

## IL-1 Inhibition and Vascular Function in CKD

Kristen L. Nowak,\* Michel Chonchol,\* Talat Alp Ikizler,<sup>†‡</sup> Heather Farmer-Bailey,\*  
Natjalie Salas,<sup>†</sup> Rafia Chaudhry,<sup>†</sup> Wei Wang,\* Gerard Smits,\* Isak Tengesdal,\*  
Charles A. Dinarello,\* and Adriana M. Hung<sup>†‡</sup>

\*Department of Medicine, University of Colorado Denver, Aurora, Colorado; <sup>†</sup>Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee; and <sup>‡</sup>Department of Medicine, Veterans Administration Medical Center, Tennessee Valley Healthcare System, Nashville, Tennessee

### ABSTRACT

Vascular endothelial dysfunction and increased arterial stiffness contribute to increased cardiovascular risk in patients with CKD who exhibit chronic systemic inflammation. Because chronic inflammation contributes to vascular dysfunction, blocking inflammation may reduce cardiovascular risk in patients with CKD. In a two-site, double-blind trial, we randomized 42 adult patients with stage 3–4 CKD who were already receiving optimal background therapy to receive either IL-1 trap rilonacept or placebo for 12 weeks. Coprimary end points included change in brachial artery flow-mediated dilation (FMD<sub>BA</sub>) and aortic pulse-wave velocity (aPWV) after 4, 8, and 12 weeks. Exploratory end points included change in high-sensitivity C-reactive protein (hsCRP), FMD<sub>BA</sub> after acute ascorbic acid infusion, and vascular endothelial cell protein expression of NADPH oxidase. Participants were 63±11 (mean±SD) years of age and 24% were women; mean eGFR was 38±13 ml/min per 1.73 m<sup>2</sup>. Compared with placebo, rilonacept improved FMD<sub>BA</sub> (baseline: 3.36%±2.06% [mean±SD], 12 weeks: 2.45%±2.29% with placebo and baseline: 3.75%±3.12%, 12 weeks: 4.86%±3.20% with rilonacept; *P*<0.01), without changing aPWV (*P*=0.56). Rilonacept also reduced hsCRP levels (median [interquartile range]) (baseline: 4.60 [1.90–8.22] mg/L, 12 weeks: 2.16 [0.92–7.38] mg/L; *P*<0.01) and endothelial cell NADPH oxidase expression (*P*<0.05). Acute infusion of ascorbic acid to inhibit superoxide production associated with a nonsignificant trend toward increased FMD<sub>BA</sub> in the placebo group (*P*=0.07) but not the rilonacept group (*P*=0.56). Rilonacept was well tolerated (five adverse events versus two with placebo). In conclusion, treatment with an IL-1 trap improved FMD<sub>BA</sub> without changing aPWV and reduced systemic inflammation in patients with CKD.

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In 1983, an “IL-1” hypothesis was first proposed, positing that monocyte release of IL-1, the master cytokine of inflammation, was the basis of numerous complications in patients on chronic dialysis.<sup>1</sup> However, the inflammatory process associated with CKD begins well before the need for chronic dialysis.<sup>2,3</sup> Circulating levels of both the proinflammatory cytokine IL-1 and its naturally occurring receptor antagonist (IL-1Ra) are elevated in CKD.<sup>4,5</sup> Furthermore, it has been shown that the inflammasome, a protein structure that perpetuates the inflammatory response and activates IL-1, is activated in CKD regardless of the etiology.<sup>6–8</sup> Increased systemic inflammation in patients with CKD is associated with increased risk of cardiovascular mortality.<sup>9,10</sup> Whether inhibiting IL-1 can

reduce cardiovascular risk in patients with CKD is currently unknown.

As much as 80% of all cardiovascular diseases (CVD) are associated with dysfunction and disorders of arteries.<sup>11</sup> Two of the greatest concerns for

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**Correspondence:** Dr. Kristen L. Nowak, Division of Renal Diseases and Hypertension, University of Colorado Denver, 12700 East 19th Avenue C281, Aurora, CO 80020, or Dr. Adriana M. Hung, Division of Nephrology, Medical Center North S-3223, 1161 21st Avenue South and Garland, Nashville, TN 32212. Email: [Kristen.Nowak@ucdenver.edu](mailto:Kristen.Nowak@ucdenver.edu) or [Adriana.hung@vanderbilt.edu](mailto:Adriana.hung@vanderbilt.edu)

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risk of CVD are the development of vascular endothelial dysfunction (impaired endothelium-dependent dilation [EDD], often assessed as impaired brachial artery flow-mediated dilation [ $\text{FMD}_{\text{BA}}$ ]), and stiffening of the large elastic arteries, typically measured as aortic pulse-wave velocity (aPWV).<sup>12</sup> Impairment in both  $\text{FMD}_{\text{BA}}$  and aPWV are evident in CKD<sup>13,14</sup> and are independent predictors of future cardiovascular events and mortality.<sup>15,16</sup>

A key mechanism contributing to the development of impaired EDD is increased inflammation.<sup>17,18</sup> The exact mechanisms by which inflammatory signaling impairs EDD in humans are not completely understood; however, the suppression of EDD is, at least in part, because of inflammation promoting increased oxidative stress.<sup>19,20</sup> Inflammatory signaling stimulates oxidant enzyme systems (e.g., NADPH oxidase) to produce reactive oxygen species, including superoxide anion.<sup>19</sup> Superoxide reduces bioavailability of nitric oxide,<sup>20</sup> which is a key mechanism in CKD-associated impairment of EDD and increased arterial stiffness.<sup>21,22</sup>

Accordingly, we performed the first randomized controlled trial of IL-1 inhibition in patients with CKD not requiring chronic dialysis. The primary aim was to determine if inhibiting IL-1 improved vascular function (increased  $\text{FMD}_{\text{BA}}$  and reduced aPWV) in patients with stage 3–4 CKD. Additionally, we assessed whether IL-1 inhibition also reduced systemic inflammation and vascular oxidative stress.

