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TSX: RVX

Apabetalone (RVX-208) reduces ACE2 expression in human cell culture systems, which could attenuate SARS-CoV-2 viral entry

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Disclosures: All authors are employees of Resverlogix

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Abstract: MP130

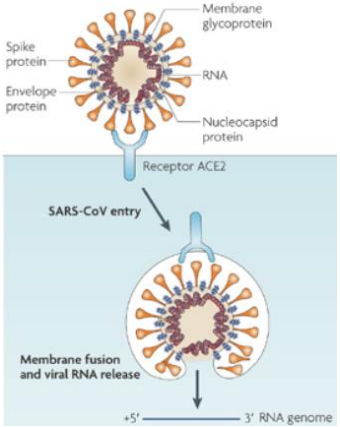
Background and Objective

Infection of human cells with SARS-CoV-2

The SARS-CoV-2 virus causes life threatening complications including acute coronary syndrome, venous thromboembolism and hyperinflammation in the lung doi.org/10.1038/s41569-020-0413-9

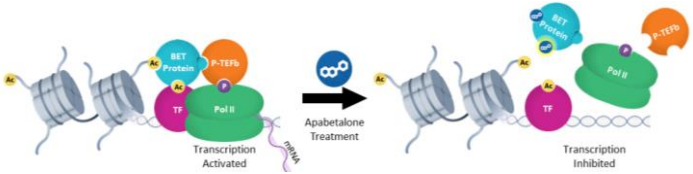
SARS-CoV-2 “Spike Protein” binds the human cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) for entry into host cells and initiation of infection; ACE2 expressing cells in the respiratory track are the first to be infected doi.org/10.1016/j.cell.2020.02.052

Recombinant ACE2 or neutralizing ACE2 antibodies reduce viral infection and replication in host cells, establishing ACE2 as a target for SARS-CoV-2 intervention doi.org/10.1016/j.cell.2020.04.004, doi.org/10.1016/S2213-2600(20)30418-5, doi: 10.1126/science.abd0831



http://europepmc.org/article/MED/19198616

Apabetalone mechanism of action



Haarhaus et. al 2019 Curr Opin Nephol Hyperten © Resverlogix 2019

Figure Legend:
BET: bromodomain and extraterminal proteins
Ac: acetylated lysine residue on DNA associated proteins
BD: bromodomain
TF: transcription factor
Yellow halo indicates selectivity of apabetalone for bromodomain 2 within BET proteins

Repurposing apabetalone for COVID-19 treatment

Apabetalone is an orally available inhibitor of BET proteins - epigenetic readers modulating gene expression by bridging acetylated histones or transcription factors with transcriptional machinery doi: 10.1371/journal.pone.0083190

Apabetalone is well-tolerated by patients and is currently in late stage clinical development for cardiovascular disease doi:10.1001/jama.2020.3308

Apabetalone has been administered to 1,934 subjects for up to two years

This study examines regulation of ACE2 expression by apabetalone in cell culture systems & the impact of treatment on binding of SARS-CoV-2 spike protein

Apabetalone Reduces ACE2 Expression in Human Calu-3 Lung Cells

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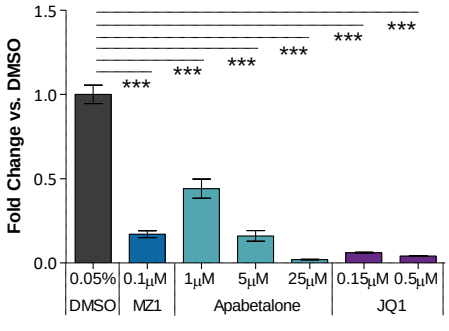
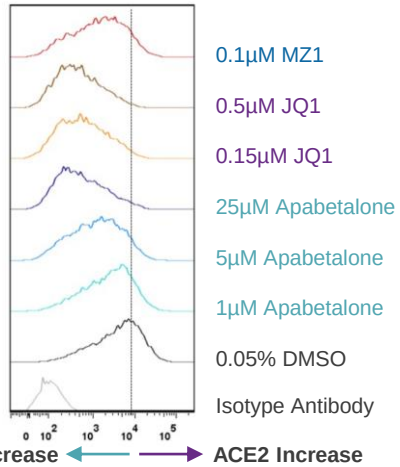
ACE2 Gene Expression
48h treatment

