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TSX: RVX

The Epigenetic Modulator Apabetalone Decreases Neuroinflammation in Blood Brain Barrier Cell Models and LPS-Treated Mice

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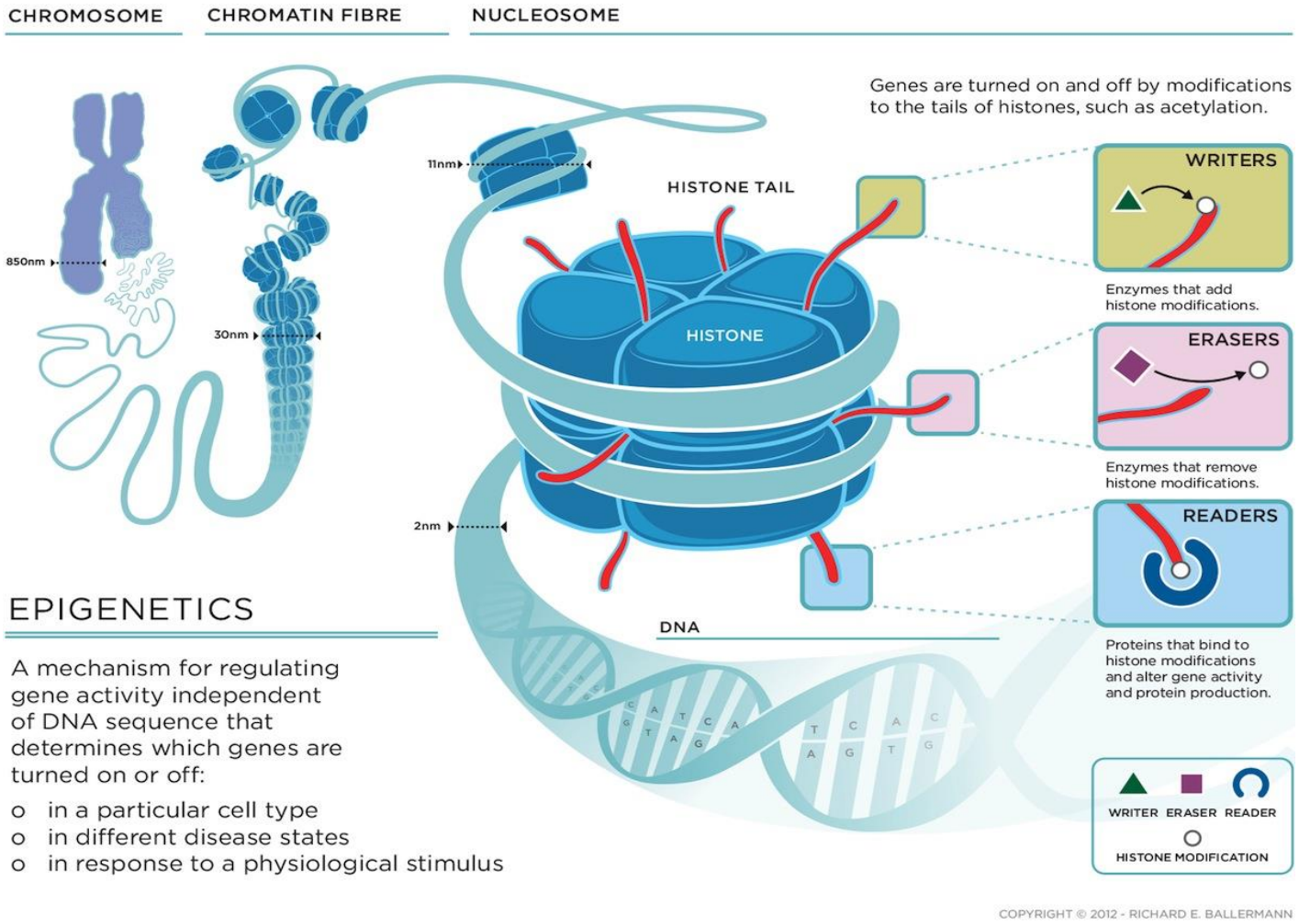
Epigenetics



The *epigenetic code* refers to secondary modifications to chromatin components that *regulate transcriptional activity*

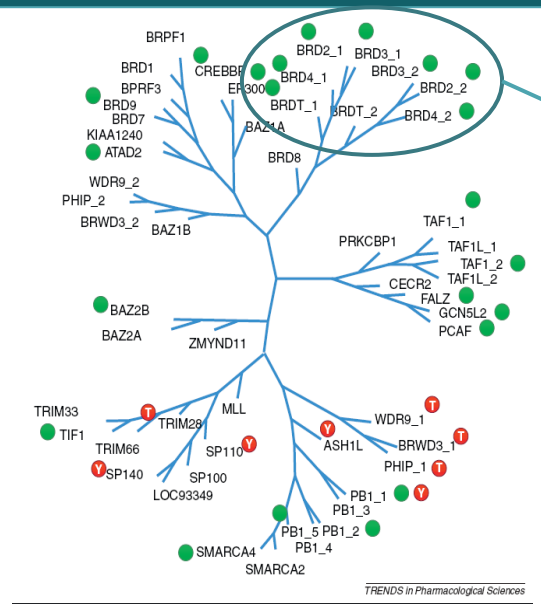
Addition, removal or recognition of these modifications is done by proteins called *writers, erasers and readers*

Acetylation of histone lysine residues by writers marks *active regions* of chromatin