## RESULTS

### Enrollment and Baseline Clinical Characteristics

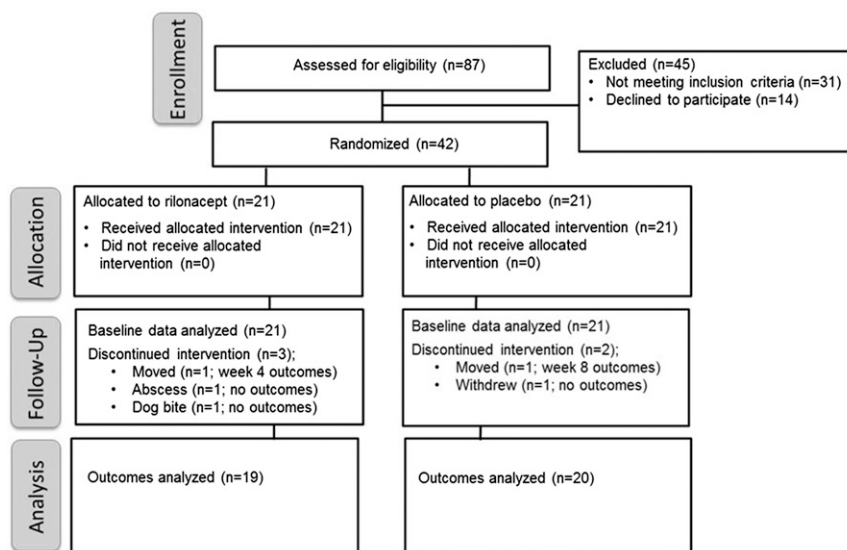
Of the 87 participants who were screened for participation in this randomized, placebo-controlled, double-blind trial, 42 were

randomized to receive either the IL-1 inhibitor, rilonacept, or placebo (Figure 1). Three participants in the rilonacept group and two in the placebo group discontinued the intervention before the final study visit at 12 weeks. These participants were still included in the analysis for the visits they completed. The reasons for study discontinuation are shown in Figure 1. Participants in each arm did not differ significantly in terms of baseline characteristics, including sex, race/ethnicity, etiology of CKD, medications, smoking status, eGFR, body mass index, BP, serum albumin, baseline serum high-sensitivity C-reactive protein (hsCRP), and baseline plasma IL-6 (Table 1).

### Effect of IL-1 Inhibition on Vascular Function

The co-primary end point,  $\text{FMD}_{\text{BA}}$ , was improved by 30% after 12 weeks in the rilonacept group ( $3.75 \pm 3.12$  versus  $4.86 \pm 3.20$  [mean  $\% \Delta \pm \text{SD}$ ]), as compared with a 27% reduction in the placebo group ( $3.36 \pm 2.06$  versus  $2.45 \pm 2.29$ ;  $P < 0.01$ ) (Figure 2A). Results were similar when presented as absolute change (Table 2). Baseline diameter and shear rate did not differ across the study, thus were not included as covariates in the model (Table 2). Endothelium-independent dilation to sublingual nitroglycerin, a measure of smooth muscle cell responsiveness to nitric oxide, was unaffected by IL-1 inhibition (Table 2).

The second coprimary end point, aPWV, did not change in the rilonacept ( $1011 \pm 289$  cm/s versus  $1023 \pm 248$  cm/s) or placebo group ( $1130 \pm 293$  cm/s versus  $1133 \pm 303$  cm/s;  $P = 0.56$ ; Figure 2B). Carotid-radial pulse-wave velocity, a measure of peripheral arterial stiffness, also did not change with the intervention (Table 2). BP remained stable throughout the intervention in both groups (Table 2). Additional secondary end points (carotid artery intimal medial thickness [cIMT], carotid artery compliance,  $\beta$ -stiffness index, and carotid systolic BP [SBP]) were also unchanged (Table 2). Of note, there



**Figure 1.** Patient enrollment, randomization, and completion (CONSORT) flow diagram. Note, data from participants who discontinued the intervention were still included in the linear mixed-effects models analysis for the visits completed.

