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TSX: RVX

Apabetalone Downregulates Fibrotic, Inflammatory and Calcific Processes in Renal Mesangial Cells: Mechanism for Reduced Cardiac Events in CKD Patients

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KIDNEY WEEK 2021

FINANCIAL DISCLOSURE

DISCLOSURES FROM PAST 12 MONTHS

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Company Name: Resverlogix

Employed by and owns stock and/or stock options in Resverlogix, who funded this study.

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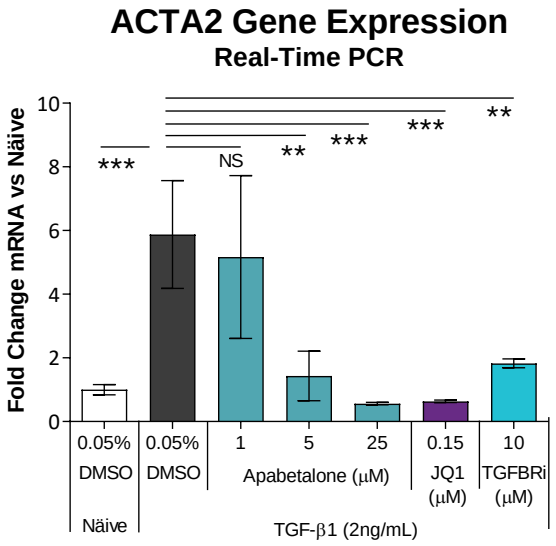
Introduction

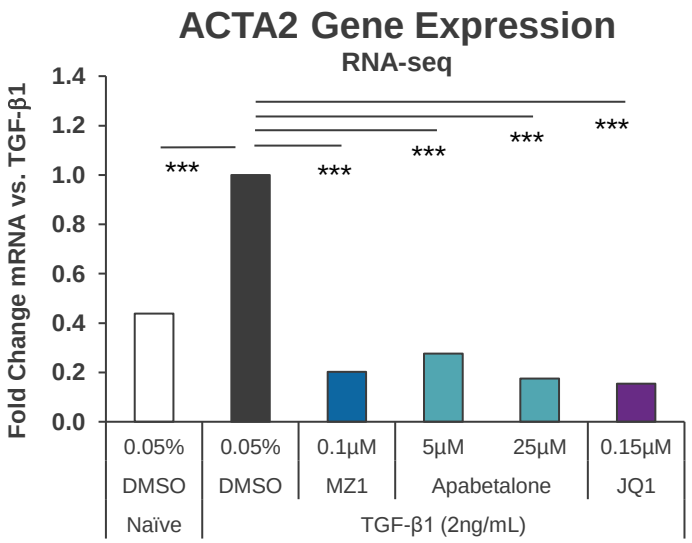
- Patients with chronic kidney disease (CKD) have elevated risk of major adverse cardiac events (MACE)
- Apabetalone is an orally available inhibitor of BET proteins – epigenetic readers that modulate expression of genes involved in fibrosis, inflammation & calcification.
  - Am J Respir Crit Care Med. 2019, 200:910-920, Cardiovasc Ther. 2020, 2020:9397109, Atherosclerosis. 2019, 280:75-84
- In the phase 3 BETonMACE trial, apabetalone reduced MACE by half in patients with CKD (eGFR < 60 mL/min/1.73m^2; HR 0.50 95% CI 0.26,0.96 p=0.04]), implying major benefit along the kidney-heart axis.
  - Clin J Am Soc Nephrol. 2021, 16:705-716
- This study examines effects of apabetalone treatment on primary human renal mesangial cells (HRMCs) in culture on pathways that contribute to renal pathology.

Apabetalone Opposes Fibrotic Activation of HRMCs

TGF-β1 is a fibrotic stimulus that promotes over-production of extracellular matrix (ECM)



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



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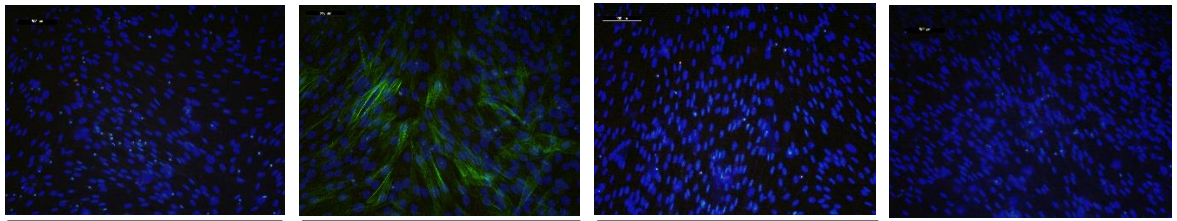
Immunofluorescence:
Green: α-SMA Blue: Nuclei