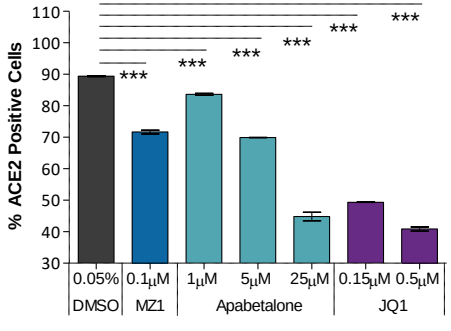


***p<0.001, ANOVA followed by Dunnett's

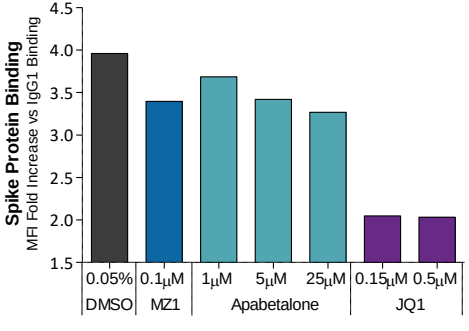
Cell Surface ACE2 Protein
72h treatment



% of ACE2 Positive Cells 72h treatment



Binding of SARS-CoV-2 Spike Protein to Calu-3 Cells
after 96h of BETi treatment



Conclusions:

Apabetalone dose dependently reduces ACE2 mRNA by >90%, cell surface ACE2 protein by >80% and percent of cells with ACE2 by >50%. Similar results with JQ1 or MZ1, BET inhibitors (BETi) with different chemical scaffolds, verify on target effects.

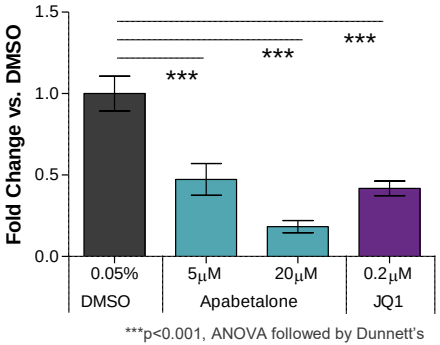
Because cells without ACE2 have limited ability to take up SARS-CoV-2, reduction in ACE2 levels suggest apabetalone could decrease SARS-CoV-2 infection of host cells (doi.org/10.1038/s41586-020-2012-7).

Reduction in Spike protein binding implies apabetalone could decrease SARS-CoV-2 association with & infection of host cells.

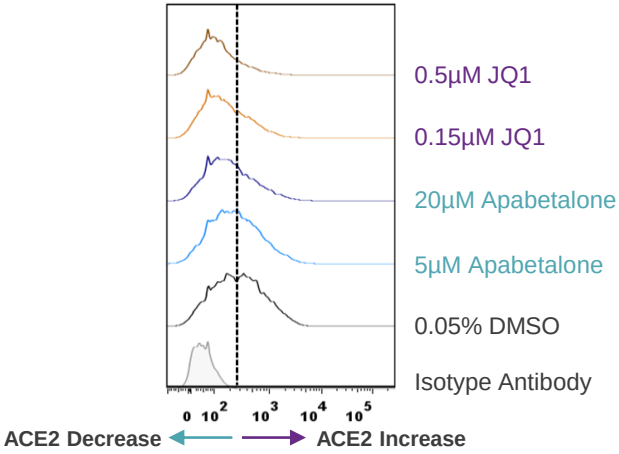
Apabetalone Reduces ACE2 Surface Abundance in Vero E6 Cells

Vero E6 are a monkey kidney epithelial cell line often used to culture live SARS-CoV-2 virus

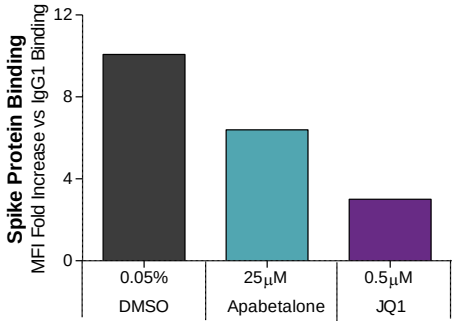
ACE2 Gene Expression
24h treatment



Cell Surface ACE2 Protein
48h treatment



Binding of SARS-CoV-2 Spike Protein to Vero E6 Cells
after 48h of BETi treatment

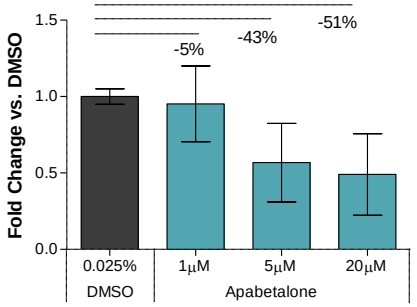


Conclusions:

- Apabetalone dose dependently reduces ACE2 gene expression and cell surface ACE2 protein levels. Consistency between apabetalone and JQ1 verify on target BETi effects.
- Apabetalone diminishes binding of the SARS-CoV-2 Spike Protein (receptor binding domain) to Vero E6 cells by ~40%.
- Reduction in Spike protein binding implies apabetalone can attenuate SARS-CoV-2 association and entry into host cells.