**Table 1.** Baseline characteristics of study participants according to study group

Clinical Characteristics	All (n=42)	Rilonacept (n=21)	Placebo (n=21)	P Value
Women, % (n)	24 (10)	24 (5)	24 (5)	1.00
Age, yr, mean±SD	63±11	61±11	65±11	0.29
Race/ethnicity, % (n)				1.00
White non-Hispanic	48 (20)	48 (10)	48 (10)	
Hispanic	29 (12)	29 (6)	29 (6)	
Black	23 (10)	23 (5)	23 (5)	
Etiology of CKD, % (n)				
Hypertension	52 (22)	57 (12)	48 (10)	0.76
Type 2 diabetes	45 (19)	48 (10)	43 (9)	1.00
Type 1 diabetes	7 (3)	5 (1)	10 (2)	1.00
ADPKD	7 (3)	5 (1)	10 (2)	1.00
Renal vascular disease	14 (3)	0 (0)	14 (3)	0.23
FSGS	5 (1)	5 (1)	0 (0)	1.00
Antihypertensive agent, % (n)	100 (42)	100 (21)	100 (21)	1.00
ACEi/ARB	67 (28)	52 (11)	81 (17)	0.10
Calcium channel blocker	50 (25)	48 (10)	53 (11)	1.00
β Blocker	40 (16)	38 (8)	43 (9)	1.00
Statin, % (n)	64 (27)	52 (11)	76 (16)	0.18
Smoking status, % (n)				0.09
Never	40 (17)	43 (9)	38 (8)	
Current	10 (4)	19 (4)	0 (0)	
Former	50 (21)	38 (8)	62 (13)	
MDRD eGFR, ml/min per 1.73 m <sup>2</sup> , mean±SD	38±13	38±14	38±12	0.73
Urine protein-to-creatinine ratio, mg/mmol, median (interquartile range)	0.27 (0.11–0.67)	0.34 (0.10–0.61)	0.21 (0.11–1.14)	0.94
BMI, kg/m <sup>2</sup> , mean±SD	32±5	32±5	31±5	0.49
SBP, mmHg, mean±SD	133±17	129±26	137±19	0.09
DBP, mmHg, mean±SD	79±10	79±9	79±12	0.65
Serum albumin, g/dl	3.88±0.33	3.88±0.31	3.89±0.35	0.73
Baseline hsCRP, mg/L, median (interquartile range)	3.8 (1.6–5.9)	4.6 (1.9–8.2)	3.3 (1.4–5.2)	0.14
Baseline IL-6, pg/ml, median (interquartile range)	13.5 (7.0–13.50)	15.0 (7.0–26.0)	9.0 (7.0–25.0)	0.60

P values are a comparison of rilonacept and placebo groups. ADPKD, autosomal dominant polycystic kidney disease; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic BP.

was no difference in the primary outcomes according to site of participation.

### IL-1 Inhibition Reduced Systemic Inflammation

Rilonacept reduced hsCRP in the rilonacept compared with placebo group (median [interquartile range]) (rilonacept: baseline, 4.60 [1.90–8.22] mg/L, week 4, 1.46 [0.89–2.14] mg/L, week 8, 1.69 [0.76–2.77] mg/L, week 12, 2.16 [0.92–7.38]; placebo: baseline, 3.3 [1.4–5.2] mg/L, week 4, 3.95 [1.78–5.05] mg/L, week 8, 2.90 [1.47–6.20] mg/L, week 12, 2.68 [1.52–5.90] mg/L;  $P<0.01$ ; Figure 3A), indicating reduced systemic inflammation. The response was consistent, with reduced hsCRP at 12 weeks in 14 out of 18 participants completing the study in the rilonacept group (Figure 3B). There was a tendency toward reduced IL-6 with rilonacept, but this was not statistically significant ( $P=0.35$ ) (Supplemental Table 1). There was no change in IL-1Ra ( $P=0.41$ ), IL-18 ( $P=0.52$ ), or IL-18 binding protein ( $P=0.13$ ), but a trend toward reduced free IL-18 ( $P=0.08$ ) (Supplemental Table 1).

### IL-1 Inhibition Reduced Vascular Oxidative Stress

In a subgroup, an acute supraphysiologic infusion of ascorbic acid known to scavenge superoxide tended to improve FMD<sub>BA</sub> at 12

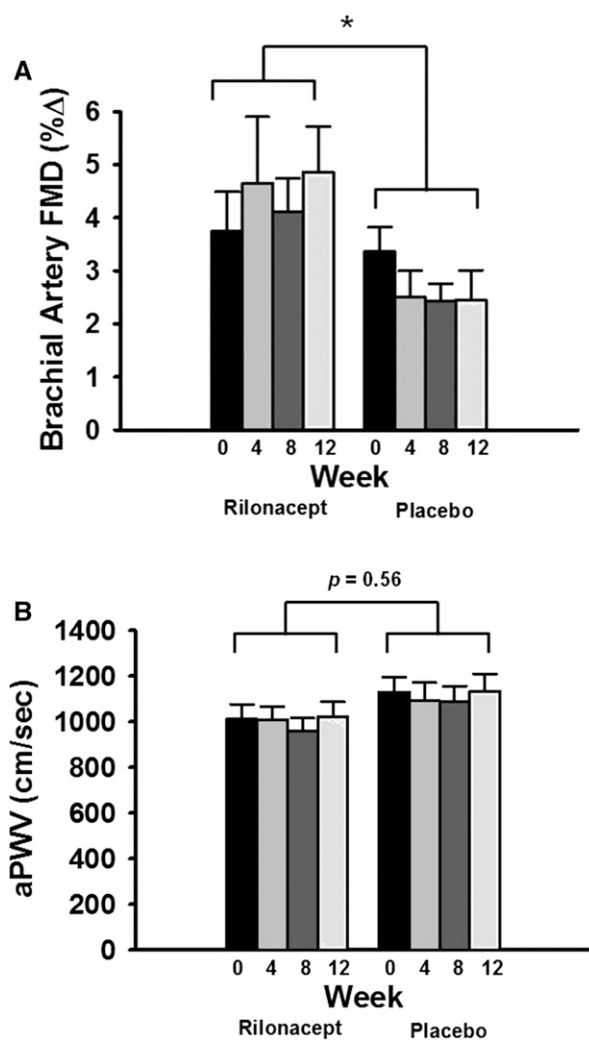
weeks in the placebo ( $P=0.07$ ) but not rilonacept group ( $P=0.56$ ; group×condition interaction  $P=0.07$ ), supporting that inhibition of IL-1 reduced vascular oxidative stress (Supplemental Figure 1). Additionally, expression of the oxidant enzyme NADPH oxidase in vascular endothelial cells collected from a subgroup of participants was reduced in the rilonacept but not placebo group at 12 weeks (rilonacept:  $-0.02\pm0.03$ ; placebo:  $+0.01\pm0.03$  (relative to human umbilical vein endothelial cell control;  $P<0.05$ ).