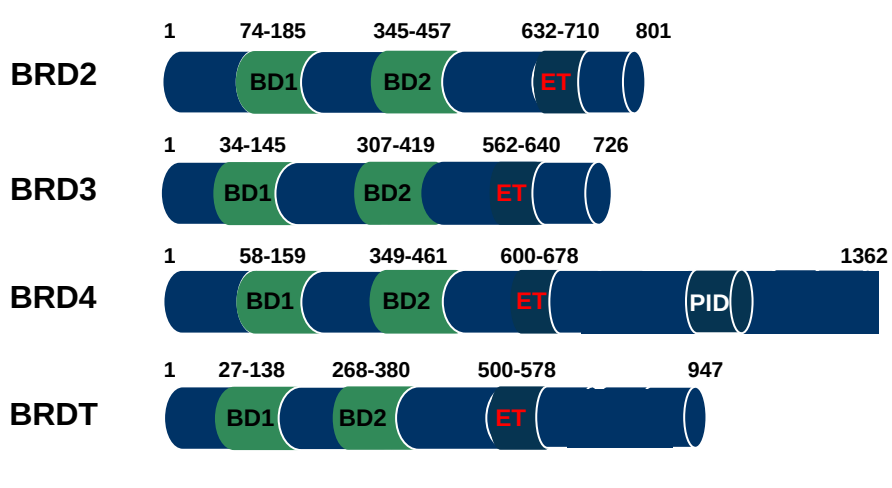
Acetylated lysines on histones are recognized by *readers called BET proteins* that recruit transcriptional regulatory factors to *activate or suppress genes*

Apabetalone (RVX-208) Is a Small Molecule Inhibitor that Competitively Inhibits BET Bromodomains

BET proteins are part of a superfamily of proteins



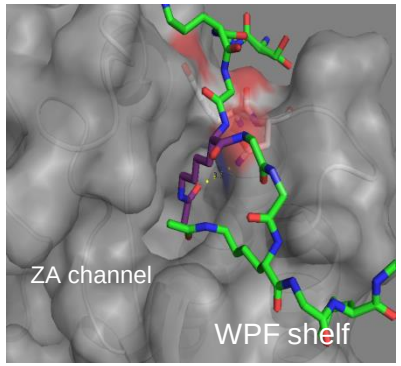
Each BET protein contains two bromodomains (BD)

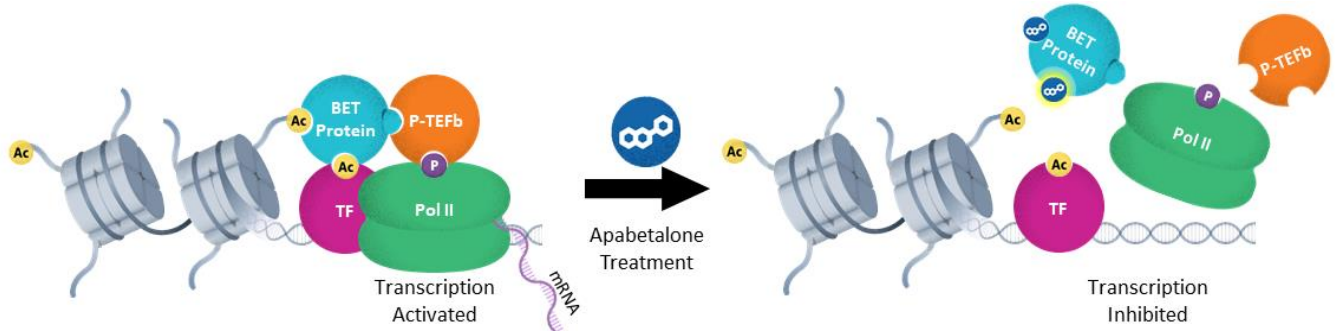


Bromodomains bind acetylated histones and transcription factors to regulate gene transcription

X-ray crystallography

Acetylated lysine (color) bound to bromodomain (grey)



