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TSX: RVX

# BET protein inhibitor apabetalone suppresses inflammatory hyper-activation of monocytes from patients with cardiovascular disease and type 2 diabetes

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Resverlogix Corp.
AHA 2020

# Disclosures


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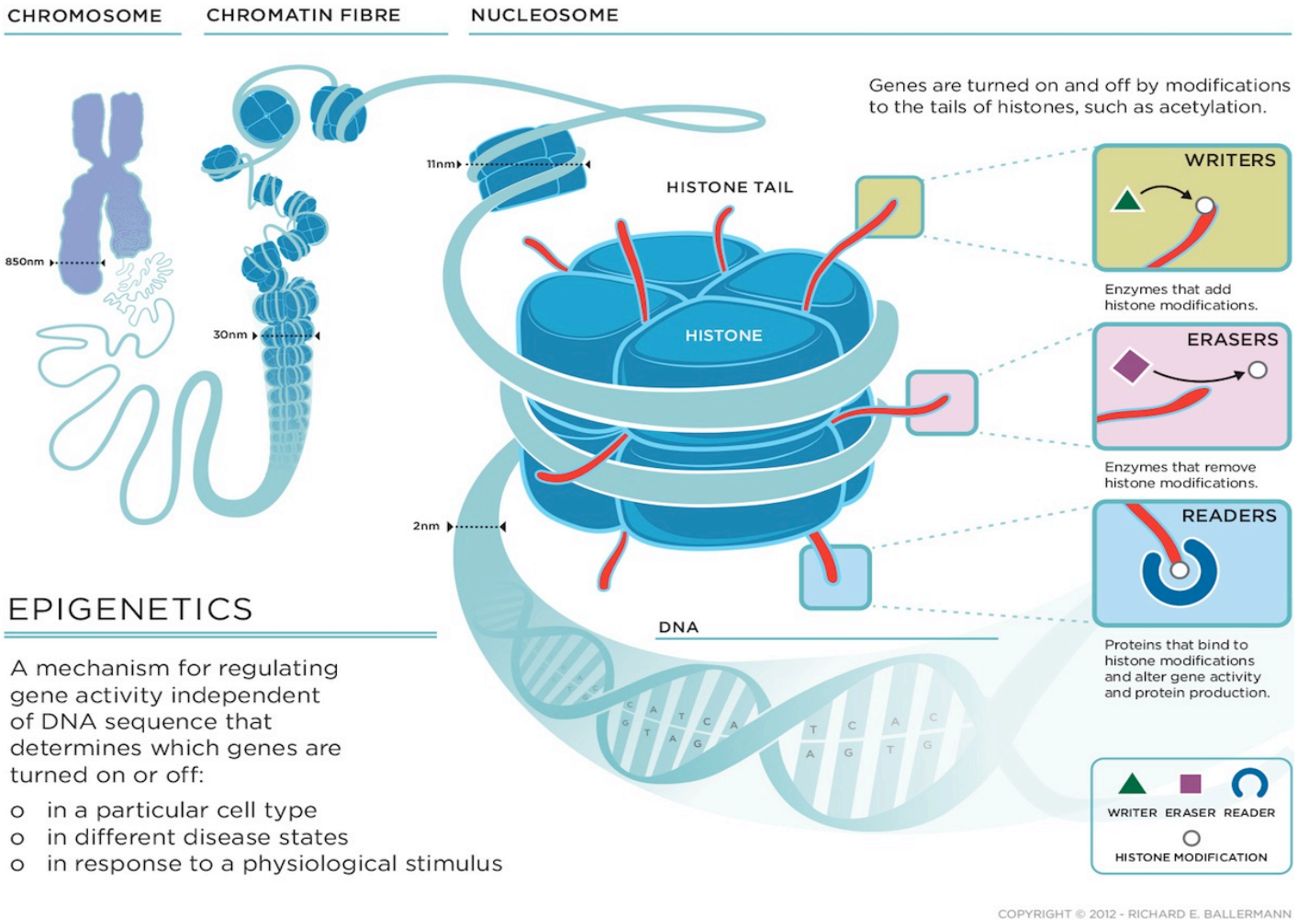
# Epigenetics




