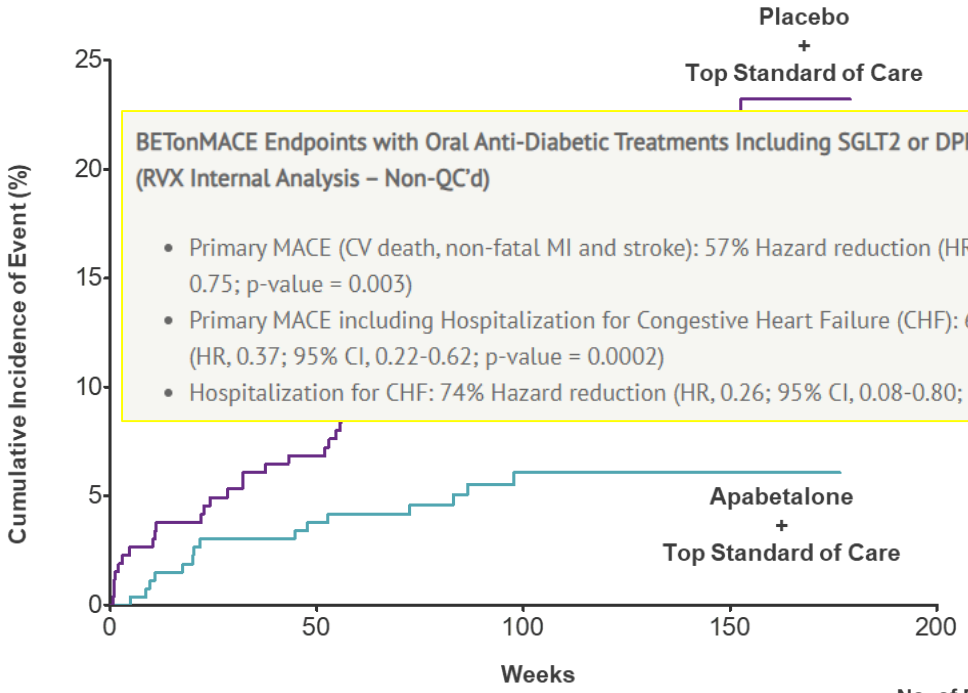
Phase 3 Clinical Trial: BETonMACE – Additional Observations

Patients With Baseline GFR <60 mL/min



No. at Risk					No. of Events
Placebo	163	127	84	19	41
Apabetalone	121	107	72	17	16

Patients Receiving SGLT2 or DPP4 Inhibitors



BETonMACE Endpoints with Oral Anti-Diabetic Treatments Including SGLT2 or DPP4 Inhibitors (RVX Internal Analysis – Non-QC'd)

- Primary MACE (CV death, non-fatal MI and stroke): 57% Hazard reduction (HR, 0.43; 95% CI, 0.25-0.75; p-value = 0.003)
- Primary MACE including Hospitalization for Congestive Heart Failure (CHF): 63% Hazard reduction (HR, 0.37; 95% CI, 0.22-0.62; p-value = 0.0002)
- Hospitalization for CHF: 74% Hazard reduction (HR, 0.26; 95% CI, 0.08-0.80; p-value = 0.02)

No. at Risk					No. of Events
Placebo	264	244	151	36	42
Apabetalone	265	253	169	41	15

On February 3, 2020, the FDA granted Breakthrough Therapy Designation for apabetalone in combination with top standard of care, including high-intensity statins, for the secondary prevention of MACE in patients with T2DM and recent ACS.

Apabetalone in Clinical Trials - Safety Analysis

- Apabetalone is well tolerated.
  - AE's and SAE's are similar in treated and placebo groups.
- Apabetalone treatment led to an increase in transaminases in 3.7% of treated subjects (ALT>5xULN) and only 3.1% greater than placebo.
  - Majority of ALT elevations occurred within a 16-week timeframe and resolved with continuing therapy for levels below 5x ULN and following therapy discontinuation for levels > 5x ULN
  - ALT elevations were not accompanied by raises in bilirubin - no Hy's law cases reported.
- Apabetalone treatment did not result in thrombocytopenia, a potential dose-limiting toxicity associated with many BETi oncology candidates.
- No additional toxicity issues have been identified that require dose scheduling, interruption or holidays.