### Renal Function

There was no change in renal function in either group, as measured by eGFR using the four-variable Modified Diet Renal Disease (MDRD) equation (rilonacept: baseline,  $38\pm14$  ml/min per 1.73 m<sup>2</sup> and 12 weeks,  $36\pm15$  ml/min per 1.73 m<sup>2</sup>; placebo: baseline,  $38\pm14$  ml/min per 1.73 m<sup>2</sup> and 12 weeks,  $38\pm12$  ml/min per 1.73 m<sup>2</sup>;  $P=0.52$ ).

### Adverse Events

Overall, rilonacept was well tolerated. Seven participants experienced any adverse event related or possibly related to the drug ( $n=5$  in the rilonacept group,  $n=2$  in the placebo group). One participant in the rilonacept group developed



**Figure 2.** Changes in FMD<sub>BA</sub> and aPWV with rilonacept and placebo. Mean group FMD<sub>BA</sub> (A) and aPWV (B) at baseline, 4 weeks, 8 weeks, and 12 weeks according to group (rilonacept or placebo). Values are mean  $\pm$  SEM; \* $P < 0.01$  (linear mixed-effects models).

an injection site reaction, and one participant in the rilonacept group developed a neck abscess/pyomyositis, which led to the investigators discontinuing study participation. No other participants discontinued the study because of an adverse event related to the drug. Three participants in the rilonacept group and two participants in the placebo group experienced symptoms consistent with an upper respiratory tract infection that required treatment with an oral antibiotic and skipping a weekly injection as a precautionary measure. One participant in the rilonacept group was discontinued from the study by the investigators after being bitten by a dog.

## DISCUSSION

Our study was the first to administer an IL-1 inhibitor to a population of patients with CKD not requiring chronic

dialysis. Our findings are the first to show that inhibiting IL-1 improves EDD, a key antecedent of CVD, in patients with moderate-to-severe CKD. We also provide the first insight in humans regarding a potential reduction in vascular oxidative stress with IL-1 inhibition. Notably, the treatment was well tolerated, with a similar adverse event profile compared with the placebo group. Overall, our findings support the conduction of future randomized controlled trials assessing the efficacy of anti-inflammatory therapy to reduce cardiovascular outcomes in high-risk patients with CKD.

After 12 weeks, FMD<sub>BA</sub> was improved by an absolute change of 1.1% with IL-1 inhibition, as compared with a reduction of 0.9% in the placebo group. Notably, this improvement was seen despite the fact that patients were already receiving optimal background therapy, including standard treatment of hypertension, diabetes, and/or hypercholesterolemia. Patients with CKD exhibit significant vascular endothelial dysfunction,<sup>13,23</sup> indicating that current therapy is not sufficient to ameliorate nontraditional cardiovascular risk factors. An improvement in FMD<sub>BA</sub> by 1.1%Δ is clinically significant, as it is similar to the magnitude of change seen according to meta-analyses of previous randomized controlled trials assessing the effect of statins and angiotensin-converting enzyme inhibitors across various populations.<sup>24,25</sup> In a recent meta-analysis of epidemiologic studies, a 1% increase was been associated with 13% reduction in risk of cardiovascular events, after adjustment for confounders.<sup>26</sup> We provide the first evidence that inhibiting IL-1 improves EDD, an independent predictor of future cardiovascular events and mortality,<sup>16,27</sup> in patients with moderate-to-severe CKD. Of note, a reduction in FMD<sub>BA</sub> in a placebo group over time has also been observed in previous trials of patients with CKD, in as little as 3 months.<sup>28–30</sup>

In contrast to FMD<sub>BA</sub>, IL-1 inhibition failed to reduce aPWV, the gold standard index of large-elastic artery stiffness. Large-elastic artery stiffness is modulated by both functional (*i.e.*, vascular tone) and structural (*i.e.*, arterial wall proteins) influences.<sup>31</sup> Inflammation may modulate either of these components; however, an intervention of relatively short duration (*i.e.*, 12 weeks) would likely only affect the functional component (*e.g.*, nitric oxide bioavailability, endothelin-1 signaling), without sufficient time to induce structural modifications to the vasculature (*e.g.*, vascular calcification, changes in collagen I, collagen III, and TGF- $\beta$ ). It is possible that in patients with moderate-to-severe CKD, the structural modifications to the vasculature are too great to be overcome by functional changes alone. Of note, randomized controlled trials with an end point of aPWV have typically failed to induce significant change in patients with CKD,<sup>28,32,33</sup> with the exception of BP-lowering interventions.<sup>34,35</sup> Similarly, short-term (30 days) inhibition of IL-1 with the IL-1Ra anakinra failed to reduce aPWV in patients with rheumatoid arthritis.<sup>36</sup> The possibility remains that a longer duration of treatment with an IL-1 inhibitor may indeed reduce aPWV.

