**Benefit of Apabetalone on Plasma Proteins in Renal Disease**

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**Introduction:** Apabetalone, a small molecule inhibitor, targets epigenetic readers termed BET proteins that contribute to gene dysregulation in human disorders. Apabetalone has *in vitro* and *in vivo* anti-inflammatory and antiatherosclerotic properties. In phase 2 clinical trials, this drug reduced the incidence of major adverse cardiac events in patients with cardiovascular disease. Chronic kidney disease is associated with a progressive loss of renal function and a high risk of cardiovascular disease. We studied the impact of apabetalone on the plasma proteome in patients with impaired kidney function.

**Methods:** Subjects with stage 4 or 5 chronic kidney disease and matched controls received a single dose of apabetalone. Plasma was collected for pharmacokinetic analysis and for proteomics profiling using the SOMAscan 1.3k platform. Proteomics data were analyzed with Ingenuity Pathway Analysis to identify dysregulated pathways in diseased patients, which were targeted by apabetalone.

**Results:** At baseline, 169 plasma proteins (adjusted *P* value <0.05) were differentially enriched in renally impaired patients versus control subjects, including cystatin C and β2 microglobulin, which correlate with renal function. Bioinformatics analysis of the plasma proteome revealed a significant activation of 42 pathways that control immunity and inflammation, oxidative stress, endothelial dysfunction, vascular calcification, and coagulation. At 12 hours postdose, apabetalone countered the activation of pathways associated with renal disease and reduced the abundance of disease markers, including interleukin-6, plasminogen activator inhibitor-1, and osteopontin.

**Conclusion:** These data demonstrated plasma proteome dysregulation in renally impaired patients and the beneficial impact of apabetalone on pathways linked to chronic kidney disease and its cardiovascular complications.

**Keywords:** bromodomain; cardiovascular disease; chronic kidney disease; epigenetics; inflammation; proteomics

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Chronic kidney disease (CKD) affects approximately 11% of the general worldwide population, and represents an important global health challenge. CKD evolves over many years, with progressive loss of renal function, which could eventually lead to renal replacement therapy (dialysis) and kidney transplantation. Over the course of the disease, there is a progressive increase in both mortality and comorbidities, primarily due to the development of cardiovascular disease (CVD). Consequently, early therapeutic intervention is essential to slow CKD progression, prevent end-stage renal disease, and improve the quality of life and longevity of patients.

CKD is characterized by heightened levels of oxidative stress, advanced glycation end-products, proinflammatory cytokines, and uremic toxins. Pathogenic signaling initiated by these factors induces changes in epigenetic marks, including methylation and acetylation on chromatin-associated proteins. BET proteins BRD2, BRD3, BRD4, and BRDT are epigenetic readers that use small interaction modules called bromodomains (BDs) to bind to acetylated lysine—tagged histones and transcription factors associated with transcriptionally active chromatin. Binding leads to the recruitment of the transcriptional machinery that drives gene expression. In disease states, abnormal signaling often results in overabundant or mislocalized acetylation marks, overexpression of BET proteins, and increased BET protein

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occupancy of gene regulatory regions.\textsuperscript{14–21} This causes changes to gene transcription patterns that control cell fate, proliferation and activation in cancer, fibrosis, autoimmunity, and inflammation.\textsuperscript{14–21} Recently, BET proteins were shown to contribute to renal disorders, including experimental polycystic kidney disease and renal inflammatory disease.\textsuperscript{22–26} Importantly, in these studies, small molecule inhibitors targeting the BDs of BET proteins countered aberrant gene transcription, which led to improved renal outcomes.\textsuperscript{22–24,26}

Apabetalone (RVX-208) is a first-in-class orally available BET inhibitor (BETi) developed to treat CVD.\textsuperscript{27–30} In biochemical assays, apabetalone targeted the second BD (BD2) of BET proteins with 20– to 30-fold selectivity.\textsuperscript{33,34} In cells, BD2 binding by apabetalone resulted in differential transcriptional effects compared with a pan-BETi that targeted both BD1 and BD2 with equal affinity.\textsuperscript{34,35} Apabetalone downregulated immune, inflammatory, and proatherosclerotic genes in \textit{ex vivo}–treated human blood cells and primary hepatocytes, as well as in a mouse model of atherosclerosis.\textsuperscript{30–38} Furthermore, apabetalone improved the plasma lipid profiles of CVD patients and significantly lowered the incidence of major adverse cardiac events in the Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation (SUSTAIN) and the ApoA-I Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation (ASSURE) phase 2 trials.\textsuperscript{30,39} Although high-potency pan-BETi are currently being tested for cancer indications,\textsuperscript{40} apabetalone was the first BETi to enter clinical trials for the treatment of CVD. Determining the scope of the \textit{in vivo} actions of apabetalone is currently an area of active investigation.\textsuperscript{27–31}