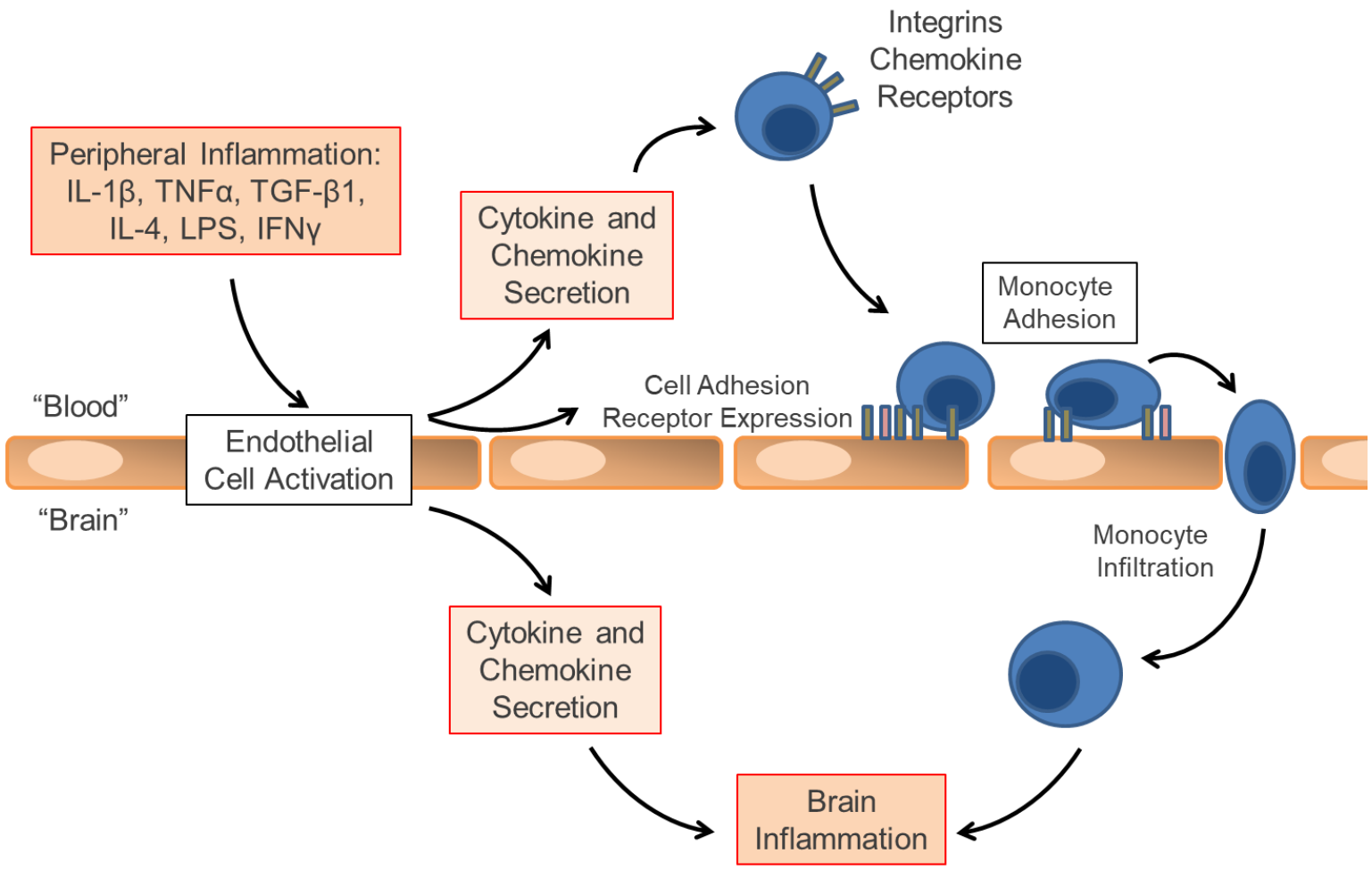


Apabetalone disrupts BD-chromatin binding

Apabetalone in Human Clinical Trials

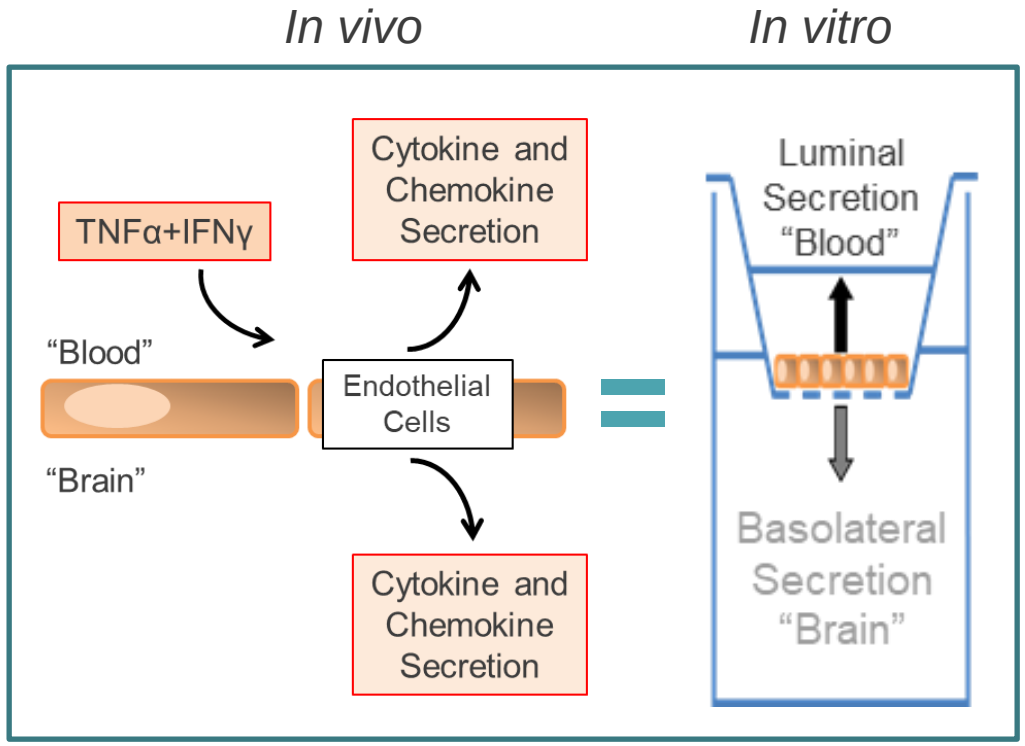
- **Apabetalone/RVX-208/RVX000222** (2-(4-(2-hydroxyethoxy)-3,5-dimethylphenyl)-5,7-dimethoxyquinazolin-4(3H)-one) was discovered in 2006.
- Tested in multiple phase 2 trials in CVD patients (endpoints: HDL, ApoA-I elevation)
- Phase 3 cardiovascular event-driven trial BETonMACE
  - ***Design***: Multi-centre, double-blind, randomized, parallel group, placebo-controlled
  - ***Patients***: 2400+ high risk type 2 diabetes with CAD, up to 104 weeks of dosing
  - ***Results****:* Apabetalone treatment showed a favorable trend on all cardiac endpoints and reached nominal statistical significance for CHF
  - On February 3, 2020, the FDA granted ***Breakthrough Therapy Designation*** to 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.
  - A follow-up phase 3 trial BETonMACE2 is currently being planned.