Summary

1.) Human diseases share a common basic mechanism

2.) BET proteins are important components of the transcriptional machinery in gene regulation.

3.) Apabetalone is a selective BET inhibitor that has the potential to significantly lower MACE in pts with DM and CVD.

Outline

1.) ~~Role of epigenetics in disease (cancer and CVD/inflammation)~~
2.) ~~Activity of Bromodomain and ExtraTerminal Proteins (BET) in epigenetics~~
3.) ~~Apabetalone (RVX-208), a selective BET inhibitor~~
    - ~~Unique BD selectivity and mechanism of action~~
    - ~~Preclinical studies; cells and animals~~
    - ~~Biomarkers and pathways~~
    - ~~Clinical data (Phase 1-3 trials)~~
    - ~~Safety of Apabetalone~~
4.) Future development of Apabetalone
5.) Summary

Apabetalone Development in Additional Indications

- **Type 2 Diabetics with CVD**
  - Phase 3 trial BETonMACE2 is planned with 5,000 - 6,000 patients with T2DM, recent ACS and low HDL levels
- **CKD patients with CVD**
  - Single apabetalone dose regulated pathways that control immunity and inflammation, oxidative stress, endothelial dysfunction, vascular calcification, and coagulation in the CKD stage IV patient plasma proteome compared to matched controls (Wasiak 2017)
  - Apabetalone treatment of the CKD subgroup in the Phase 3 trial BETonMACE resulted in a HR 0.50 with CI 0.26-0.96, p=0.032 (Ray 2020)
  - BETonMACE2 intends to enroll at least 25% of patients with an eGFR <60 mL/min/1.7m^2
  - Phase 2 trial planned
- **Vascular Dementia**
  - Preclinical work suggest potential benefit in vascular dementia and neuroinflammation
  - Apabetalone treatment of BETonMACE patients with a baseline MoCA<22 significantly improved MoCA scores compared to placebo
  - Phase 2 trial planned
- **Pulmonary Arterial Hypertension (PAH)**
  - Apabetalone reversed the PAH phenotype in isolated PAH microvascular endothelial cells and smooth muscle cells in vitro, and in diverse PAH rat models (Van der Feen 2019)
  - Currently enrolling 10 well-characterized PAH patients stable for >4 months on standard PAH-therapies in a 16 week Phase 2 trial
- **Fabry Disease (FD)**
  - Ex vivo treatment of FD patient PBMCs with apabetalone inhibits pro-inflammatory responses initiated via the TLR4 pathway in stimulated monocytes and neutrophils
  - Phase 2 trial planned
- **Facioscapulohumeral dystrophy (FSHD)**
  - Apabetalone prevents DUX4 activation and the transcription of downstream disease associated genes. FSHD animal models in development to assess efficacy
- **COVID-19**
  - ACE2 is a cell surface receptor used by 2019-nCoV to gain entry into host cells. Apabetalone reduces circulating ACE2 protein levels in patients, and downregulates ACE2 gene transcription by 40-90% in vitro
  - Phase 2 trial planned

Summary

1.) Human diseases share a common basic mechanism

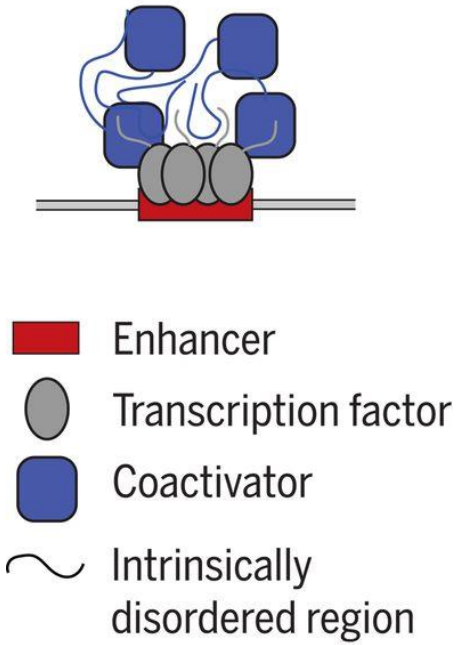
2.) BET proteins are important components of the transcriptional machinery in gene regulation.

3.) Apabetalone is a selective BET inhibitor used chronically has the potential to significantly lower MACE in pts with DM and CVD.