CKD is characterized by a strong immune and inflammatory component that contributes to accelerated endothelial dysfunction, vascular inflammation, atherosclerosis, and calcification.\textsuperscript{31–42} CKD patients often succumb to cardiovascular complications because current therapies fail to treat the key pathogenic mechanisms underlying disease progression.\textsuperscript{31} To evaluate the potential of apabetalone as a CKD therapeutic, we examined the pharmacokinetics of apabetalone in a phase 1, open-label, parallel group study of patients with an estimated glomerular rate (eGFR) of \(<30\) ml/min per 1.73 m\(^2\). Using a novel aptamer-based proteomics approach coupled to bioinformatics, we identified proteins enriched in the plasma from patients with impaired kidney function, relative to control subjects. Furthermore, we demonstrated that a single dose of apabetalone downregulated plasma markers of endothelial dysfunction, atherosclerosis, vascular inflammation, calcification, fibrosis, hemostasis, and chronic inflammation, countering the pathogenic signaling in CKD patients. These findings suggest that apabetalone treatment might benefit CKD patients.

### METHODS

#### Patient Selection

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. The phase 1 apabetalone pharmacokinetic study consisted of 2 cohorts of 8 subjects each who received a single 100-mg oral dose of apabetalone after a meal. Cohort 1 consisted of subjects with stage 4 or 5 CKD who were not on dialysis, with an eGFR of \(<30\) ml/min per 1.73 m\(^2\) and a mean eGFR of \(<1.5\) ml/min per 1.73 m\(^2\). Cohort 2 consisted of control subjects matched to the renally impaired subjects in age, weight, and sex, as well as an eGFR of \(\geq60\) ml/min per 1.73 m\(^2\), with mean eGFR of 78.5 ml/min per 1.73 m\(^2\). Additional characteristics of the cohorts are provided in Table 1.

#### Apabetalone Pharmacokinetics and Safety

Blood samples for determination of plasma concentration of apabetalone were collected at the following time points:

<table>
<thead>
<tr>
<th>Sample Time (h)</th>
<th>Cohort 1 renal impaired</th>
<th>Cohort 2 controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.5 (171)</td>
<td>3.4 (171)</td>
</tr>
<tr>
<td>1</td>
<td>2.8 (151)</td>
<td>2.8 (151)</td>
</tr>
<tr>
<td>2</td>
<td>3.0 (152)</td>
<td>3.0 (152)</td>
</tr>
<tr>
<td>3</td>
<td>3.0 (152)</td>
<td>3.0 (152)</td>
</tr>
<tr>
<td>4</td>
<td>3.1 (153)</td>
<td>3.1 (153)</td>
</tr>
<tr>
<td>6</td>
<td>3.3 (154)</td>
<td>3.3 (154)</td>
</tr>
<tr>
<td>8</td>
<td>3.3 (154)</td>
<td>3.3 (154)</td>
</tr>
<tr>
<td>24</td>
<td>3.3 (154)</td>
<td>3.3 (154)</td>
</tr>
</tbody>
</table>

**Table 1.** Demographic and clinical characteristics of patients enrolled in the phase 1 pharmacokinetics study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cohort 1 renal impaired ((n = 8))</th>
<th>Cohort 2 controls ((n = 8))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>55.9 ± 16.5</td>
<td>52.6 ± 16.5</td>
</tr>
<tr>
<td>Male sex</td>
<td>5 (63)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>White race</td>
<td>8 (100)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>8 (100)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>28.1 ± 5.1</td>
<td>25.5 ± 3.0</td>
</tr>
<tr>
<td>Median clinical chemistry measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>36.0 ± 4.1</td>
<td>39.8 ± 3.1</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>113.1 ± 60.4</td>
<td>73.0 ± 16.2</td>
</tr>
<tr>
<td>CKD-EPI (ml/min per 1.73 m(^2))</td>
<td>18.8 ± 5.9</td>
<td>78.4 ± 10.6</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>306.3 ± 104.9</td>
<td>87.9 ± 10.3</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/L)</td>
<td>21.9 ± 10.5</td>
<td>6.1 ± 1.9</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mm Hg)</td>
<td>132.8 ± 26.4</td>
<td>129.0 ± 21.7</td>
</tr>
<tr>
<td>Diastolic (mm Hg)</td>
<td>67.4 ± 14.8</td>
<td>71.5 ± 12.7</td>
</tr>
</tbody>
</table>