**Table 2.** Hemodynamic factors

Hemodynamic Factors	Rilonacept		Placebo		P Value
	Baseline	12 Wk	Baseline	12 Wk	
FMD <sub>BA</sub> , mmΔ	0.14±0.10	0.19±0.12	0.13±0.07	0.11±0.09	<0.001
Baseline brachial artery diameter, mm	3.99±0.80	3.97±0.74	3.94±0.68	4.13±0.59	0.36
Peak shear rate, s <sup>-1</sup>	740±334	716±311	716±180	710±245	0.82
Brachial artery dilation to NTG, %Δ	17.9±10.4	18.3±4.8	17.6±8.0	13.5±5.2	0.09
Brachial artery dilation to NTG, mmΔ	0.66±0.33	0.70±0.23	0.65±0.25	0.54±0.19	0.07
Brachial SBP, mmHg	130±18	133±17	141±18	134±20	0.84
Brachial DBP, mmHg	75±8	74±8	79±12	75±9	0.74
clMT, mm	0.79±0.28	0.77±0.23	0.83±0.34	0.88±0.35	0.37
CR-PWV, cm/s	959±129	876±182	987±241	1013±151	0.19
Carotid artery compliance, (mm/mmHg)×10 <sup>-1</sup>	0.08±0.05	0.08±0.04	0.06±0.03	0.06±0.02	0.22
Carotid β-stiffness index, A.U.	13.1±10.1	11.7±5.6	14.6±7.9	13.5±3.2	0.08
Carotid SBP, mmHg	131±19	128±24	135±19	128±26	0.98

Data are mean±SD. Brachial BPs were measured in the supine position during the tonometry assessment. CR-PWV, carotid artery compliance, carotid β-stiffness index, and carotid SBP were only measured in a subgroup of participants from the Denver site (see Concise Methods). P values are group effect from linear mixed-effects models (model also included 4-week and 8-week data) for all variables. NTG, nitroglycerin; DBP, diastolic BP; CR-PWV, carotid-radial pulse-wave velocity; A.U., arbitrary units.

The decision to administer an IL-1 trap was due to the increasing recognition that the inflammasome plays a key role in chronic inflammatory conditions such as CKD.<sup>37</sup> Circulating levels of both IL-1 and IL-1Ra are elevated in CKD.<sup>4</sup> We have previously shown that the administration of an IL-1Ra in patients on chronic dialysis effectively reduced CRP by >50%.<sup>38</sup> Similarly, in our study rilonacept effectively reduced hsCRP by >50%, supporting the role of the inflammasome in the chronic inflammatory response seen in CKD. This type of response has not been elicited with administration of a TNF-α antagonist in a CKD population.<sup>39</sup> The inflammasome is a group of intracellular protein complexes including the nucleotide-binding domain, leucine-rich-containing family, pyrin-domain containing 3 subfamily member itself. The activation and subsequent assembling of inflammasome control the production of important proinflammatory cytokines including IL-1β and IL-18. Circulating IL-6 levels tended to decrease with the intervention, however they did not reach statistical significance, which may be explained by the high variability of these measurements within each group. Additionally, there was a tendency for a reduction in free IL-18, which is intriguing as IL-1 is hypothesized to drive caspase-1, which is required for processing of the inactive IL-18 precursor to an active cytokine. An imbalance of IL-18 to IL-18 binding protein leads to elevated levels of circulating free IL-18, which has been implicated in mediating kidney diseases.<sup>40</sup>

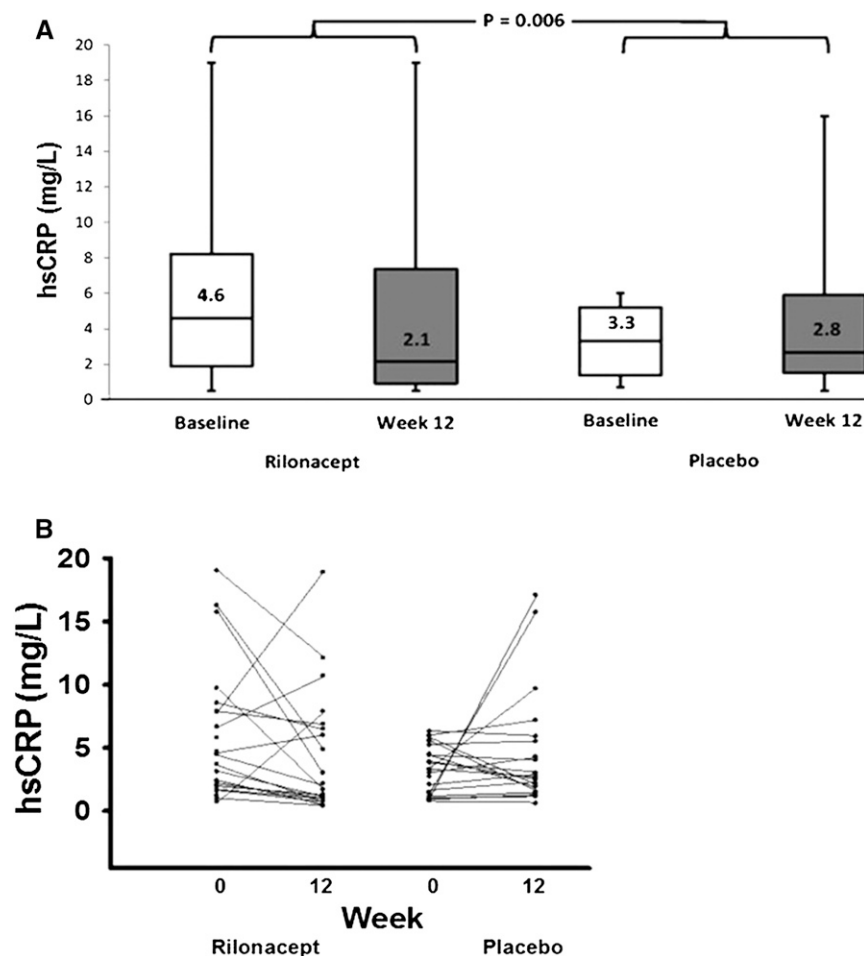
Feedback between inflammation and oxidative stress is well documented.<sup>41</sup> Reactive oxygen species stimulate proinflammatory gene transcription and protein expression *via* the activation of redox-sensitive transcription factors such as NF-κB,<sup>42</sup> and proinflammatory cytokines including IL-1 can further promote production of reactive oxygen species *via* mechanisms including activation of the angiotensin II type 1 receptor and NADPH oxidase.<sup>19,43</sup> Short-term inhibition of IL-1 with the IL-1Ra anakinra has been previously shown to reduce circulating markers of oxidative stress, while also