Naïve	2ng/mL TGF-β1	TGF-β1 + 5μM Apabetalone	TGF-β1 + 25μM Apabetalone

Method:

HRMCs from donors without kidney dysfunction were stimulated 24 hours with pro-fibrotic **TGF-β1** ±

- **Apabetalone**
- **JQ1:** BET inhibitor [BETi] with chemical scaffold different than apabetalone
- **MZ1:** BETi that promotes degradation of BET proteins

Gene expression measured by real-time **PCR** or **RNA-seq**

Robust induction of smooth muscle actin gene expression (ACTA2) with TGF-β1 stimulation as expected; white vs **black** bars

- Marker of fibrotic activation

An **inhibitor of TGF-β receptors** reduced or abolished the response to TGF-β, indicating expected signal transduction pathways mediate this pro-fibrotic stimulation (**light blue** bar)

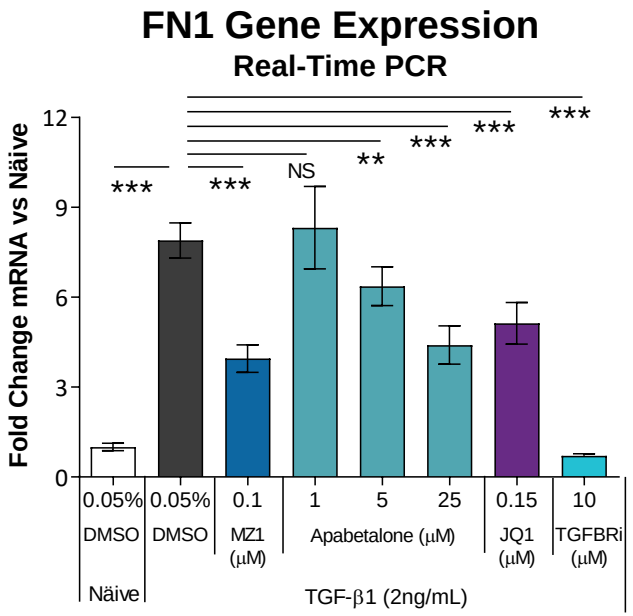
Apabetalone, **JQ1** and **MZ1** all suppress TGF-β stimulated ACTA2 expression, confirming on-target BETi effects

Apabetalone blocked TGF-β induced α-SMA protein production, indicating block to fibrotic state