**Comorbidities**

- Hypertension 5
- Hypo/hyperthyroidism 2
- Diabetes 1
- Hyperlipidemia 1
- Peripheral vascular disease 1
- Allergy 1
- Autoimmune 1
- Genetic disorder 1
- Gastrointestinal 1

Values are number (%), mean ± SD, or number. BMI, body mass index; CKD-EPI, Chronic Kidney Disease-Epidemiology equation.
points: baseline and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 34, and 48 hours postdose. Total voided urine for determining urinary excretion of apabetalone was collected over the following intervals: baseline (single-void), 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours postdose. Plasma and urine pharmacokinetic samples were shipped to and processed by Intertek Pharmaceutical Services (San Diego, California). Safety assessments included monitoring of adverse events, vital signs, clinical laboratory findings, 12-lead electrocardiograms, and physical examination.

SOMAscan Analysis
Patient plasma samples collected at baseline and at 6, 12, 24, 48 hours after the single apabetalone dose underwent proteomic analysis using the SOMAscan 1.3k platform (SomaLogic, Boulder, Colorado). SOMAscan uses modified aptamers to simultaneously detect 1305 proteins. The assay can quantify proteins that span over 8 logs in abundance (from femtomolar to micromolar), which allows direct analysis of plasma samples without depletion of abundant proteins. The SOMAscan assay transforms protein concentrations into DNA aptamer concentrations that are quantifiable on a DNA microarray. Changes in relative fluorescent units, which are directly proportional to the amount of target protein in the initial sample, were expressed relative to baseline.

Statistical Analysis
To assess the differential protein levels at baseline between cohort 1 and cohort 2, the Mann-Whitney test was used when data were not normally distributed; otherwise Student’s t-tests were used. The Benjamini-Hochberg false discovery rate (FDR) multiple testing correction was used to generate FDR-adjusted \( P \) values. An adjusted \( P \) value < 0.05 was considered significant. Shapiro-Wilk tests were used to determine data distribution.

Pathway Analysis
QIAGEN’s Ingenuity Pathway Analysis software (IPA; QIAGEN, Redwood City, California; www.qiagen.com/ingenuity) was used to interpret the proteomics data.

RESULTS
Pharmacokinetics of Apabetalone in Control and Stage 4 or 5 CKD Patients
In this study, we compared 8 subjects with eGFRs ranging from 9 to 29 ml/min per 1.73 m² (cohort 1, CKD stage 4 or 5, and median creatinine clearance 18.8 ml/min per square meter) and 8 subjects with eGFR ranging from 66 to 109 ml/min per 1.73 m² (cohort 2, age-, sex-, weight-matched, and median creatinine clearance 78.4 ml/min per square meter). In addition to stage 4 or 5 CKD, cohort 1 also displayed comorbidities that commonly accompany renal disease, including CVD, diabetes, and hypothyroidism (Table 1). Baseline plasma samples were obtained from both cohorts to assess clinical biochemistry parameters. Comparison of apabetalone pharmacokinetics following a single dose revealed that the maximum (peak) serum concentration, area under the curve, time to reach maximum (peak) serum concentration (Tmax), and terminal half-life were similar for both the renally impaired and matched subjects (Supplementary Table S1). Minimal amounts of apabetalone were excreted in the urine of either cohort (Supplementary Table S2), which is consistent with the elimination of apabetalone through hepatic clearance. No significant adverse events occurred during the study in the control or the CKD cohorts (data not shown).