improving FMD<sub>BA</sub> in patients with rheumatoid arthritis.<sup>36</sup> Our study is the first to evidence that IL-1 inhibition likely reduces oxidative stress directly at the level of the vasculature in patients with CKD not requiring dialysis.

Indeed, infusion of a supraphysiologic dose of ascorbic acid (vitamin C), a well established method for acutely reducing oxidative stress-related suppression of EDD,<sup>44,45</sup> tended to improve FMD<sub>BA</sub> in the placebo but not rilonacept group at 12 weeks, suggesting that IL-1 inhibition reduced vascular oxidative stress. Furthermore, the rilonacept group exhibited reduced vascular endothelial cell protein expression of the oxidant enzyme NADPH oxidase. IL-1 signaling can stimulate NADPH oxidase to produce reactive oxygen species, including superoxide anion, leading to subsequent reduction in NO bioavailability and impairment in EDD.<sup>19,20</sup>

Chronic inflammation has been recognized for over a decade as a critical nontraditional risk factor for CVD in patients with nondialysis-dependent CKD.<sup>2</sup> However, clinical trials of anti-inflammatory therapies in patients with kidney disease have, to date, been nonspecific therapies with pleiotropic effects, including statins, vitamin D therapy, and sevelamer. Despite long scientific interest in such trials, direct anticytokine therapies had not been tested in patients with nondialysis-dependent CKD before this study. Our results are of great clinical significance, as they demonstrate that IL-1 inhibition improves surrogate markers of CVD. Thus, future randomized controlled trials assessing the efficacy of IL-1 inhibition for improving cardiovascular outcomes in patients with CKD are indicated.

The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study is an ongoing, multinational, phase 3, four arm, randomized, placebo-controlled trial evaluating whether the IL-1 monoclonal antibody canakinumab reduces cardiovascular events (recurrent myocardial infarction, stroke, and cardiovascular death) in stable patients with coronary artery disease and high hsCRP (>2 mg/L) despite usual care,



**Figure 3.** Change in hsCRP with rilonacept and placebo. (A) Median (interquartile range) group levels of hsCRP at baseline and 12 weeks according to group (rilonacept or placebo). (B) Individual subject levels of hsCRP at baseline and 12 weeks. For A, middle line indicates median, borders of the box indicate the 25th and 75th percentiles, and whiskers indicate the 5th and 95th percentiles.  $P < 0.01$  (linear mixed-effects models).

including optimal statin therapy (Clinicaltrials.gov identifier: NCT01327846).<sup>46</sup> Completion is expected in 2017. However, patients with severe CKD ( $\text{eGFR} < 30 \text{ ml/min per } 1.73 \text{ m}^2$ ) will be excluded from enrollment, and results will not be applicable to patients with advanced CKD, a group at the highest risk for poor outcomes.

In addition to the novelty of administering an IL-1 inhibitor to patients with nondialysis-dependent CKD, there are several other important strengths of this study. Compliance was extremely high and well documented, as injections were given in person at the research centers and missed only when clinically indicated (e.g., upon suspicion of an upper respiratory infection). The study design of performing vascular measurements every 4 weeks provided important insight into the time course of the vascular effects of IL-1 inhibition. Additionally, we obtained unique insight into the physiologic mechanisms by which IL-1 inhibition improved vascular

endothelial function, using novel translational research techniques evaluating vascular oxidative stress. We recognize the limitations that the trial included only a limited number of patients, which may have limited our ability to detect a significant change in IL-6, and that we evaluated surrogate cardiovascular end points. Additionally, the short duration may have limited the ability to detect changes in large-elastic artery stiffness.

In conclusion, 12 weeks of treatment with the IL-1 trap rilonacept improved vascular endothelial function without changing large-elastic artery stiffness in patients with stage 3–4 CKD. This was associated with a reduction in systemic inflammation and vascular oxidative stress. Our results support conducting future randomized controlled trials assessing the efficacy of IL-1 inhibition for improving cardiovascular outcomes in patients with CKD.