Apabetalone counters pro-fibrotic activation of TGF-β stimulated HRMCs

Anti-Fibrotic Effects of Apabetalone

Fibronectin (FN1) is a component of the ECM that contributes to fibrosis



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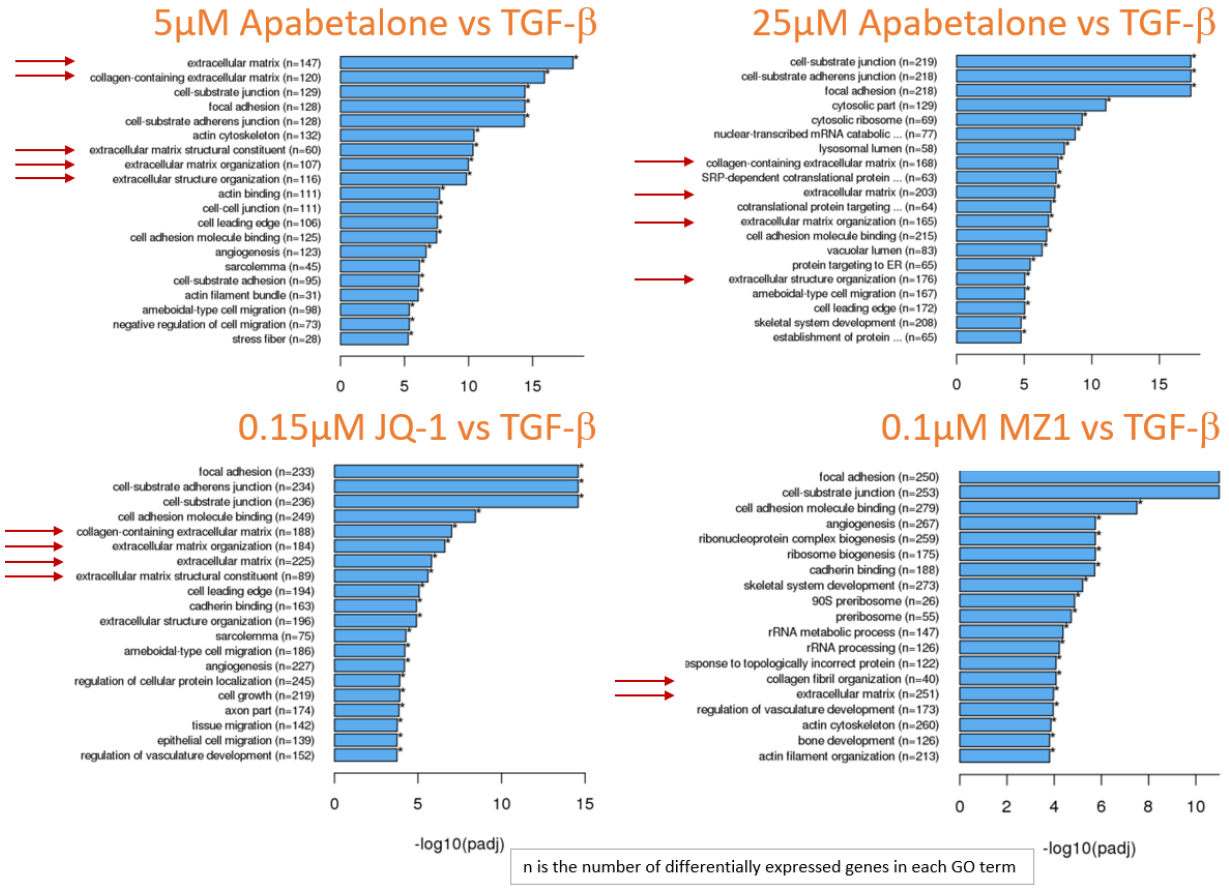
Apabetalone counters TGF-β stimulated production of a pro-fibrotic ECM component in HRMCs

Gene Ontology (GO) analysis of differential gene expression



GO analysis of RNA-seq shows multiple gene sets associated with ECM (red arrows) in the top 20 affected by BET inhibitors, supporting anti-fibrotic properties

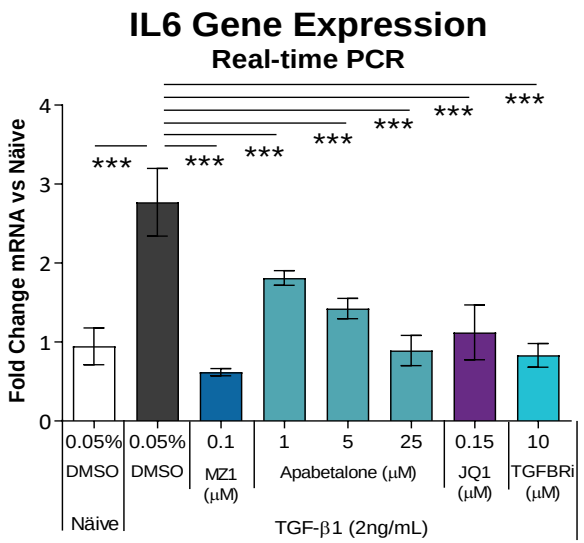
Anti-Inflammatory Effects of Apabetalone

Method:

HRMCs from donors without kidney dysfunction were stimulated 24 hours with pro-fibrotic **TGF-β1 (top)** or **LPS (bottom)** ±

- **Apabetalone**
- **JQ1:** BET inhibitor [BETi] with chemical scaffold different than apabetalone
- **MZ1:** BETi that promotes degradation of BET proteins