Plasma Proteome Profiling in CKD Patients
To gain insight into the molecular mechanisms that underlie CKD and its comorbidities, the proteomic composition of plasma samples from late-stage CKD (cohort 1) and control (cohort 2) subjects was assessed at baseline with the SOMAscan 1.3k platform (Figure 1a). Two hundred eighty-eight proteins were differentially expressed between the 2 cohorts (difference >10%, \( P < 0.05, \) FDR <0.2) (Figure 1b and Supplementary Table S3), forming a database for functional pathway analysis. Upon correction for multiple testing, 167 proteins remained significantly upregulated, whereas 2 proteins were downregulated in the CKD cohort (difference >10%, \( P < 0.005, \) FDR <0.05) (Figure 1b and Supplementary Table S3, column F).

CKD progression is accompanied by an overall increase in the plasma concentration of low-molecular-weight (LMW; <45 kDa) proteins due to a decline in the filtration capacity of the kidneys. Analysis showed that 173 of the 288 CKD cohort-enriched proteins were LMW proteins. These LMW proteins showed a greater fold enrichment in the CKD cohort compared with proteins of >45 kDa (2.3 ± 1.2 vs 1.7 ± 0.76, respectively; \( p = 0.000024; \) Student’s...
Many of the enriched LMW proteins are well-established biomarkers that correlate with kidney function, including cystatin C (3.1-fold), β2-microglobulin (5-fold), neutrophil gelatinase-associated lipocalin (4.6-fold), liver fatty acid-binding protein (2.7-fold), and fibroblast growth factor 23 (1.8-fold) (Supplementary Table S3 and references therein). Numerous uremic toxins, as classified by the European Toxin Work Group, were also upregulated in the CKD cohort (Supplementary Table S4). Other enriched proteins included cytokines and their soluble receptors, adhesion molecules, metalloproteases, and complement and coagulation proteins, as well as factors involved in calcification, metabolism, and oxidative stress (Supplementary Table S3).

Gene ontology analysis of the CKD cohort-associated proteins (FDR < 0.2) showed significant enrichment of 53 terms in the molecular function category (Supplementary Table S5). The top 21 enriched terms (Table 2) were related to ligand-receptor signaling, with 9 linked to cytokine and chemokine pathways. IPA, with its manually curated database from peer-reviewed literature, further predicted not only the impact of disease on cellular functions (P values), but also the direction of change by calculating the activation z-score (Supplementary Table S6). Analysis showed that 234 “disease or function” annotations were increased in the CKD cohort (z score > 2), whereas 6 annotations were decreased (z score < -2). In particular, top pathways (Table 3) which referred to immune and inflammatory cell migration, proliferation, differentiation, activation, and viability, were all upregulated in plasma from the CKD cohort compared with the control cohort. The IPA analysis also predicted a marked upregulation of 42 canonical pathways (z score > 2) and a downregulation of

![Figure 1. SOMAscan analysis of the plasma proteome. Study design flowchart (a). Proteins differentially expressed in the CKD cohort are plotted according to their fold enrichment (b). The degree of significance is color coded (see legend). Note that p values tend to be highly significant for highly enriched proteins. Fold enrichment as a function of protein molecular weight (c). Note that the majority of enriched proteins have a molecular weight of < 45 kDa. Protein enrichment also tends to be greater for proteins with molecular weight < 45 kDa.](#)
2 pathways (z-score: $<-2$), with functions in immune and inflammatory responses, endothelial dysfunction, coagulation, renin-angiotensin system, calcification, and oxidative stress (Table 4). Thus, our bioinformatics analysis highlighted many of the underlying dysfunctional pathways that contribute to CKD and its comorbidities.

### Apabetalone Downregulates Markers and Pathways Upregulated in Plasma From Renally Impaired Cohort

To enable assessment of the CKD cohort’s response to apabetalone, target engagement was evaluated at multiple time points postdose. At 12 hours, the known BETi target engagement marker lymphocyte activation gene 3 (LAG3)\textsuperscript{18,23} was maximally downregulated (Supplementary Figure S1). Recovery of LAG3 plasma expression was observed following drug clearance (Supplementary Figure S1). Therefore, the impact of apabetalone on plasma protein levels in the CKD cohort was analyzed 12 hours postdose (Table 5).