## CONCISE METHODS

### Study Participants

Eligible participants were enrolled at two sites (the University of Colorado Denver Anschutz Medical Campus and the Tennessee Valley Healthcare System/Vanderbilt University Medical Center) between September 2012 and September 2014 (the trial concluded according to enrollment determined by power calculations described below). Patients eligible for inclusion were men and women aged 18–80 years with stage 3–4 CKD ( $\text{eGFR}$  with the MDRD prediction equation<sup>47</sup>  $15\text{--}60 \text{ ml/min per } 1.73 \text{ m}^2$  and stable renal function in the past 3 months) and evidence of chronic inflammation ( $\text{hsCRP} > 2.0 \text{ mg/L}$  and  $< 30 \text{ mg/L}$  [to identify chronic inflammation rather than acute inflammation/infection] on at least two consecutive weekly determinations). Initially, inclusion criteria required serum albumin  $< 3.5 \text{ g/dl}$ ,  $\text{hsCRP} > 4.0 \text{ mg/L}$ , urine protein excretion  $< 3.5 \text{ g/24 hr}$ , and  $\text{eGFR}$  of  $20\text{--}60 \text{ ml/min per } 1.73 \text{ m}^2$ ; however, these criteria were amended very early in the study to increase recruitment ( $n=40$  participants were enrolled under these new criteria). All participants were on an optimal, stable, antihypertensive, diabetic, and lipid-lowering regimen as appropriate for at least 1 month before inclusion.

Patients were excluded if they had: urine protein excretion  $> 5 \text{ g/d}$ , advanced CKD requiring chronic dialysis, active infection (acute or chronic [within 3 months] or antibiotic therapy [within 1 month]), or history of recurrent infection), life expectancy  $< 1$  year, history of severe congestive heart failure (ejection fraction  $< 35\%$ ), hospitalization in the last month, known malignancy or malignancy within the

past year, HIV, active chronic hepatitis B, hepatitis C, risk for tuberculosis, chronic conditions that may have interfered with hsCRP or immune function (*i.e.*, severe arthritis, lupus, or inflammatory bowel disease), body mass index  $\geq 40$  kg/m<sup>2</sup> (for accuracy of vascular measurements), were taking immunosuppressant agents in the past 12 months, or were pregnant, nursing, or planning to become pregnant.

### Study Design

A 12-week, randomized, placebo-controlled (1:1 allocation), parallel group, double-blind study with the IL-1 inhibitor rilonacept was conducted at each site's Clinical and Translational Research Center, except for the Vanderbilt visits that were for medication administration only (performed at Veterans Affairs clinics). Randomization (without blocking) occurred using a computer-generated procedure generated and kept by a statistician. After initial screening, subjects meeting inclusion criteria had baseline vascular measurements performed, as described below. Measurements were made under supine, overnight fasted (water only) conditions, following standard recommendations including 24-hour abstention from physical activity and a climate controlled room.<sup>12,48</sup> Participants were then randomized by the study coordinator according to the statistician-generated random allocation sequence, and received an initial loading dose of either rilonacept or matching placebo, with dosing continuing once weekly for 12 weeks. All investigators, coordinators, and patients were blinded to group assignment, with only the nursing staff not affiliated with the study and the statistician aware of the randomization. A different statistician performed all statistical analyses. Measurements were repeated after 4, 8, and 12 weeks of the intervention. The coprimaries were change in FMD<sub>BA</sub> and aPWV in the rilonacept as compared with placebo group.

### Procedures

#### *Rilonacept and Weekly Visits*

Rilonacept is a soluble IL-1 decoy receptor that binds IL-1, acting as a trap to neutralize IL-1 $\beta$  or IL-1 $\alpha$  before it can bind to cell-surface receptors. Participants received an initial loading dose of 320 mg of rilonacept (or placebo injection with identical inactive ingredients), delivered as two 2 ml subcutaneous injections of 160 mg on the same day at two different injection sites. Both the active and placebo were supplied by Regeneron pharmaceuticals in prefilled vials. Dosing continued with once weekly injections of 160 mg, administered as a single 2 ml subcutaneous injection, or a 2 ml placebo injection. At each weekly visit, vital signs were measured and patients were carefully evaluated for symptoms and signs of upper respiratory infection or other infection before receiving the injection.

#### *Vascular Measurements*

FMD<sub>BA</sub> was determined using duplex ultrasonography (University of Colorado used a Vivid 7 Dimension; GE Healthcare, Waukesha, WI; and Vanderbilt University used an iU22; Phillips, Bothell, WA) with electrocardiogram-gated end-diastolic ultrasound images analyzed by a single blinded analyst using a commercially available software package (Vascular Analysis Tools 5.8.1; Medical Imaging Applications), as described in detail previously.<sup>12,44,49</sup> Doppler flow of the brachial artery was also measured and peak shear rate was calculated

as a potential covariate.<sup>12,44,49</sup> Endothelium-independent dilation (brachial artery dilation to 0.4 mg of sublingual nitroglycerin) was assessed as a standard index of smooth muscle cell sensitivity to exogenous nitric oxide.<sup>12,44,49</sup>

aPWV was measured as described in detail previously.<sup>12,50,51</sup> Briefly, a transcutaneous custom tonometer (University of Colorado used a Noninvasive Hemodynamics Workstation; Cardiovascular Engineering Inc., Norwood, MA; and Vanderbilt University used a Sphygmocor; AtCor Medical, Itasca, IL) was positioned at the carotid, brachial, radial, and femoral arteries to noninvasively assess aPWV and carotid-radial pulse-wave velocity (an index of peripheral stiffness [Denver site only]). Distance between the suprasternal notch and femoral artery was measured using a custom raised ruler (Noninvasive Hemodynamics Workstation) or tape measure (all other distances). The distance from the suprasternal notch to the carotid was subtracted from the distance between the two recording sites, and aPWV was calculated as the distance divided by time between the feet of the waveforms, recorded at each site, as described previously.<sup>52</sup>