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

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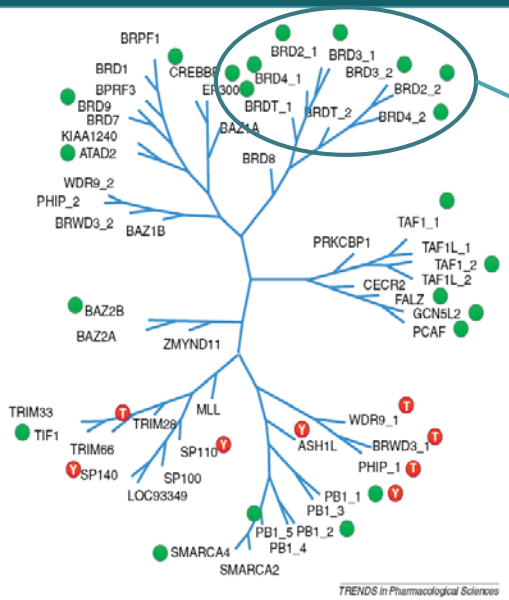
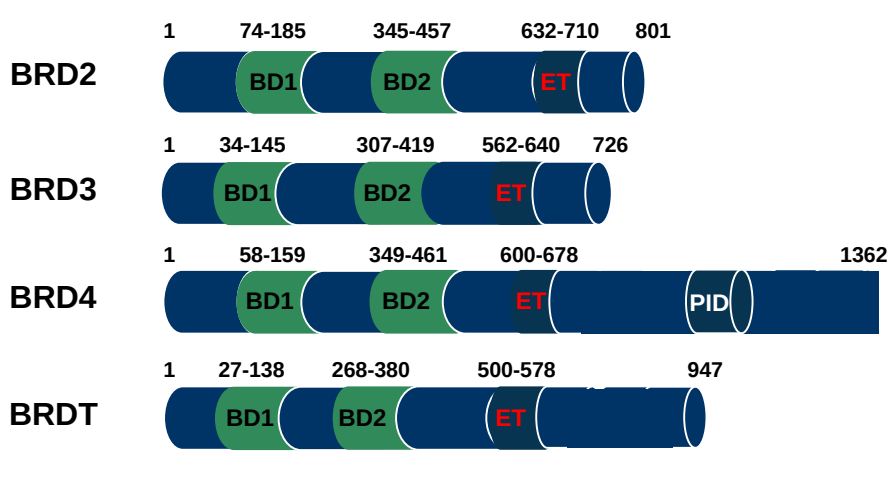
## BET proteins are part of a superfamily of proteins



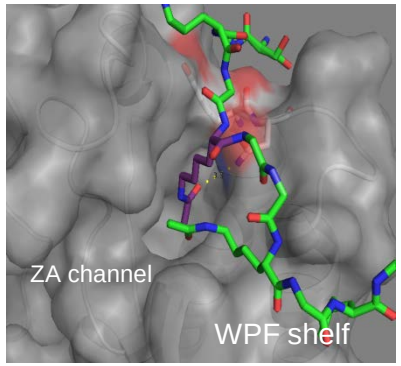

## Each BET protein contains two bromodomains (BD)

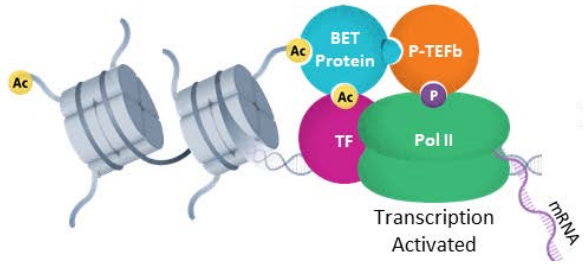



## Bromodomains bind acetylated histones and transcription factors to regulate gene transcription

***X-ray crystallography***

Acetylated lysine (color) bound to bromodomain (grey)