Numerous plasma proteins with physiological functions relevant for CKD and CVD were downregulated by apabetalone in the CKD cohort. Circulating proatherogenic, inflammatory cytokines interleukin (IL)-6, IL-1, IL-17, IL-12, and IL-23, as well as the IL-15 receptor subunit \( z \), decreased in abundance with apabetalone treatment (10%–29%) (Table 5). Apabetalone reduced levels of metalloproteases (MMP-3, MMP-10) and extracellular matrix proteins (fibronectin, osteopontin)
(11%–25%). Markers of fibrinolysis were also downregulated, including plasminogen activator inhibitor-1, tissue-type plasminogen activator, and urokinase-like plasminogen activator (42%, 28% and 18%, respectively), as well as a marker of platelet activation and vascular inflammation, P-selectin (23%). Furthermore, apabetalone decreased the abundance of activated fragments of complement proteins, including anaphylatoxin C5α (12%) (Table 5). In contrast, apabetalone had limited effects on disease biomarkers in the control cohort (Table 5).

To gain insight into the functional effects of apabetalone, the impact of apabetalone on the IPA canonical pathways upregulated in plasma from the CKD cohort was assessed (Table 4). Remarkably, apabetalone reversed the activation of 33 of 42 pathways in the CKD cohort, with the greatest impact on pathways that were most upregulated in the CKD patients (Table 4). These canonical pathways regulate inflammation through cytokine signaling, acute phase response, T-cell, and dendritic cell responses. Further, apabetalone downregulated other pathways relevant to CKD pathophysiology: vascular calcification (bone morphogenetic protein signaling), fibrosis (integrin-linked protein kinase signaling), apoptosis (death cell receptor signaling), angiogenesis (vascular endothelial growth factor and pigment epithelium-derived factor signaling), nitric oxide, and reactive oxygen species signaling. Moreover, intracellular signaling pathways converging on the transcription factor nuclear factor-κB (NF-κB) were also down-regulated by apabetalone, whereas the peroxisome proliferator–activated receptor pathway was upregulated, countering pathogenic signaling in CKD.50,51 Finally, pathways that contribute to comorbidities, such as hypertension, diabetes, and cardiac hypertrophy, were also downregulated by apabetalone (Table 4). Overall, apabetalone simultaneously modulated multiple pathways that contribute to CKD pathology and associated vascular complications.

### DISCUSSION

Changes in plasma protein composition are a central feature of CKD pathophysiology and its associated comorbidities. Until recently, however, attempts to study the plasma proteome have been hampered by the complexity of the plasma protein composition and inherent limitations of mass spectrometry.52–54 SOMAselect is a powerful protein discovery tool that
can simultaneously detect 1305 proteins representative of various cellular functions.44 Here, a total of 169 proteins (adjusted P < 0.05) were identified as differentially expressed in plasma from control and renally impaired cohorts. Approximately 60% were LMW proteins (<45 kDa), consistent with the impairment of kidney filtration in late stage CKD.35,46 The plasma proteome presented here shows little overlap with previously published mass spectrometry driven studies (Supplementary Table S3, column J).52–54 In contrast, our data largely corroborates the results of a previous SOMAscan study,44 in which a smaller panel (614 aptamers) was used to assess stages 3 to 5 CKD patient plasma composition. Forty-five of the 60 markers identified by Gold et al.,44 were also detected in this study (Supplementary Table S3, column J). Differences may be due to cohort size and composition. Many of the enriched proteins are well established CKD biomarkers that correlate with kidney function and CKD or CVD outcomes (Supplementary Table S3, columns H and I). These proteins play a role in inflammation, vascular calcification, thrombosis, cell adhesion, extracellular matrix remodeling, oxidative stress, and metabolism. Interestingly, several of the enriched proteins we identified have not been previously linked to CKD or CVD. Establishing the functional relevance of these novel CKD cohort-enriched proteins and determining their prognostic value presents exciting avenues for future studies.