Additionally, as secondary indices of arterial stiffness, the tonometry assessment in conjunction with ultrasound imaging of the carotid artery also provided blinded assessment of carotid artery compliance, carotid artery  $\beta$ -stiffness index, and carotid SBP in a subgroup of participants from the Denver site ( $n=8-13$ /group per visit), as described previously.<sup>53,54</sup> cIMT was also measured as a secondary outcome in all participants.<sup>53,54</sup>

The influence of oxidative stress on FMD<sub>BA</sub> was assessed in a subgroup of participants from the Denver site by infusing a supraphysiologic dose of ascorbic acid or isovolumic saline, and measuring FMD<sub>BA</sub> during the drip infusion when peak plasma concentrations occurred, as described previously.<sup>44,45</sup> ( $n=7-13$ /group per visit).

Vascular endothelial cells were obtained immediately before other vascular measurements from the intima of an antecubital vein of an arm not designated for future dialysis access in a subgroup of participants from the Denver site ( $n=5-8$ /group per visit). Cells were recovered, fixed, and slides were prepared and frozen for later staining, positive identification of endothelial cells, assessment of nuclear integrity, and quantification of protein expression of NADPH oxidase (p47<sup>phox</sup>; EMD Millipore, Billerica, MA) by a blinded analyst using immunofluorescence, as described previously.<sup>12,44,55</sup>

#### *Laboratory Analysis*

Serum hsCRP was measured every 4 weeks using the immunoturbidimetric method. eGFR was calculated every 4 weeks using the MDRD equation.<sup>47</sup> The MDRD equation was used rather than the Chronic Kidney Disease Epidemiology Collaboration equation, as all participants had a diagnosis of CKD by their primary nephrologist and their eGFR was  $<60$  ml/min per 1.73 m<sup>2</sup>.<sup>2,56</sup> Plasma IL-6, IL-1Ra, IL-18, and IL-18 binding protein were measured by ELISA (Bio-Techne, Minneapolis, MN). Free IL-18 was calculated as described previously.<sup>57</sup>

### Outcome Measures

The coprimaries end points were change in FMD<sub>BA</sub> and aPWV in the rilonacept as compared with placebo group. Secondary outcomes were the change in hsCRP, IL-6, FMD<sub>BA</sub> after acute infusion of

ascorbic acid, and vascular endothelial cell protein expression of NADPH oxidase. Exploratory outcomes included change in IL-1Ra, IL-18, IL-18 binding protein, free IL-18, carotid artery compliance, carotid artery  $\beta$ -stiffness index, carotid SBP, cIMT, and eGFR.

### Statistical Analyses

Differences in baseline variables between groups were assessed using *t* tests, Fisher exact test, rank-based tests, or chi-squared tests. The changes in FMD<sub>BA</sub> and aPWV (coprimary outcomes) in response to treatment were analyzed using mixed-effects linear regression models. The primary interest was the main effect of group with all time points included in the model, including baseline measurements, thus comparing the difference between groups over time. The interaction between group and linear time (difference in slope) was of secondary interest, and the term was dropped from the model as no interaction approached significance (FMD<sub>BA</sub>:  $\beta=0.01$ ,  $P=0.99$ ; aPWV:  $\beta=3.75$ ,  $P=0.90$ ). This statistical approach was also used to evaluate change in secondary outcomes, exploratory outcomes, and potential covariates. Skewed variables (inflammatory markers) were analyzed using a rank-based test and some were mathematically transformed to approach normality when appropriate. To assess the effect of the intervention of vascular oxidative stress, the model included both group (rilonacept versus placebo) and condition (saline versus ascorbic acid) as main effects.  $\alpha$  for the two coprimary end points was set at 0.025 (two-sided), with no adjustment for secondary outcomes, as they were considered exploratory. All data are reported as mean  $\pm$  SD or median (interquartile range), unless otherwise noted.

A sample size of 15 subjects per group was calculated on the basis of 80% power at an  $\alpha$  level of 0.025 (two-sided) in order to detect a difference in the primary outcome with the smallest effect size (aPWV: effect size of 1.19, mean  $\pm$  SD difference of  $143 \pm 120$  cm/s; FMD<sub>BA</sub>: effect size of 1.28, mean  $\pm$  SD difference of  $2.4 \pm 1.9\%$ ), on the basis of previously published data assessing the effect of an intervention on FMD<sub>BA</sub> and aPWV.<sup>44,50</sup> To account for potential drop-out and experimental failure of about 25%, 21 participants were randomized to each group.

### Study Approval

All procedures were approved by the Institutional Review Board of the University of Colorado Denver, the Tennessee Valley Healthcare System, and Vanderbilt University Medical Center, and adhere to the Declaration of Helsinki. The nature, benefits, and risks of the study were explained to the volunteers and their written informed consent was obtained before participation. The trial was registered at Clinicaltrials.gov (identifier: NCT01663103).

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### DISCLOSURES

None.

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