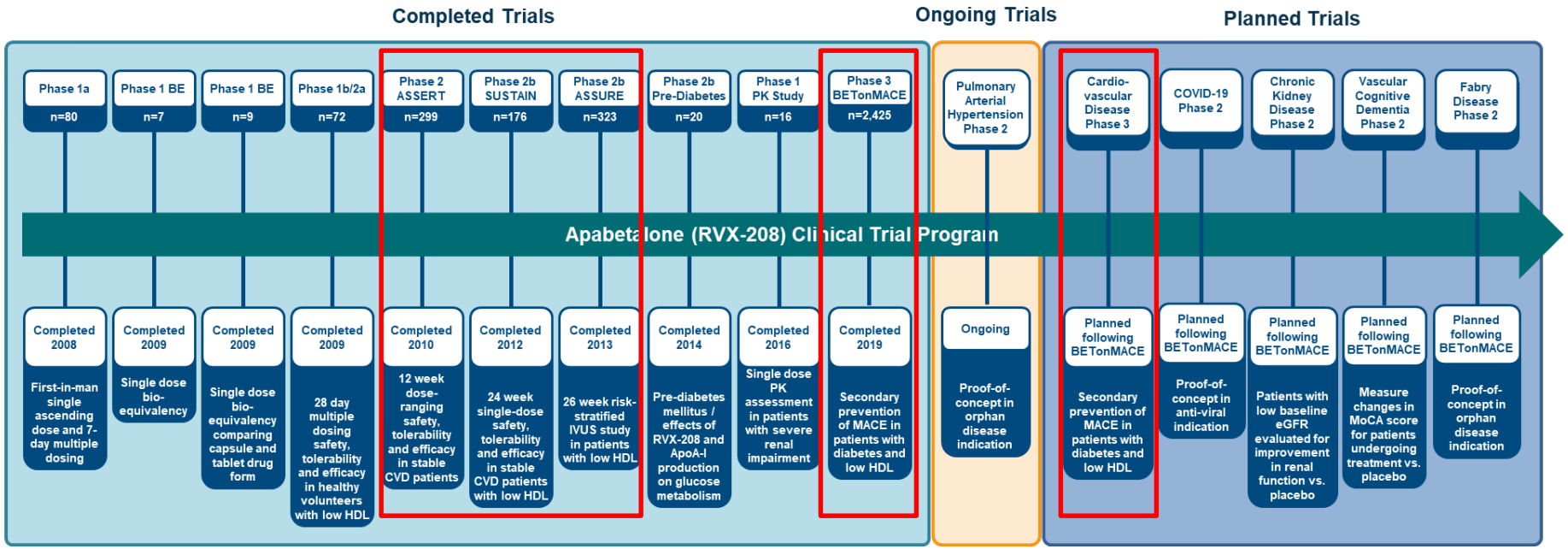



## Apabetalone disrupts BD-chromatin binding

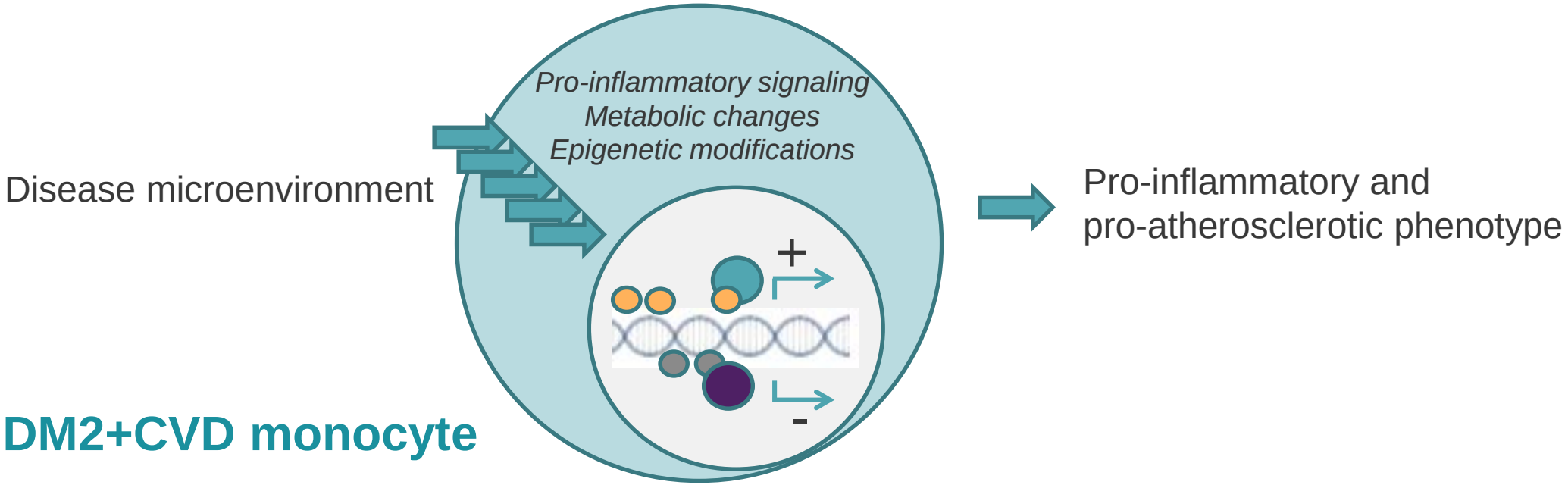
# Apabetalone in Human Clinical Trials

- **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.