Analysis of plasma from renally impaired patients revealed that a single dose of apabetalone rapidly downregulated multiple CKD and CVD protein markers (Table 5), as well as associated molecular pathways (Table 4). In contrast, in the control cohort, apabetalone had no significant effect on pathway activation or repression (as determined by the IPA z-score; data not shown), or on the abundance of most circulating disease markers (Table 5). This differential sensitivity to a BET protein inhibitor highlights the dysregulation of BET protein-dependent pathways in human CKD, which is consistent with published data from cell and animal models.21,23,26

Inflammation contributes to the onset and progression of renal disease.31 Indeed, 40% of the 288 proteins upregulated in the CKD cohort are directly or indirectly linked to inflammation (Supplementary Table S3). The most prevalent among these are cytokines and chemokines known to be dysregulated in CKD and its comorbidities.35 In plasma from the CKD cohort, there was an increase in circulating cytokines (Supplementary Table S3), and pathways controlled by IL-6, IL-17, oncostatin M, IL-22, IL-8, and granulocyte-macrophage, colony-stimulating factor were predicted to be activated (Table 4). Significantly, a single dose of apabetalone reduced the plasma protein levels of multiple cytokines and chemokines, including IL-6, IL-12, IL-17, and IL-23 (Table 5). In addition, apabetalone was predicted to counter the CKD cohort-associated upregulation of cytokine pathways, including IL-6, IL-8, and IL-17 (Table 4). Essential components of the innate and adaptive immune system55,57 were among the top upregulated pathways in the CKD cohort, including the dendritic cell maturation and Th1 signaling pathways (Table 4, and Supplementary Figures S2 and S3). Extensive cross-talk occurred between these 2 pathways, with activated dendritic cells stimulating inflammatory Th1 lymphocyte responses, which, in turn, promoted dendritic cell maturation.58 Apabetalone had a significant impact on both pathways (z scores: −4.2 and −3.3, respectively) (Table 4, and Supplementary Figures S2 and S3), which was consistent with published data30,38.

Inflammatory cytokines such as IL-6 induce the acute phase response (APR) protein expression and secretion by the liver.39 Multiple APR proteins were upregulated in plasma from the CKD cohort potentially due to increased IL-6 signaling (as predicted by IPA) (Supplementary Table S3 and Supplementary Figures S4 and S5). APR signaling pathway was ranked the sixth most activated pathway in CKD plasma (Table 4). APR proteins are markers of disease progression and become highly enriched in the plasma of stage 3 and 4 CKD patients.53,54 Interestingly, this enrichment is greater in plasma of patients with CKD than in the plasma of CVD patients,53 which indicates that inflammation is exacerbated in patients with advanced kidney failure.61 Of note, the APR pathway was robustly downregulated by apabetalone (z score: −3.46) (Table 4 and Supplementary Figure S5), in line with previously published data.30,37,38

Consistent with the susceptibility of CKD patients to thrombosis and bleeding,60 the coagulation system pathway was upregulated in plasma from the CKD cohort (Table 4). Several circulating hemostatic factors were predictive of CVD events.51,62 Apabetalone significantly decreased markers of the fibrinolysis pathway (including plasminogen activator inhibitor-1, urokinase-like plasminogen activator, and tissue-type plasminogen activator) (Table 5), which are associated with disturbances in hemostasis61 and atherosclerosis64 in CKD patients. Inflammation and coagulation can activate the complement cascade, propagating and amplifying proinflammatory signaling and damage.65 In agreement with previous studies,37 apabetalone reduced the plasma concentration of activated C3 fragments and of the anaphylatoxin C5a, a marker that has been correlated with CVD outcomes.66–68 To date, no adverse events related to
coagulation nor complement system dysregulation have been observed in clinical trials with apabetalone\(^3\) (and unpublished data).

Increased acetylation of gene promoters and transcription factors such as STAT3 and NF-kB have been detected in cultured renal cells and animal models of inflammatory renal disease.\(^{23,69–71}\) These aberrant acetylated lysine residues become the target of BET proteins (e.g., BRD4) that then drive transcription of inflammatory genes.\(^{23,71}\) Apabetalone downregulated the abundance of multiple NF-kB target proteins in plasma from the CKD cohort, including IL-6, IL-1\(\alpha\), IL-12, IL-23, MMP-3, and plasminogen activator inhibitor-1 (Table 5), and decreased the predicted activation of the NF-kB signaling pathway (z-score: \(-3.5\) (Table 4 and Supplementary Figure S6)). These data corroborate previously reported inhibition of NF-kB-dependent pathways by BETi.\(^{16,23}\)

Current therapies fail to counter progressive systemic inflammation, endothelial dysfunction, vascular calcification, fibrosis, and atherosclerosis in patients with CKD.\(^3\) The plasma proteome study reported here demonstrated that apabetalone might oppose many of these pathogenic pathways in patients with CKD (and its accompanying comorbidities) by rapidly down-regulating plasma markers associated with these dysfunctions. Considering its favorable pharmacokinetic properties in CKD patients and a well-established safety profile,\(^{29,32}\) apabetalone is a potential therapeutic agent capable of modulating molecular pathways associated with risk factors that drive CKD progression and its cardiovascular complications.