Gene expression measured by real-time PCR



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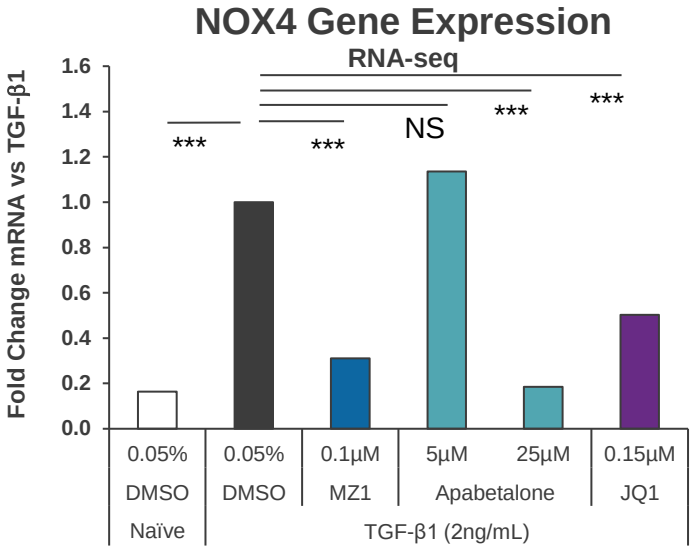

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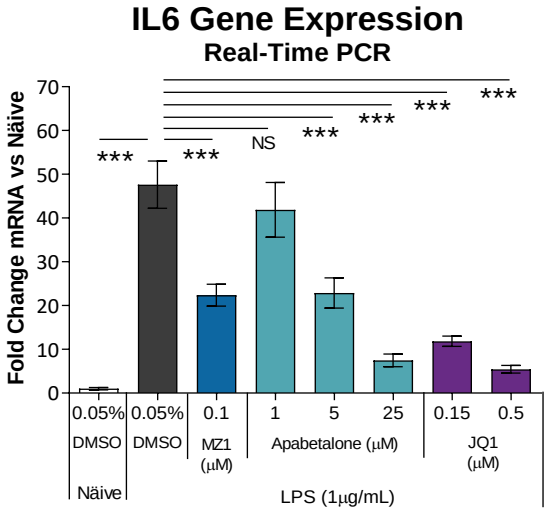

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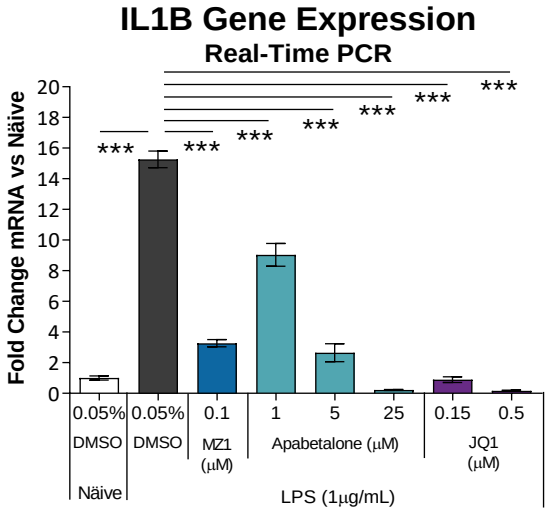

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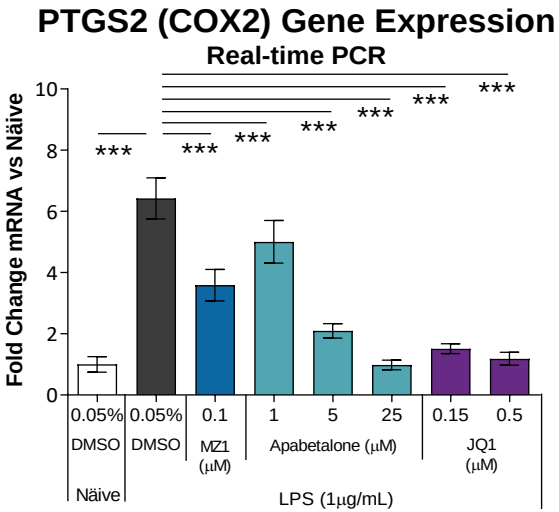