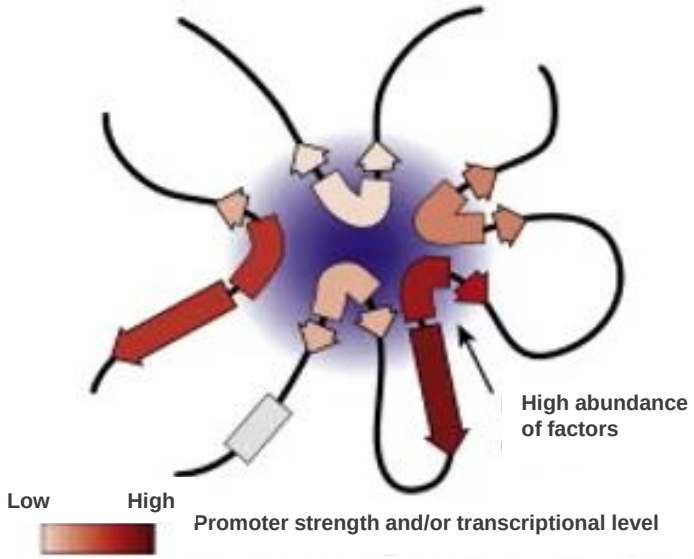
4.) Apabetalone is currently being tested in other disease states.

Typical-, Super-, and Active-Enhancers in Regulating Gene Activity

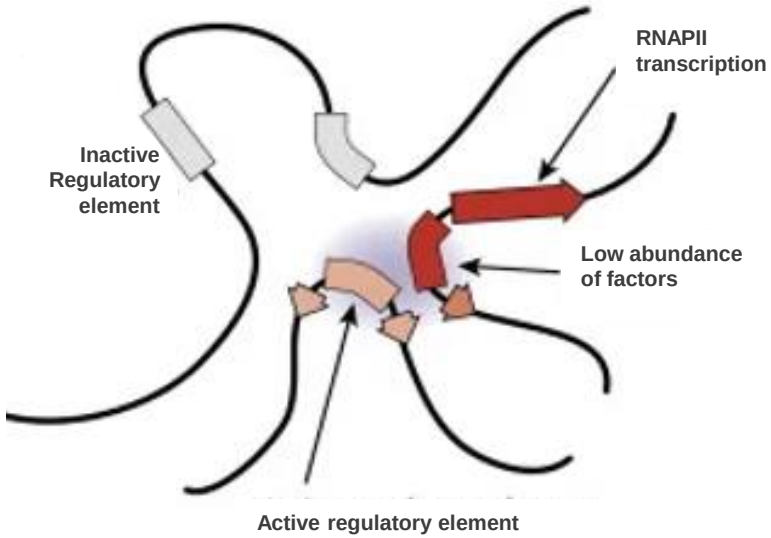
1.) Typical enhancer



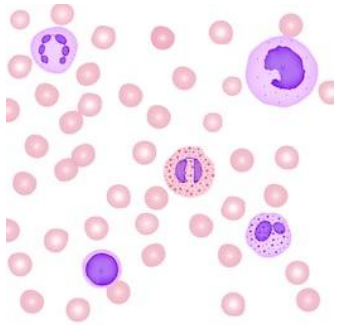
2.) Super-enhancer



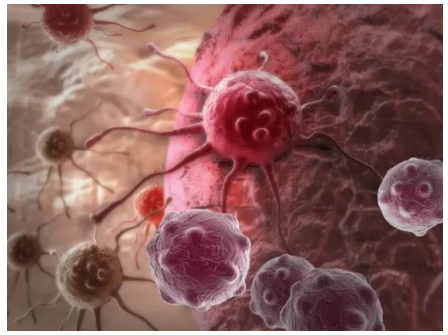
3.) Active enhancer



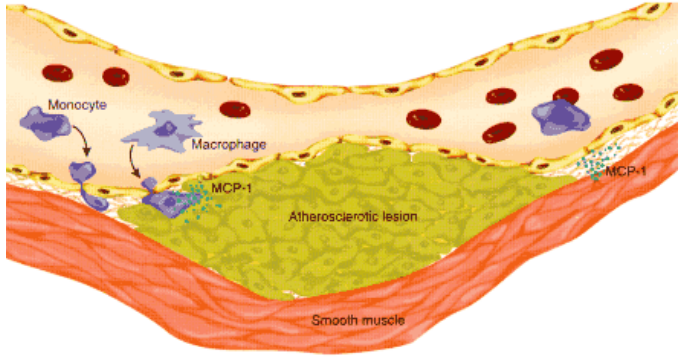
Benjamin R. Sabari et al. Science 2018;361:eaar3958