Endothelial Dysfunction and Monocyte Infiltration Contribute to Neuroinflammation



- Peripheral inflammation associated with chronic disease **causes brain endothelial cells to express proinflammatory molecules** and to lose their barrier properties, allowing for **monocyte infiltration** into the brain.
- Endothelial proinflammatory activation is ascribed to **epigenetic regulation of gene transcription by BETi**.
- Hypothesis: Epigenetic modulators such as **apabetalone can "correct" the pro-inflammatory phenotype** of brain endothelial cells.

Apabetalone Counters Cytokine Expression in Brain Endothelial Cells

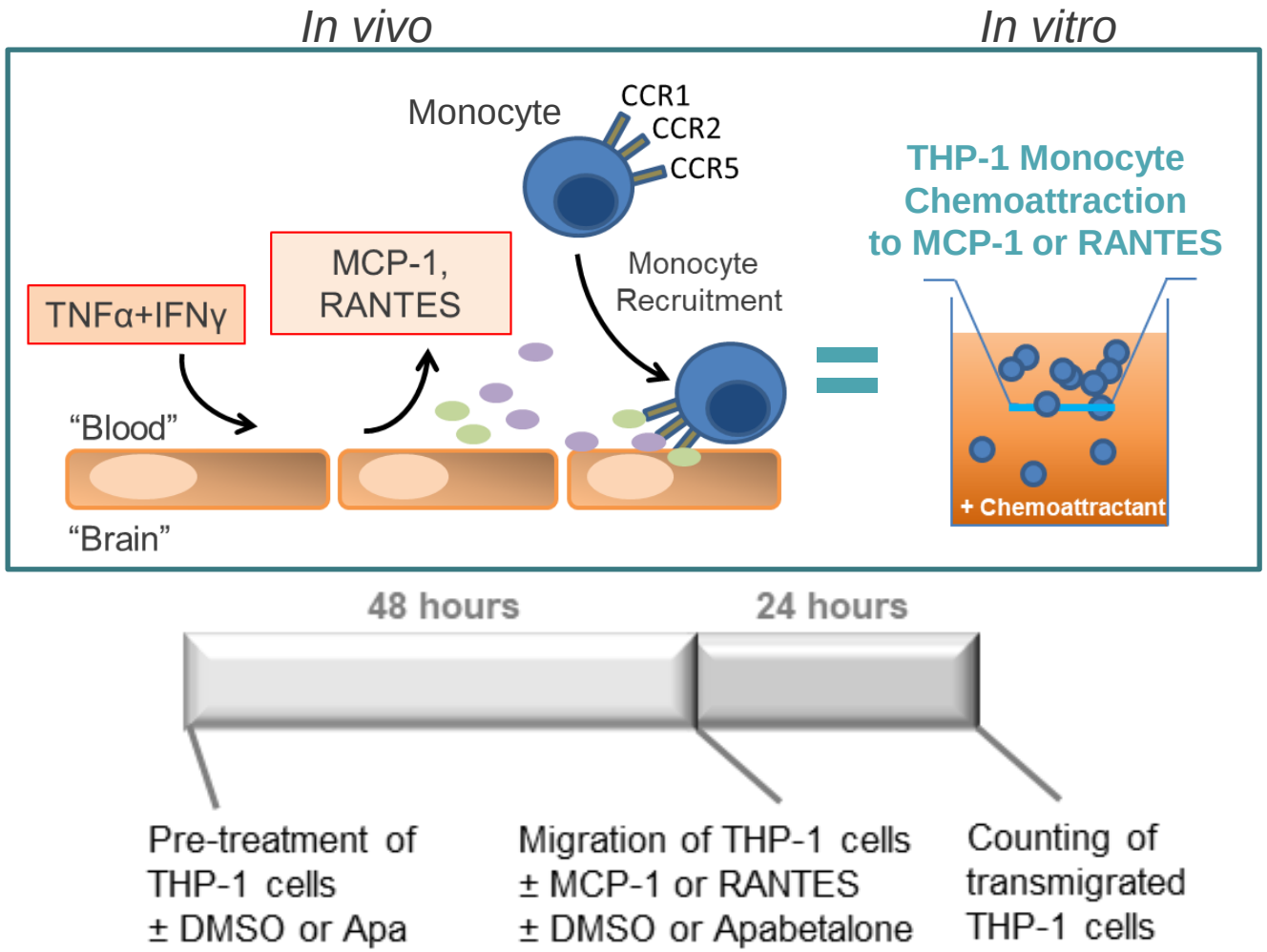


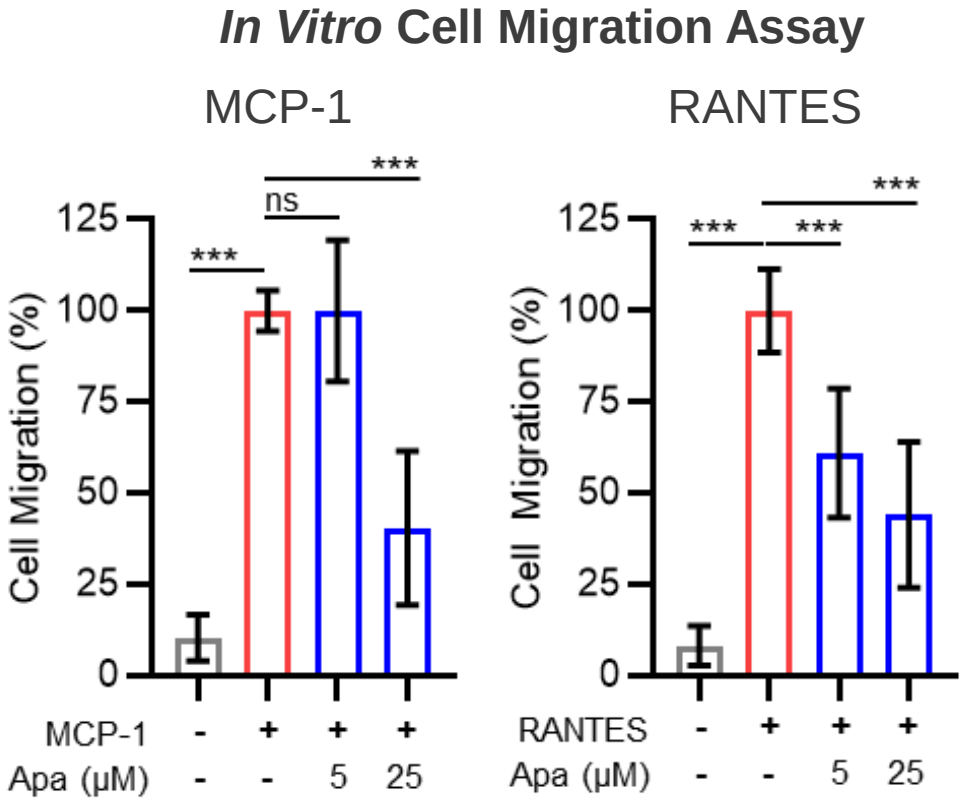
TNFα+IFNγ Stimulation (4h) of hCMEC/D3 cells