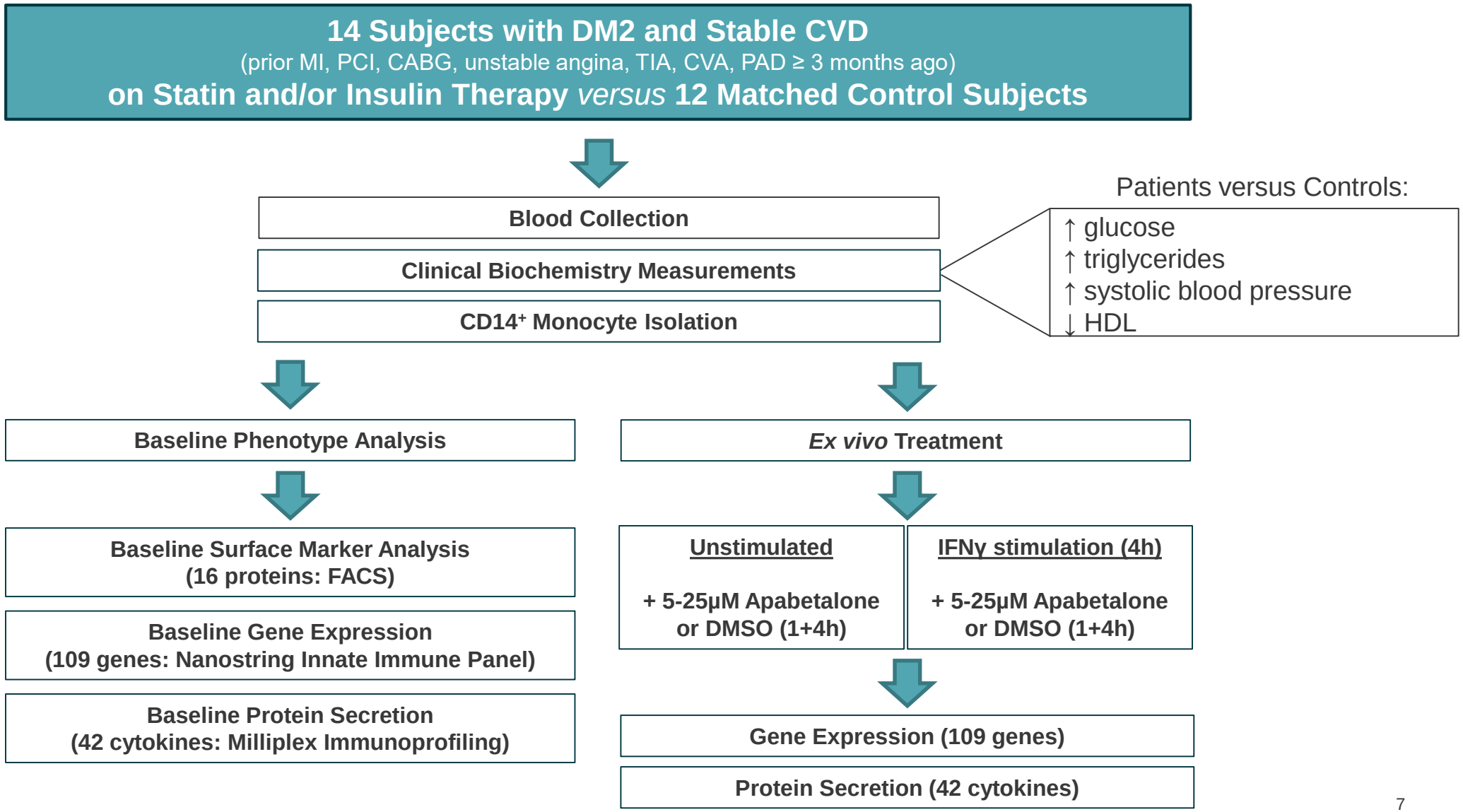


# Lowering Monocyte Inflammation In Patients With Type 2 Diabetes and CVD with Apabetalone

- In diabetes and CVD, microenvironmental factors can trigger pro-inflammatory signaling in monocytes that leads to cytokine production and vascular wall invasion which, in turn, can promote atherosclerosis.
- This “hyper-activation” is partially ascribed to **epigenetic reprogramming**.
- ***Hypothesis**: **Epigenetic modulators such as apabetalone would “correct” the pro-inflammatory hyper-activation of circulating monocytes**.*