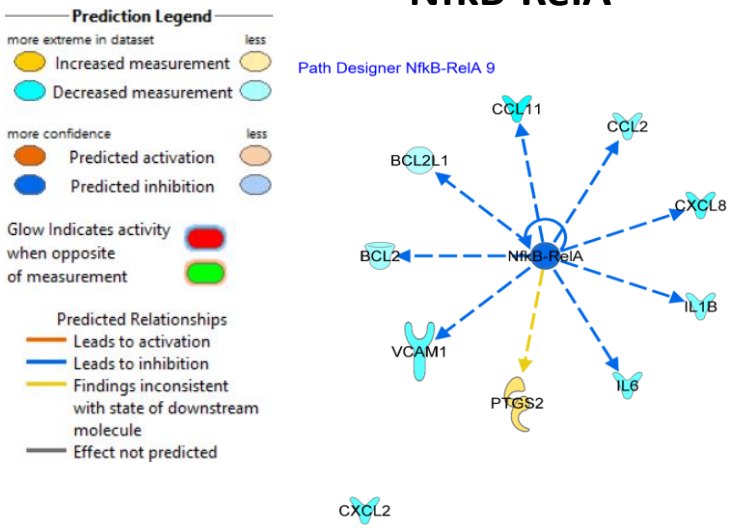
****p< 0.001, ANOVA followed by Dunnett's*

Apabetalone suppresses inflammatory gene expression stimulated with TGF-β or LPS

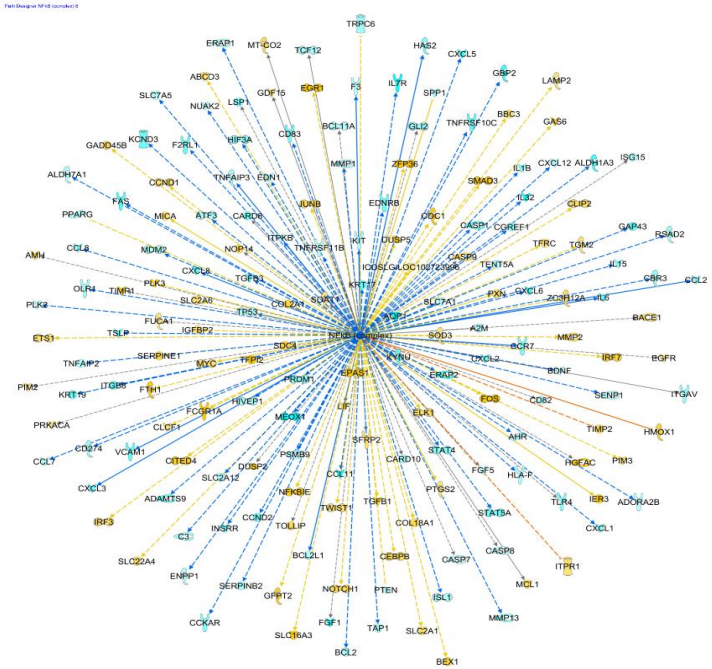
Upstream Regulator of Inflammation is Inhibited by Apabetalone

Upstream Regulator Blue – inhibition Orange – activation Green p<0.05	Human Renal Mesangial Cells (HRMC) 24h BETi + 2ng/mL TGF-β								
	5μM Apabetalone			25μM Apabetalone			0.15μM JQ1		
	Predicted Activation State	z-score	p-value	Predicted Activation State	z-score	p-value	Predicted Activation State	z-score	p-value
NfkB-RelA	No prediction	-1.18	4.49E-03	Inhibited	-2.533	4.10E-03	Inhibited	-2.00	4.98E-03
NFkB complex	No prediction	-0.443	4.63E-13	Inhibited	-3.142	1.25E-09	Inhibited	-2.00	4.98E-03

NfkB-RelA



NFkB complex



Differential gene expression by RNA-seq

Ingenuity Pathway Analysis (IPA) predicts apabetalone inhibits NF-κB, a master upstream regulator of inflammatory processes

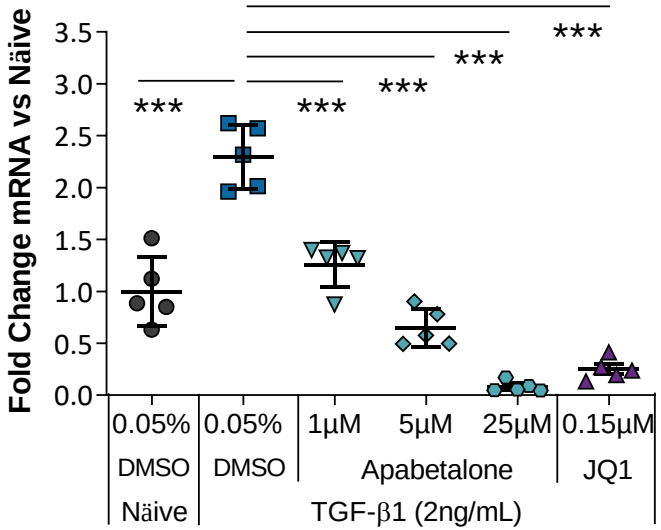
Apabetalone counters inflammatory TGF-β stimulation of HRMCs, and may suppress inflammation associated with renal pathology

NfkB-RelA and NFkB complex are upstream regulators of inflammation. Both were predicted to be inhibited by apabetalone.