**DISCLOSURE**

SW, LMT, CH, SCS, DG, RJ, MS, JOJ, NCW, and EK are employees and shareholders of Resverlogix Corp. which supported the study financially. KK-Z is an advisory board member of Resverlogix Corp. The other author declared no competing interests.

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**AUTHORSHIP**

RR, RJ, MS, JOJ, NCW, KK-Z, and EK participated in the clinical trial design. SW, LMT, EK, and CH analyzed the data and interpreted the results. SW and SCS wrote the manuscript. MS, JOJ, NCW, EK, LMT, and DG critically reviewed the manuscript. The final version was approved by all authors.

**SUPPLEMENTARY MATERIAL**

| Table S1. Pharmacokinetic assessment of the renal impaired cohort 1 and matched control cohort 2 following a single apabetalone dose (100 mg). |
| Table S2. Urine output measured as the fractional excretion (Fe%) of apabetalone for the renal impaired cohort 1 and matched control cohort 2 following a single apabetalone dose (100 mg). |
| Table S3. Complete annotated list of 288 proteins enriched in CKD (\(P < 0.05\), Mann-Whitney test). Base-line comparison of cohort 1 versus cohort 2 (enrichment: red indicates upregulation, blue indicates down-regulation), and its statistical significance are shown in columns D, E, and F. References supporting links to renal, cardiovascular or inflammatory diseases and diabetes are listed columns H and I). Plasma proteomic studies that identified protein enrichment in CKD patients are cited (column J). |
| Table S4. Uremic toxins enriched in the plasma from CKD cohort relative to the control cohort. |
| Table S5. Gene ontology term molecular function analysis of proteins enriched in CKD plasma: complete list of 53. |
| Table S6. Ingenuity Pathway Analysis disease or functions analysis of proteins enriched in CKD plasma: complete list. |
| Figure S1. Target engagement time course. Apabetalone (blue circles and line) and LAG3 (orange diamonds and line) serum concentrations measured at discrete time intervals following a single apabetalone dose. Representative data from a single patient. |
| Figure S2. Ingenuity Pathway Analysis canonical pathway analysis: dendritic cell maturation. Predicted signaling in CKD plasma (A). Predicted changes in response to apabetalone 12 hour postsingle dose (B). Yellow and turquoise: measured upregulation or downregulation in protein abundance. Orange and blue: predicted upregulation or downregulation. Pink outline: proteome data. |
| Figure S3. Ingenuity Pathway Analysis canonical pathway analysis: Th1 signaling. Predicted signaling in CKD plasma (A). Predicted changes in response to single apabetalone 12 hour postdose (B). Yellow and turquoise: measured upregulation or downregulation in protein abundance. Orange and blue: predicted upregulation or downregulation. Pink outline: proteome data. |
| Figure S4. Ingenuity Pathway Analysis canonical pathway analysis: interleukin-6 signaling. Predicted signaling in CKD plasma (A). Predicted changes in response to single apabetalone 12 hour postdose (B). Yellow and turquoise: measured upregulation or downregulation in protein abundance. Orange and blue: predicted upregulation or downregulation. Pink outline: proteome data. |
| Figure S5. Ingenuity Pathway Analysis canonical pathway analysis: acute phase response signaling. Predicted signaling in CKD plasma (A). Predicted changes in...
response to single apabetalone 12 hour postdose (B). Yellow and turquoise: measured upregulation or downregulation in protein abundance. Orange and blue: predicted upregulation or downregulation. Pink outline: proteome data.

**Figure S6.** Ingenuity Pathway Analysis canonical pathway analysis: nuclear factor-kb signaling. Predicted signaling in CKD plasma (A). Predicted changes in response to single apabetalone 12 hour postdose (B). Yellow and turquoise: measured upregulation or downregulation in protein abundance. Orange and blue: predicted upregulation or downregulation. Pink outline: proteome data.

Supplementary material is linked to the online version of the paper at www.kireports.org.

**REFERENCES**