		Gene Expression		Luminal Secretion		Basolateral Secretion	
Gene	Protein	Vehicle Fold Induction	Apabetalone % Reduction	Vehicle Fold Induction	Apabetalone %Reduction	Vehicle Fold Induction	Apabetalone % Reduction
CCL7	**MCP-3**	**115**	**92**	**305**	**93**	**58**	**83**
CX3CL1	**Fractalkine**	**1863**	**98**	**101**	**89**	**16**	**87**
CSF2	**GM-CSF**	**11**	**97**	**11**	**85**	6.5	82
CCL2	**MCP-1**	**107**	**94**	**53**	**68**	**29**	**74**
IL6	**IL-6**	**11**	**72**	**49**	52	**15**	46
CXCL8	**IL-8**	**9**	**74**	**16**	**41**	**8.5**	**39**
CXCL10	**IP-10**	**17448**	**86**	**4450**	30	**3435**	**26**
CCL5	**RANTES**	**32**	**77**	**21**	21	**6.2**	**44**

Apabetalone treatment reduced bilateral secretion of cytokines in brain endothelial cells, indicating that BET inhibition can counter propagating of proinflammatory signals on either side of the BBB.

Apabetalone Reduces Monocyte Chemoattraction

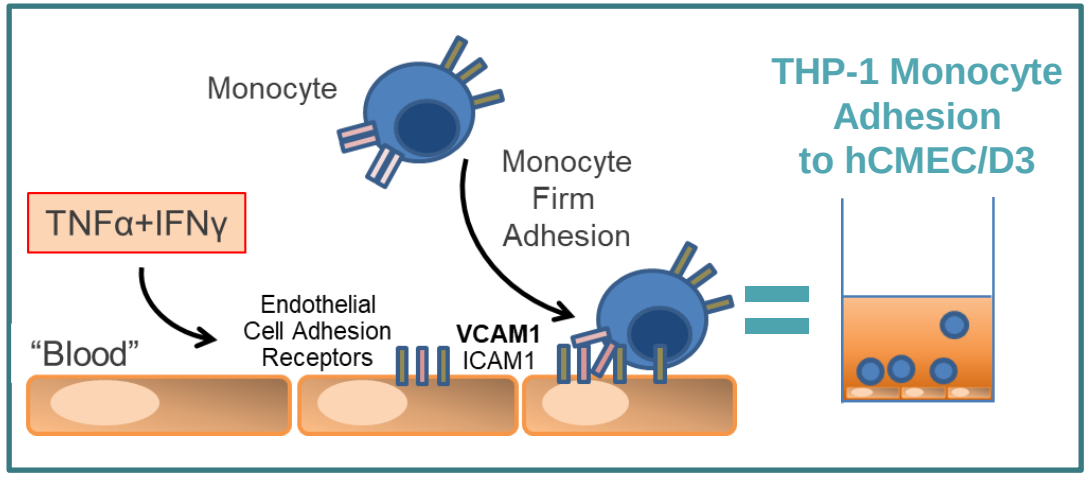


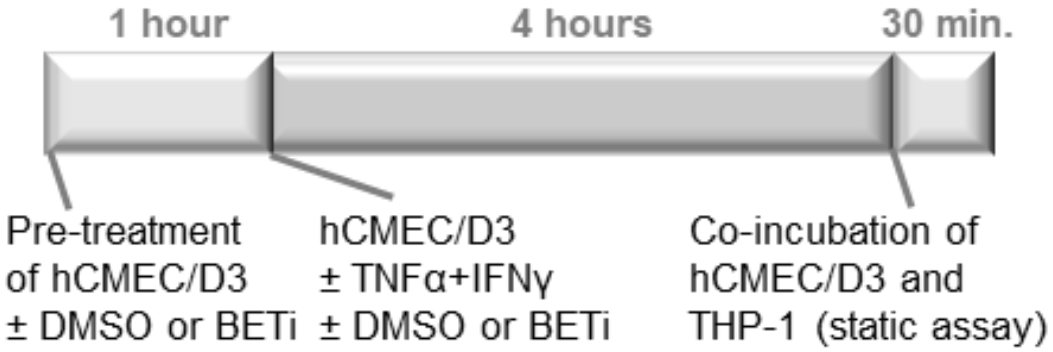


Apabetalone treatment reduced chemokine receptor expression and monocyte migration towards chemokines *in vitro*.