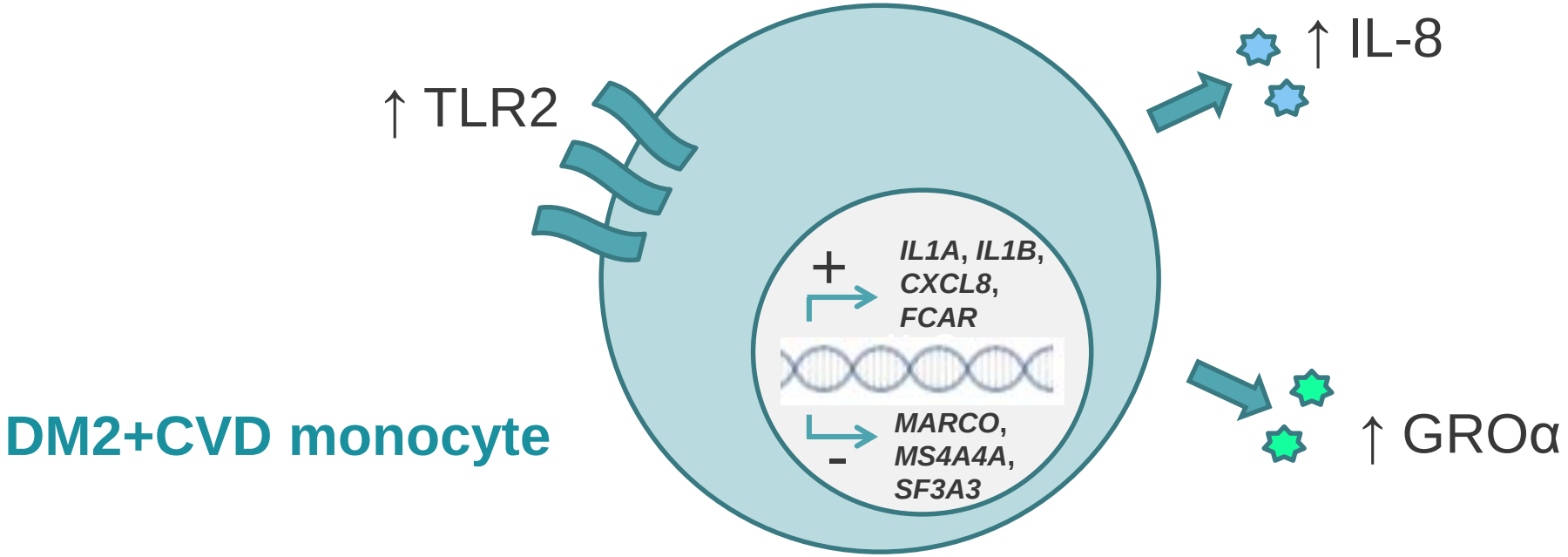


# Lowering Monocyte Inflammation In Patients With Type 2 Diabetes And CVD with Apabetalone



# Comparison of Baseline Characteristics of Non-Stimulated DM2+CVD vs. CONTROL monocytes

- **Surface marker expression**: Pro-inflammatory pattern recognition receptor **TLR2** was expressed at higher levels on the surface of DM2+CVD monocytes vs. controls
- **Gene expression**: Pro-inflammatory genes ***IL1A*, *IL1B*, *CXCL8 (IL8)*, *FCAR*** were upregulated in DM2+CVD monocytes vs. controls, whereas genes associated with an anti-inflammatory phenotype ***MARCO*, *MS4A4A* and *SF3A3*** were downregulated
- **Protein secretion**: Cytokines **IL-8 and GROα** were secreted at higher levels in DM2+CVD monocytes vs. controls

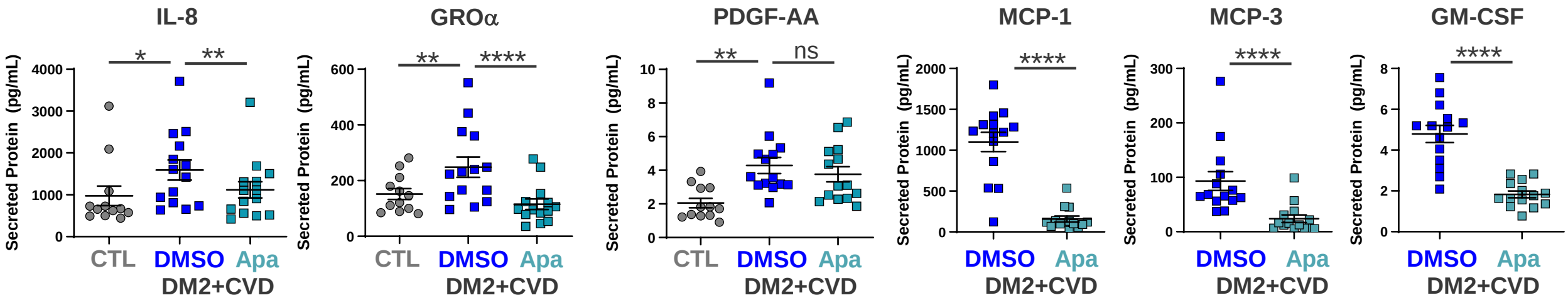



**Monocytes from DM2+CVD** patients on SoC therapy have higher expression of pro-inflammatory genes and proteins at baseline indicating ***in vivo*** **pro-inflammatory hyper-activation**