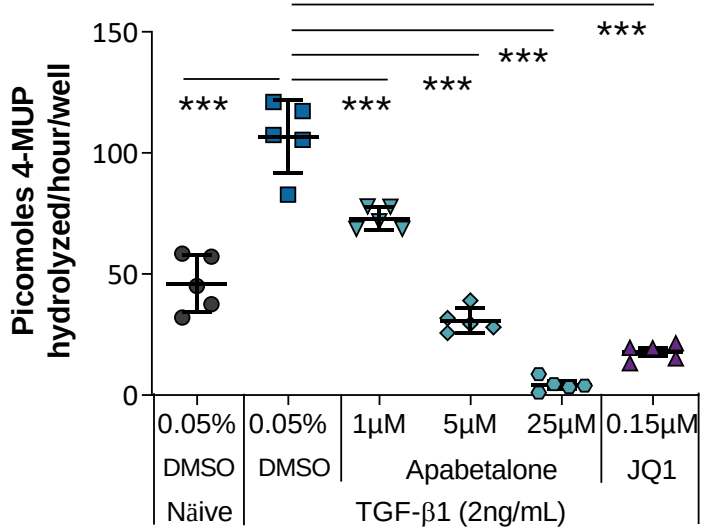
Apabetalone May Reduce Calcification

Tissue non-specific alkaline phosphatase (TNALP; gene symbol ALPL) is a key driver of calcification – a process associated with kidney dysfunction *Curr Opin Nephrol Hypertens. 2020, 29:4–15, Nat Rev Nephrol. 2017, 13:429-442*

ALPL gene expression



TNALP enzyme activity



Apabetalone downregulates ALPL expression & enzyme activity in HRMCs, which may suppress calcification prevalent in CKD

Apabetalone's Effects on Canonical Pathways of Energy Metabolism

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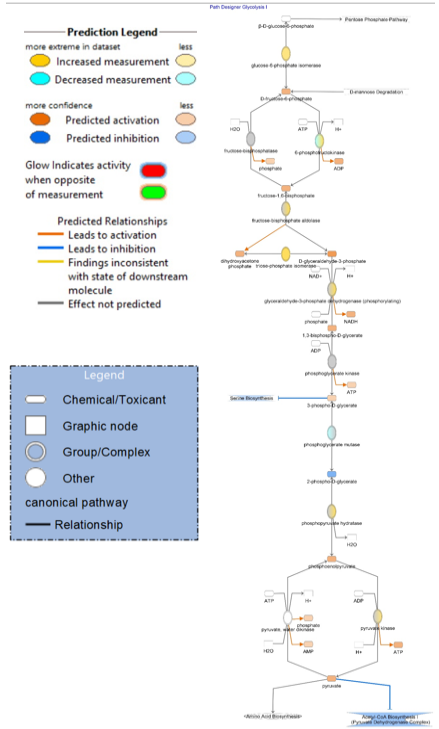
Canonical Pathways Yellow – activation ; Green p<0.05	Human Renal Mesangial Cells (HRMC) 24h BETi + 2ng/mL TGF-β					
	5μM Apabetalone		25μM Apabetalone		0.15μM JQ1	
	z-score	p-value	z-score	p-value	z-score	p-value
Glycolysis I	2.12	3.47E-01	2.31	3.65E-01	2.33	1.00E+00
Oxidative Phosphorylation	3.50	1.00E+00	5.73	2.95E-03	5.81	1.41E-02
NRF2-mediated oxidative stress response	1.62	1.51E-05	2.29	7.94E-12	1.24	5.01E-12

A: Molecule Activity Predictor (MAP) Diagram: Effect of 25μM Apabetalone on the Glycolysis I Pathway