Apabetalone Reduces Endothelial Adhesion Molecule Expression and Monocyte Adhesion to Brain Endothelial Cells

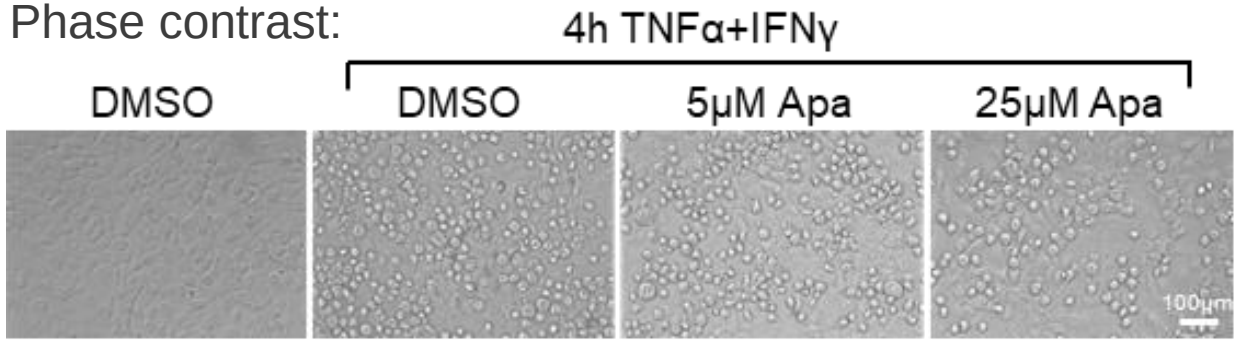
In vivo *In vitro*



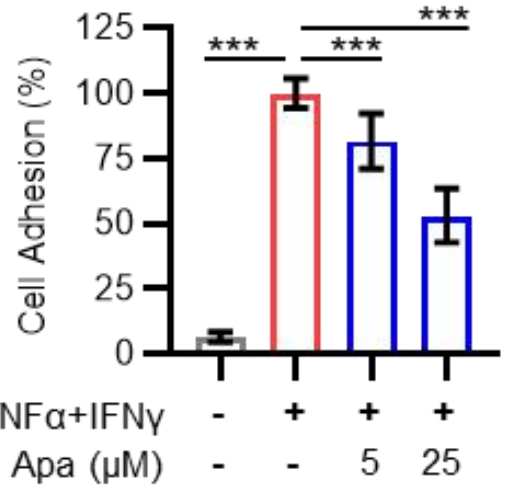


THP-1 – hCMEC/D3 Adhesion Assay

Phase contrast:



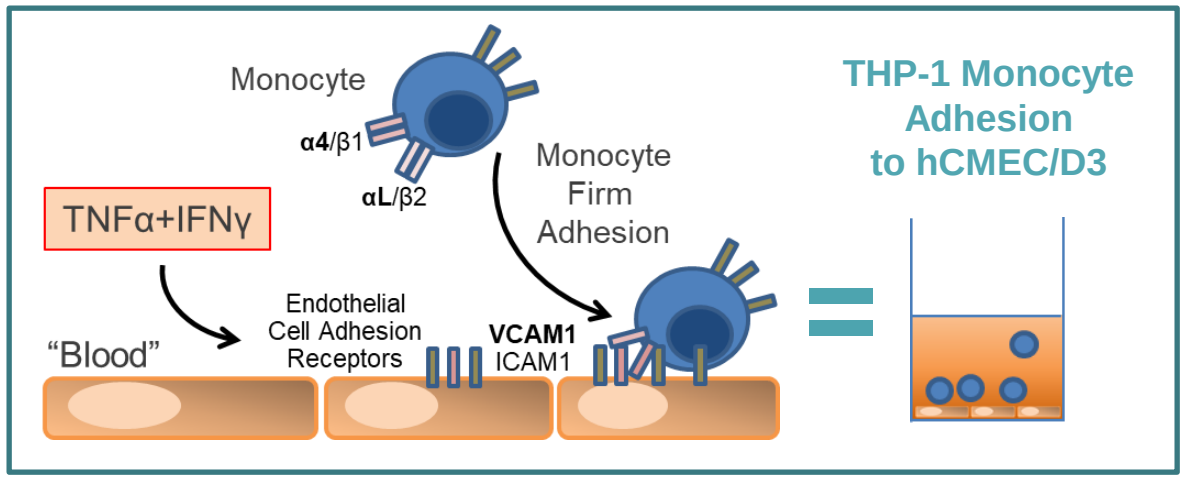
Quantification:

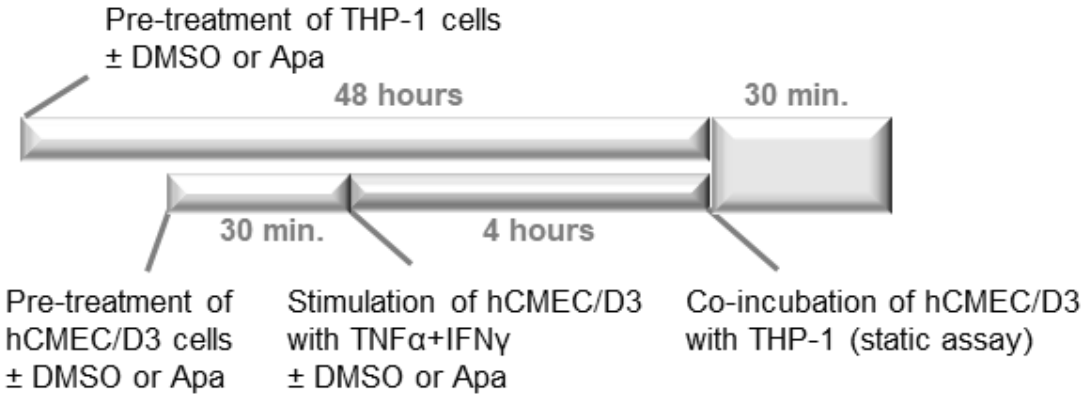


Apabetalone treatment reduced cytokine induced VCAM1 expression in endothelial cells and their adhesion to THP-1 cells