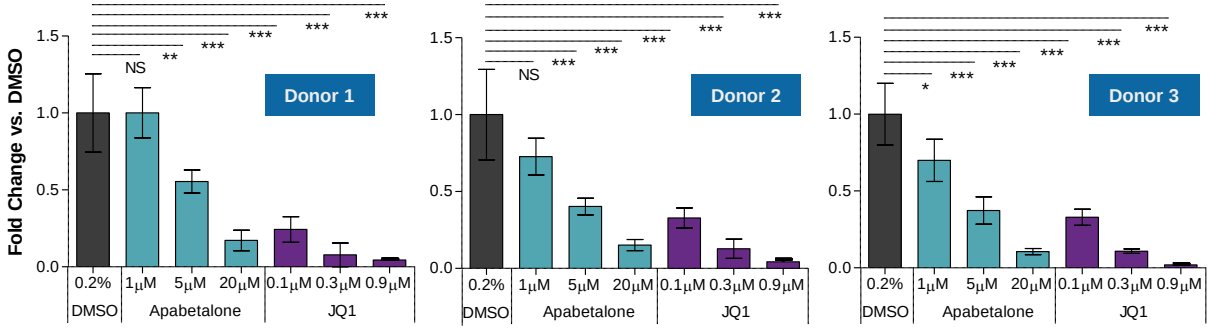
Apabetalone Downregulates ACE2 Gene Expression in Multiple Cell Systems

Human Renal Proximal Tubule Epithelial Cells (RPTEC) 18h treatment



Primary Human Hepatocytes (PHH)

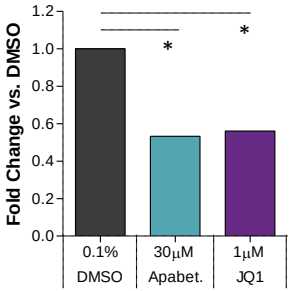
48h treatment; 3 different donors; real-time PCR



***p<0.001, **p<0.01, *p<0.05, NS = not significant ANOVA followed by Dunnett's

PHH Microarray

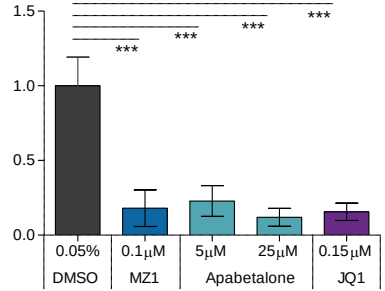
48h treatment



*p<0.05 one way ANOVA

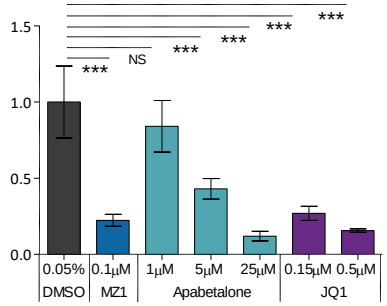
HepG2 Hepatocytes

24h treatment



Huh-7 Hepatocytes

96h treatment



***p<0.001, NS = not significant ANOVA followed by Dunnett's

Conclusions:

In RPTEC, apabetalone downregulated ACE2 mRNA by >50%

Apabetalone dose dependently reduced ACE2 gene expression in liver cells: HepG2, Huh-7 or PHH from 3 independent donors by up to 90%

Comparator BETi JQ1 or MZ1 also downregulate ACE2 expression, indicating on target BETi effects