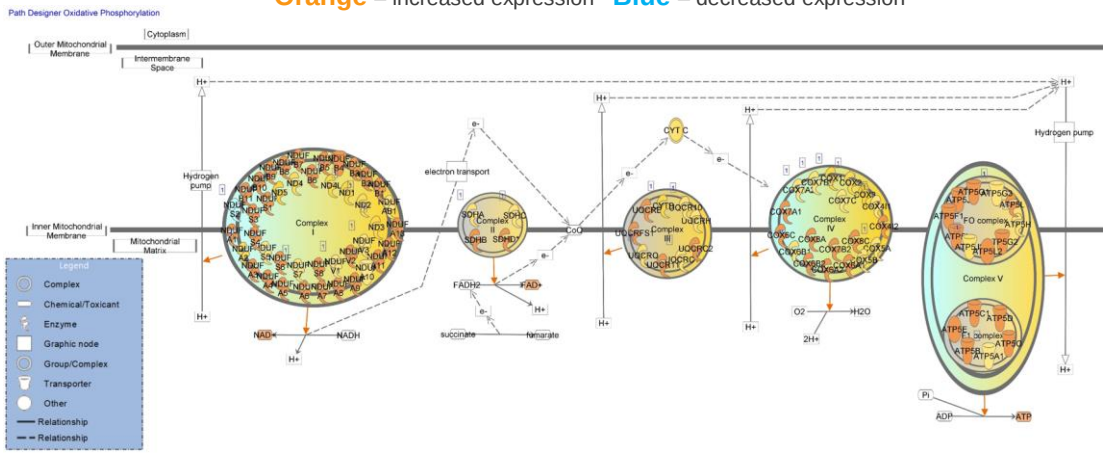
Orange – increased expression **Blue** – decreased expression



IPA predicts apabetalone activates the Glycolysis I pathway in HRMC, indicating increased glucose utilization. This may allow adaptation to high glucose in T2DM

B: MAP Diagram: Effect of 25μM Apabetalone on the Oxidative Phosphorylation Pathway

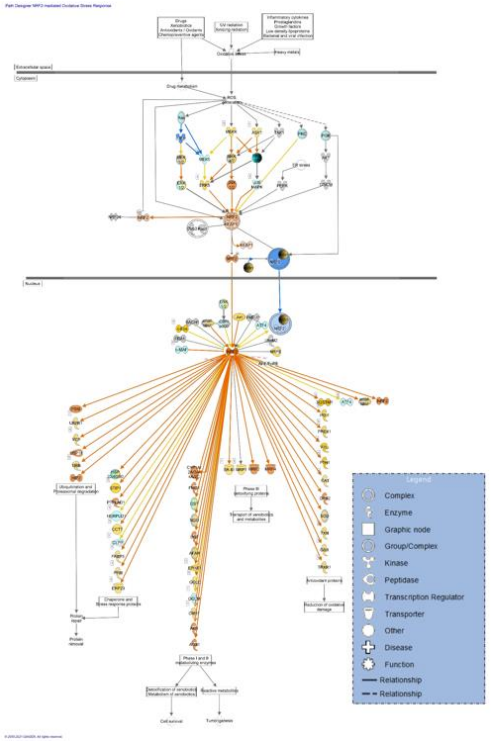
Orange – increased expression **Blue** – decreased expression



Downstream, a robust activation of the Oxidative Phosphorylation pathway was predicted with apabetalone treatment, intuitively because of increased flux of pyruvate into the Krebs cycle.

C: MAP Diagram: Effect of 25μM Apabetalone on the NRF2-Mediated Oxidative Stress Response

Orange – increased expression **Blue** – decreased expression



Elevated ROS accompanies oxidative phosphorylation. IPA predicts apabetalone activates the NRF2-mediated oxidative stress response pathway, a protective adaptation

Canonical pathways of energy metabolism predict apabetalone increases glucose utilization, and may allow kidney cells to cope with high glucose in T2DM to ameliorate diabetic nephropathy

Conclusions

- Apabetalone downregulates fibrotic, inflammatory and calcific pathways in HRMCs, which are associated with renal dysfunction.
- Changes in energy metabolism pathways predicted apabetalone facilitates adaptation to high glucose in the kidney in diabetic conditions that lead to diabetic nephropathy.
- Our results provide mechanistic insight into profound reductions in MACE in CKD patients receiving apabetalone in the phase 3 BETonMACE trial.
- The effect of apabetalone on MACE in patients with diabetes and CKD will be further evaluated in the upcoming phase 3 trials.