Apabetalone Reduces the Expression of Monocyte Adhesion Receptors and their Adhesion to Endothelial Cells

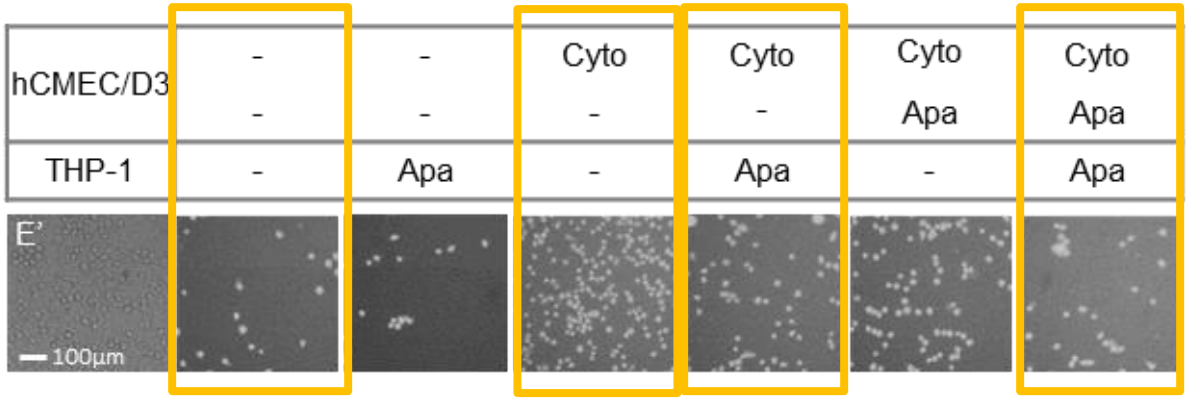
In vivo *In vitro*



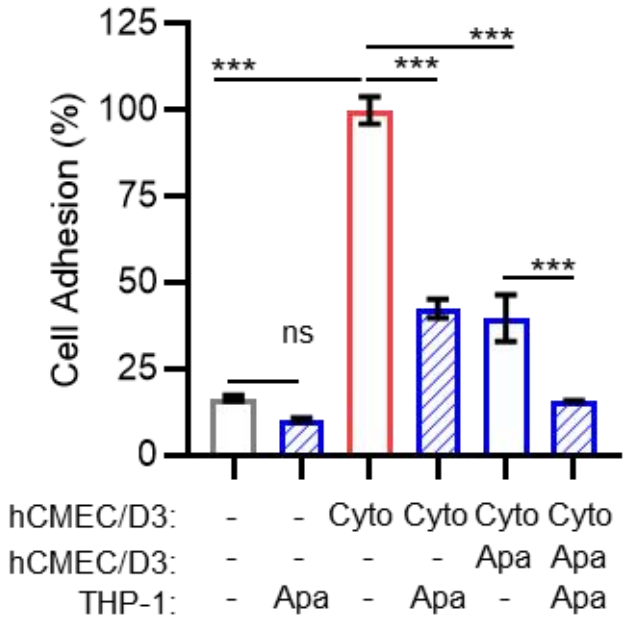


THP-1 – hCMEC/D3 Adhesion Assay

Fluorescence:

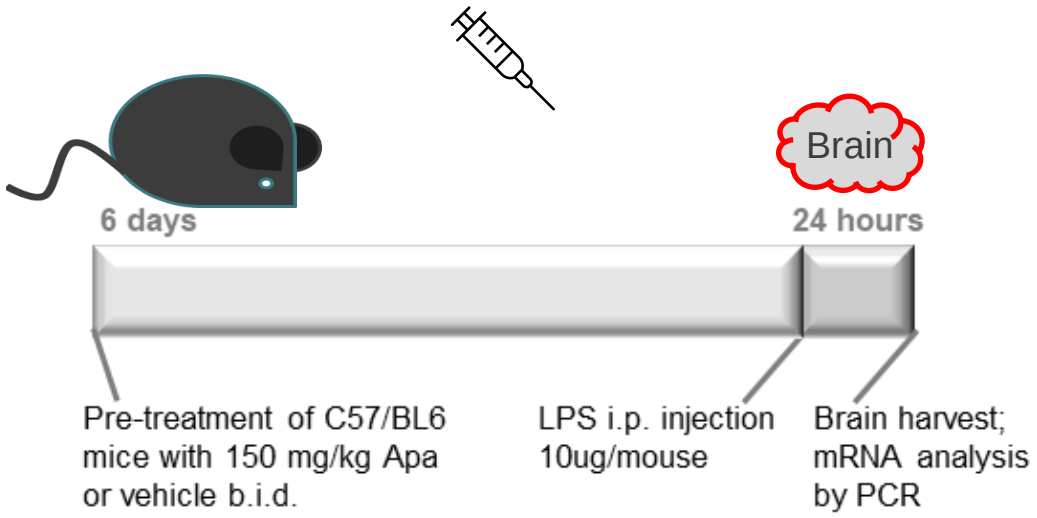


Quantification:



Apabetalone treatment reduces monocyte adhesion to endothelial cells

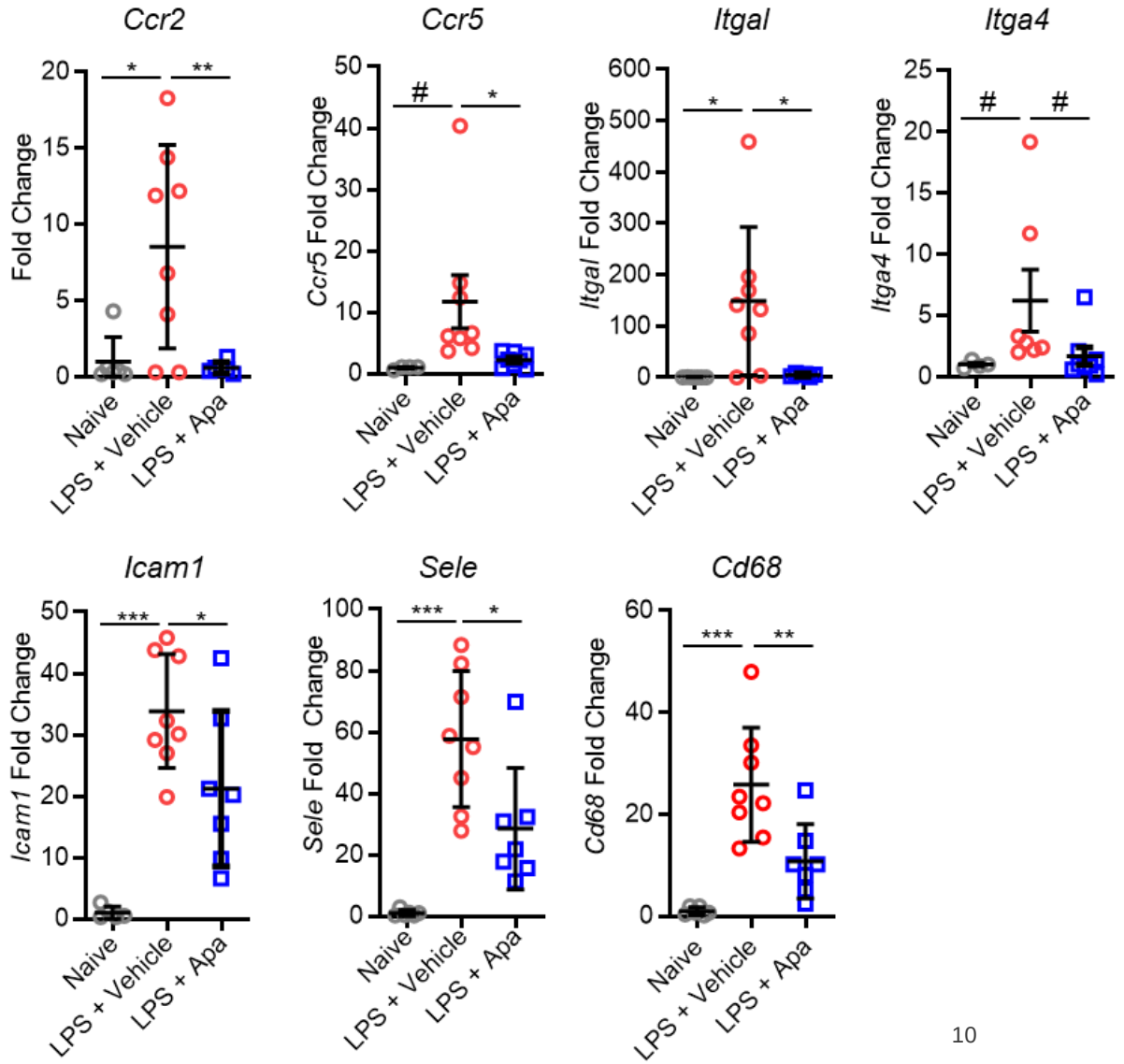
Apabetalone Reduces the Expression of Endothelial and Leukocyte Inflammation Markers in the Mouse Brain



Pharmacokinetics

Tissue	Apabetalone
Brain	2.4 µM
Plasma	32 µM
Brain : Plasma Ratio	**0.07**

Gene Expression in the Mouse Brain



Peripheral activity of apabetalone reduces endothelial activation in the brain

Pro-Inflammatory Monocyte Hyper-Activation Is Sensitive to BET Inhibition: Summary

- Monocytes from DM2+CVD patients exhibit **pro-inflammatory hyper-activation** at baseline.
- Monocytes from DM2+CVD patients **are hyper-responsive to IFNγ** upon *ex vivo* stimulation.
- This pro-inflammatory hyper-activation indicates that **diseased monocytes are "primed"** to **produce pro-inflammatory molecules** in patients which may contribute to disease progression.
- **Apabetalone attenuates monocyte hyper-activation** by downregulating key inflammatory genes and secreted cytokines in both non-stimulated and stimulated cells.
- Pro-inflammatory gene transcription **is more sensitive to BET inhibitor treatment** in monocytes from DM2+CVD patients than control monocytes, indicating that BET proteins are driving maladaptive gene expression in a diseased state.
- Findings support the development of apabetalone as a **therapy for high risk CVD patients** with epigenetic dysregulation of the innate immune response.

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Clinical Team: San Francisco, CA, USA

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Thank you for your attention!

Select Publications:

- **Wasiak 2020** BET protein inhibitor apabetalone (RVX-208) suppresses pro-inflammatory hyper-activation of monocytes from patients with cardiovascular disease and type 2 diabetes. **Clinical Epigenetics.**
- **Wasiak 2020** Epigenetic Modulation by Apabetalone Counters Cytokine-Driven Acute Phase Response In Vitro, in Mice and in Patients with Cardiovascular Disease. **Cardiovasc Ther.**
- **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 2018** 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.**