A BRD4 Enhancer Close to the ACE2 Gene May Regulate Expression

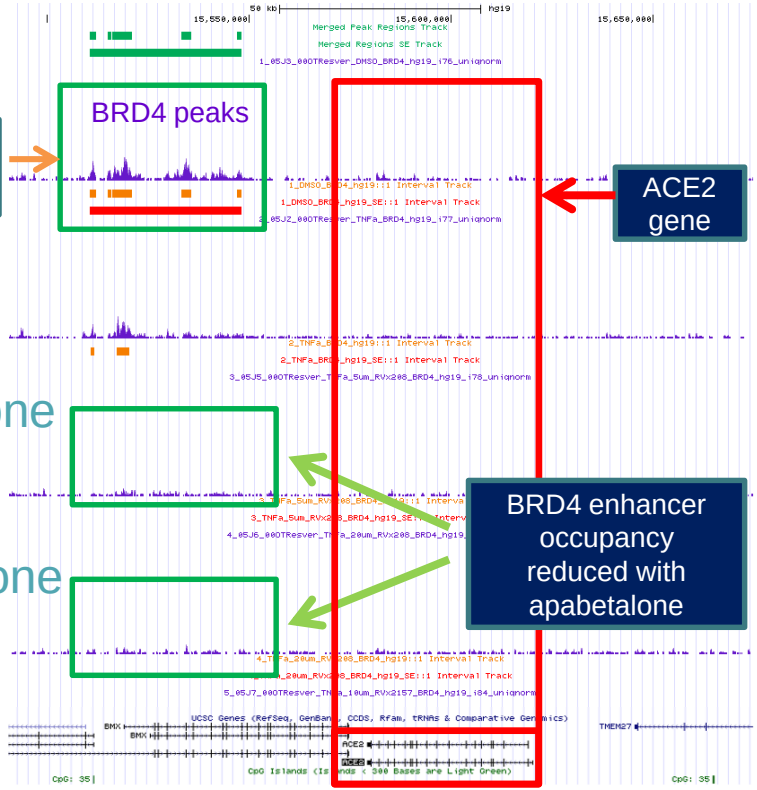
DMSO

BRD4 enhancer in proximity to ACE2 gene

TNFα

5μM Apabetalone + TNFα

20μM Apabetalone + TNFα



Observations: Human Aortic Endothelial Cells

BRD4 is a BET protein that regulates gene transcription and molecular target of apabetalone.

A BRD4 enhancer exists in proximity to the ACE2 gene locus.

Apabetalone reduces BRD4 in proximity to the ACE2 gene. This may be the mechanism of BETi regulation of ACE2 gene expression.

Summary and Conclusions

Apabetalone treatment reduces ACE2 gene expression, cell surface ACE2 protein levels and binding of SARS-CoV-2 spike protein (receptor binding domain) to (a) human lung Calu-3 cells and (b) monkey kidney epithelial Vero E6 cells.

Reduction in SARS-CoV-2 spike protein binding implies apabetalone can attenuate SARS-CoV-2 association and infection of host cells.

ACE2 gene expression is downregulated by apabetalone in various cell types including Calu-3, Vero E6, RPTEC, PHH, HepG2, and Huh-7, suggesting apabetalone may reduce SARS-CoV-2 infection in multiple organs.

ACE2 gene expression is regulated by BET proteins.

- *Demonstrated by consistent downregulation with BET inhibitors apabetalone, JQ1 and MZ1.*

ACE2 expression may be regulated by a BRD4 enhancer in close proximity to the ACE2 gene.

- *BRD4 is a BET protein that regulates gene expression and is a molecular target of apabetalone.*
- *Apabetalone reduced BRD4 chromatin occupancy in an enhancer in proximity to the ACE2 gene in human aortic endothelial cells.*

The impact of apabetalone on SARS-CoV-2 life cycle is under investigation.

This study provides mechanistic support for a SARS-CoV-2 clinical trial to reduce COVID-19 symptoms and complications with apabetalone in infected patients.

- *Apabetalone has a well established safety profile and may be rapidly repurposed for SARS-CoV-2 treatment.*

More about apabetalone treatment for cardiovascular disease is presented in sessions LF.APS.10 and AT.AOS.549

Methods

- Real-time PCR: mRNA was isolated from treated cells using Catcher PLUS kits (Life Technologies). TaqMan assays (Life Technologies) were used to determine abundance of the ACE2 transcript relative to the endogenous control cyclophilin in the same sample using the RNA Ultrasense One-step qRT-PCR kit. Data was acquired on a ViiA-7 Real-Time PCR apparatus (Applied Biosystems). Analysis was performed as $2^{(C_T \text{ cyclophilin} - C_T \text{ ACE2})}$ and results were normalized to DMSO treated samples.

- Microarray: Primary human hepatocytes (Life Technologies) were plated in 24 well format at 500,000 cells/well, then overlaid with Matrigel™ as recommended by the supplier. Cells were treated with apabetalone at 30µM or DMSO alone (0.1%) for 48hrs. Total RNA was extracted with the mirVana™ kit (Ambion) and sent to Asuragen Inc. (Austin, TX) for microarray analysis using Affymetrix Human Genome U133 Plus 2.0 Array.

- Flow cytometric analysis of ACE2 protein: Treated cells as indicated in figures were stained with Alex Fluor™647 coupled goat-anti-human ACE2 antibodies or Alex Fluor™647 coupled goat IgG. Cell surface ACE2 protein levels were measured by flow cytometry and results normalized to cells stained with an isotype antibody.

- SARS-CoV-2 spike protein binding assay: Cells treated as indicated in figures were incubated with recombinant SARS-CoV-2 Spike Protein-RBD-Fc chimeric protein or human IgG1-Fc control (R&D Systems), followed by PE conjugated anti-human Fc antibodies (Life Technologies). SARS-CoV-2 spike protein binding was quantified by flow cytometry.

- BRD4 ChIP-seq: Human Aortic Endothelial Cells (HAECs) were pretreated with 0.025% DMSO, 5 or 20µM apabetalone for one hour, then stimulated with TNFα for an additional 1 hour. Chromatin occupancy of BET protein BRD4 was determined by ChIP-seq (Active Motif) and data visualized on the UCSC Genome Browser.