# Apabetalone Attenuates Baseline Pro-inflammatory "Hyper-Activation" in Monocytes from DM2+CVD Patients on SoC Therapy

- **Gene expression (1+4h)**: Apabetalone downregulates genes overexpressed in DM2+CVD monocytes:
  ↓ *IL1A*, *CXCL8 (IL8)*, *FCAR*

- **Protein secretion (24h)**: Apabetalone downregulates secretion of cytokines and chemokines in DM2+CVD monocytes:
  ↓ IL-8 and GROα (overexpressed in DM2+CVD monocytes)
  ↓ MCP-1, MCP-3 and GM-CSF




Monocytes from DM2+CVD patients have higher expression of pro-inflammatory genes and proteins at baseline. **This "hyper-activation" is attenuated *ex vivo* by apabetalone treatment.**

# Apabetalone Downregulates Pro-Inflammatory Gene Signatures More Potently in DM2+CVD Patient Monocytes than in Control Monocytes

**1. Nanostring Gene Expression Data: 25μM Apabetalone vs. DMSO**

- DM2+CVD monocytes: 53 genes
- Control monocytes: 46 genes (out of 109; >20%Δ; adj. p<0.05)

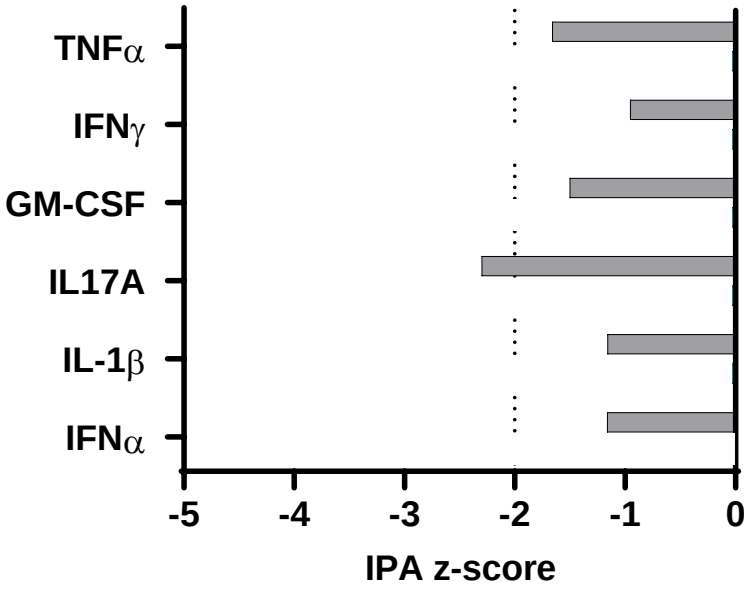
**2. Analysis of Transcriptional Gene Signatures using IPA software:**

- Gene lists were uploaded into IPA software®
- z-scores determine the transcriptional impact of apabetalone on pro-inflammatory pathways

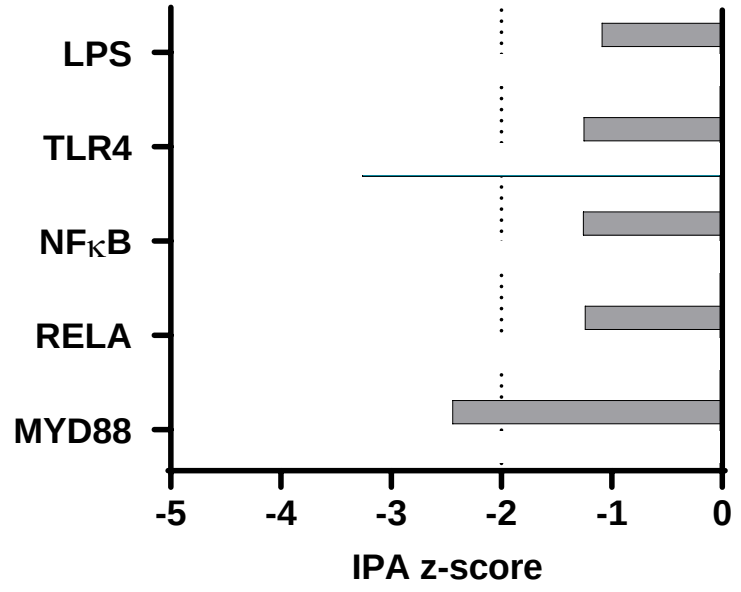
Apabetalone's effect on transcriptional signatures in:

Control monocytes  DM2+CVD monocytes

**IPA® Upstream Regulators (Cytokines)**

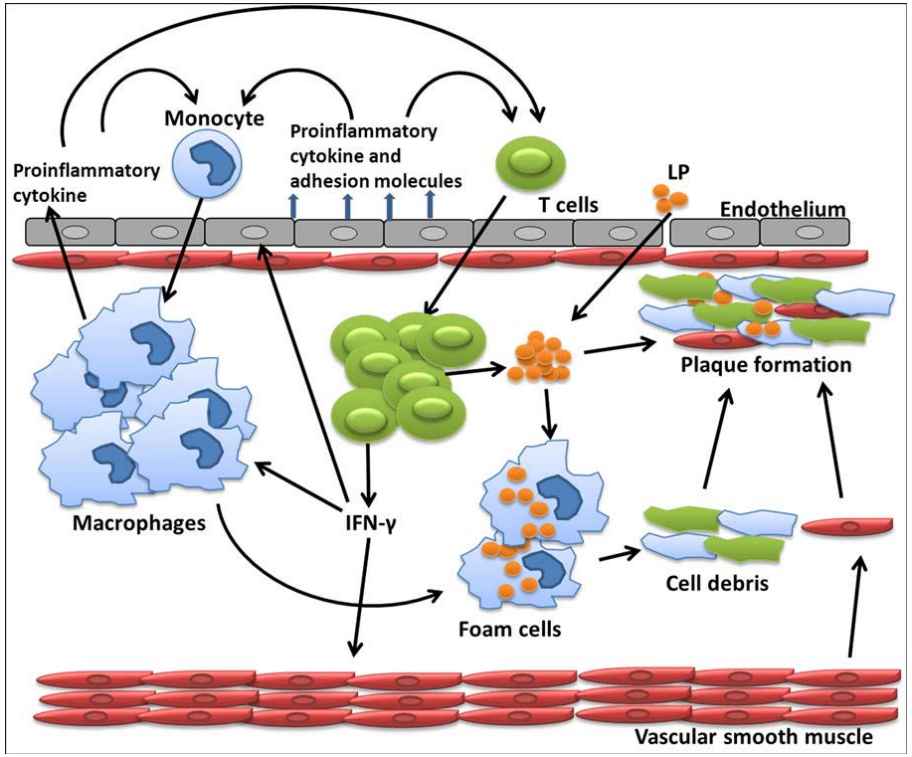



**IPA® Upstream Regulators (TLR Signaling)**




- Apabetalone treatment causes a **more robust suppression of inflammatory gene signatures in DM+CVD monocytes** as compared to controls (more negative z-scores).
- This data suggests that transcriptional activity of **BET proteins** is greater in DM2+CVD monocytes than in control monocytes.

# IFNγ Differentiates Monocytes into M1 "Classically Activated" Pro-Inflammatory Tissue Macrophages




*Lin et al., 2013, Adv. Biosci. Biotech.*

**IFNγ promotes transcription of genes encoding:**

- Enzymes
- Kinases
- Metabolic regulators
- Chromatin regulators
- Transcription regulators

**Cellular function**

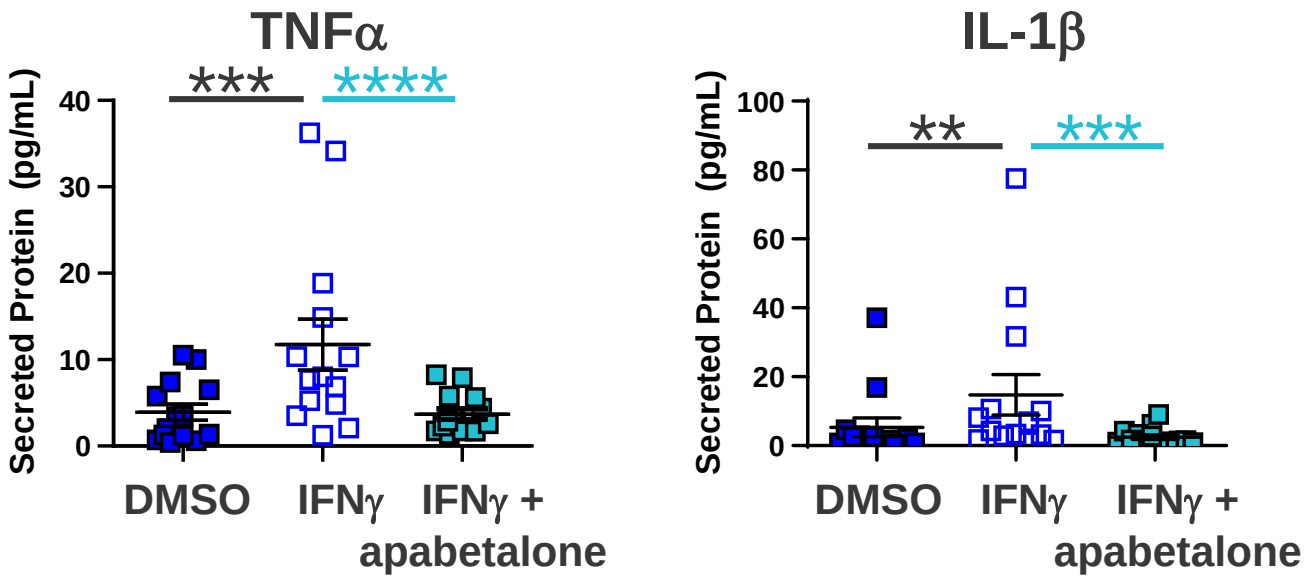
- Transcription (IRFs)
- Antigen presentation
- Cell recruitment
- Antimicrobial responses