Normal Cells



Cancer Cell



CVD Cells

Acknowledgements

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Clinical Team: San Francisco, California, USA

- Jan O. Johansson
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Select Publications:

- **Ray 2020** Effect of apabetalone added to standard therapy on major adverse cardiovascular events in patients with recent acute coronary syndrome and Type 2 diabetes: a randomized clinical trial. **JAMA**
- **Ray 2019** Effect of selective BET protein inhibitor apabetalone on cardiovascular outcomes. **Am Heart J**
- **Tsujikawa 2019** Apabetalone (RVX-208) reduces vascular inflammation in vitro and in CVD patients by a BET-dependent epigenetic mechanism**. Clinical Epigenetics**.
- **Gilham 2019** Apabetalone downregulates factors and pathways associated with vascular calcification. **Atherosclerosis.**
- **Shishikura 2019** The Effect of Bromodomain and Extra-Terminal Inhibitor Apabetalone on Attenuated Coronary Atherosclerotic Plaque: Insights from the ASSURE Trial. **Am J Cardiovasc Drugs.**
- **Haarhaus 2019** Apabetalone lowers serum alkaline phosphatase and improves cardiovascular risk in patients with cardiovascular disease**. Atherosclerosis.**
- **Haarhaus 2019** Pharmacologic epigenetic modulators of ALP in CKD **Curr Opin Nephol Hyperten**
- **Kulikowski 2018** Apabetalone Mediated Epigenetic Modulation is Associated with Favorable Kidney Function and Alkaline Phosphatase Profile in Patients with Chronic Kidney Disease. **Kidney Blood Press Res.**
- **Nicholls 2018** Selective BET Protein Inhibition with Apabetalone and Cardiovascular Events: A Pooled Analysis of Trials in Patients with Coronary Artery Disease. **Am J Cardiovasc Drugs.**
- **Wasiak 2017** Benefit of Apabetalone on Plasma Proteins in Renal Disease.**Kidney Int Rep.**
- **Wasiak 2017** Downregulation of the Complement Cascade In Vitro, in Mice and in Patients with Cardiovascular Disease by the BET Protein Inhibitor Apabetalone (RVX-208). **J Cardiovasc Transl Res.**
- **Gilham 2016** RVX-208, a BET-inhibitor for treating atherosclerotic cardiovascular disease, raises ApoA-I/HDL and represses pathways that contribute to cardiovascular disease. **Atherosclerosis.**
- **Wasiak 2016** Data on gene and protein expression changes induced by apabetalone (RVX-208) in ex vivo treated human whole blood and primary hepatocytes. **Data Brief.**
- **Nicholls 2016** Effect of the BET Protein Inhibitor, RVX-208, on Progression of Coronary Atherosclerosis: Results of the Phase 2b, Randomized, Double-Blind, Multicenter, ASSURE Trial. **Am J Cardiovasc Drugs.**
- **Jahagirdar 2014** A novel BET bromodomain inhibitor, RVX-208, shows reduction of atherosclerosis in hyperlipidemic ApoE deficient mice. **Atherosclerosis.**
- **McLure 2013** RVX-208, an inducer of ApoA-I in humans, is a BET bromodomain antagonist. **PLoS One.**
- **Nicholls 2012** ApoA-I induction as a potential cardioprotective strategy: rationale for the SUSTAIN and ASSURE studies. **Cardiovasc Drugs Ther**
- **Nicholls 2010** Efficacy and safety of a novel oral inducer of apolipoprotein a-I synthesis in statin-treated patients with stable coronary artery disease a randomized controlled trial**. J Am Coll Cardiol.**
- **Bailey 2010** RVX-208: a small molecule that increases apolipoprotein A-I and high-density lipoprotein cholesterol in vitro and in vivo [published correction appears in **J Am Coll Cardiol**