IFNγ treatment confers to monocytes a pro-inflammatory M1-like phenotype characterized by enhanced cytokine and chemokine production, phagocytosis, and intracellular killing of microbial pathogens.

# Apabetalone Counters Pro-Inflammatory Hyper-Response to IFNγ in DM2+CVD Monocytes

**Gene expression (1+4h)**: Apabetalone downregulates genes hyper-activated by IFNγ in DM2+CVD monocytes encoding cytokines ***CCL7, CCL8, TNF*** and NF-κB signaling proteins ***RELA and MYD88***

**Protein secretion (24h)**: Apabetalone suppressed IFNγ-induced **IL-1β and TNFα secretion** in DM2+CVD monocytes




Apabetalone **reduces pro-atherogenic genes and proteins** in IFNγ stimulated monocytes from DM2+CVD patients.

# Apabetalone Counters IFNγ Signaling More Potently in Monocytes from DM2+CVD Patients

**Gene expression:**
Apabetalone robustly **downregulates IFNγ targets** in **DM2+CVD monocytes**

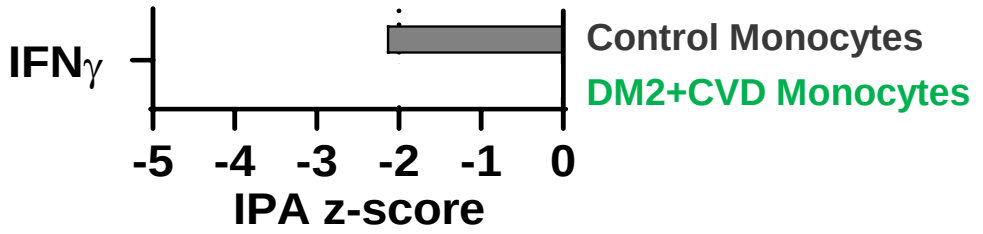
| Gene | IFNγ 4h | IFNγ+Apa | Gene | IFNγ | IFNγ+Apa |
|---|---|---|---|---|---|
| **Chemokines** | | | **Pattern Recognition Signaling** | | |
| *CCL2* | *2.2* | -91% | *TLR8* | *6.8* | -68% |
| *CCL7* | *1.75* | -90% | *LY96* | *2.1* | -67% |
| *CXCL1* | *0.38* | -88% | *TLR1* | *1.5* | -60% |
| *CCL8* | *135* | -85% | *FPR2* | *1.67* | -58% |
| *CXCL9* | *297* | -80% | *TICAM2* | *2.7* | -44% |
| *CCR1* | *1.4* | -79% | *RELA* | *1.43* | -42% |
| *CXCL10* | *755* | -61% | *MYDD88* | *1.41* | -40% |
| **oxLDL Receptor** | | | **ROS Production** | | |
| *MSR1* | *3.36* | -79% | *CYBB* | *1.77* | -42% |

Statistics: Two-Way RM ANOVA, Tukey's test. Significant $p<0.05$

**Pathway analysis**: IFNγ transcriptional signature is **preferentially inhibited** by apabetalone **in DM2+CVD monocytes**

Apabetalone's effect on IFNγ signature in:

Control monocytes  DM2+CVD monocytes




Note: IPA z-score<-2 predicts pathway downregulation

Cytokine-stimulated DM2+CVD monocytes **are more sensitive to BET inhibition** by apabetalone than control monocytes, indicating that aberrant pro-inflammatory gene transcription is BET dependent in diseased cells.

# 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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***Select Publications:***


- **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.**
- **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**