Methods:

Study design and patient selection:

In this first-in-man, open-label single center, dose-escalation trial, we enrolled adult patients with significant RVD estimated by renal artery Doppler ultrasound velocity acceleration (average Peak systolic Velocity >300 cm/sec), and/or MR/CT angiography with evident atherosclerotic stenosis (>60% lumen occlusion and/or post-stenotic dilation), and hypertension (systolic hypertension >155 mm Hg and/or the use of at least two BP drugs) and/or with declining kidney function. Exclusion criteria included serum creatinine >2.5 mg/dL, diabetes mellitus, allergy to furosemide or iodinated contrast, pregnancy, kidney transplant, malignancy, recent history of cardiovascular event (myocardial infarction, stroke, congestive heart failure within 6 months) or deep venous thrombosis within 3 months of enrollment. Enrolled patients were admitted into the Clinical Research Unit at St. Mary's Hospital, in Rochester, MN between June 2013 and April 2016. All patients received medical therapy consisting of agents blocking the renin-angiotensin system during these studies (angiotensin-converting–enzyme (ACE) inhibitors or angiotensin-receptor blockers). Twenty-eight patients with RVD were included in the analysis: fourteen were treated with standardized medical therapy plus a single intra-arterial infusion of autologous MSCs in the renal artery (MSC-treated group), of whom, seven were assigned to low dose (1.0×10^5 cells/kg) and seven patients to a higher dose (2.5×10^5 cells/kg), based on our animal studies[8]. Compared to other published studies in other diseases, these doses are considered low[10-13]. When bilateral stenoses were identified (n=6), only the kidney with most severe stenosis was infused with MSC. The other fourteen patients were treated with standardized

medical therapy alone (medically treated group). Informed, written consent was obtained as approved by the institutional review board of the Mayo Clinic. Autologous MSC preparation and administration were performed under an Investigational New Drug application approved by the Food and Drug Administration (IND 15082).

MSCs' isolation, preparation and safety evaluation:

Six weeks before planned administration, a subcutaneous abdominal fat biopsy (approximately 0.5-2 g) was obtained under sterile conditions from RVD patients in an outpatient surgical suite. Enzymatic digestion of the fat tissue was followed by selection and expansion of plastic adherent cells. Cells were cultured in Advanced MEM (Gibco Cat#12492021) supplemented with human platelet lysate (PLTMax®, Mill Creek Life Sciences) over a 2-week time period using good manufacturing practices as previously described until a sufficient number of cells were obtained for each treatment protocol [14]. Isolated MSCs were characterized by immunostaining and fluorescence-activated cell sorting (FACS) analysis to determine cellular phenotype for MSC markers (all MSCs expressed HLA-ABC, CD44, CD90, CD29, CD73 and CD105 markers, but not HLA-DR, CD45, CD14 or CD34 markers). **Release criteria** for administration after final passage included sterility testing using anaerobic and aerobic culture, endotoxin, mycoplasma, karyotype, and FACS for surface markers characteristic of MSC.

Interventions and Inpatient Study Protocol:

Patients participated in this study during a 3-day inpatient protocol on two occasions (3 months apart) in the clinical research unit as previously described[4]. Dietary intake was regulated at 150 mEq of sodium with an isocaloric diet prepared on site. The first study

day included measurement of urinary sodium excretion and GFR by iothalamate clearance (iothalamate meglumine, Conray, Mallinckrodt) over three 30-minute timed collection periods after oral hydration (20 mL/kg)[15, 16]. Single kidney-GFR was determined by apportioning the measured Iothalamate clearance by percentage of blood flow for each kidney. Blood pressure was measured by automated oscillometric recordings including 3 values taken 3 times daily (an automated oscillometric unit, Omron blood pressure, measured blood pressure at 5, 7, and 9 minutes after a 5-minute rest).

Renal oxygenation measured by Blood Oxygen Level Dependent (BOLD) MRI:

On the second day, BOLD MRI examinations were performed on a [GE Twin Speed Signa EXCITE] 3.0T system (GE Medical Systems, Waukesha, WI) using a 12-channel torso phased array coil [17]. BOLD imaging consisted of a 2D fast spoiled gradient echo sequence with multiple echo times (TEs). Parametric images of R2* were generated by fitting signal intensity versus TE data to an exponential function on a voxel-by-voxel basis and solving for R2* [18]. After the first BOLD acquisition, in order to assess oxygen-transport dependent tubular function, furosemide (20 mg) was administered intravenously and flushed with 20 mL of saline. BOLD measurements for each kidney were repeated 15 minutes later. BOLD MRI analysis could not be performed in two patients (one in each group) due to excessive susceptibility artifacts from the adjacent colon. Analysis of BOLD data was performed by drawing parenchymal regions of interest (ROIs) on 2-4 slices through the midpole hilar region of each kidney on representative T2*-weighted images, and then transferring the ROI to the corresponding R2* parametric image, as previously described[1].

Cortical and medullary tissue perfusion and blood flow measured by Multidetector Computed Tomography (MDCT):

On the third study day, the common femoral vein was cannulated with a 6F sheath and blood samples drawn from the right and left renal veins with a 5F pigtail Cobra catheter (Cook, Inc, Bloomington, IN). The catheter was then advanced into the right atrium for central venous injection of contrast for flow studies using MDCT. For assessment of perfusion MDCT imaging was obtained using a dual-source 64-slice helical MDCT scanner (SOMATOM Definition, Siemens Medical Solutions) after a bolus injection of iopamidol 370 (0.5 mL/kg up to a maximum of 40 mL). Fifteen minutes after completion of the perfusion study, a kidney volume study (5-mm thick slices) was performed in the helical mode to determine both cortical and medullary regional volumes. To calculate regional perfusions and volumes, images were reconstructed and displayed with the Analyze™ software package (Biomedical Imaging Resource, Mayo Clinic, MN, USA). ROIs were selected from cross-sectional images from the aorta, renal cortex, and medulla. Average tissue attenuation in each region was plotted over time and fitted by curve-fitting algorithms to obtain measures of renal perfusion and function, as described previously[19].

Single MSC infusion, laboratory testing, and safety monitoring:

After completing CT imaging, patients were returned to the angiography suite where standard aortic cannulation was performed through the femoral artery. Heparin (4000 units) was administered intravenously and the stenotic renal artery then selectively cannulated and imaged, after which MSCs were manually infused distal to stenosis

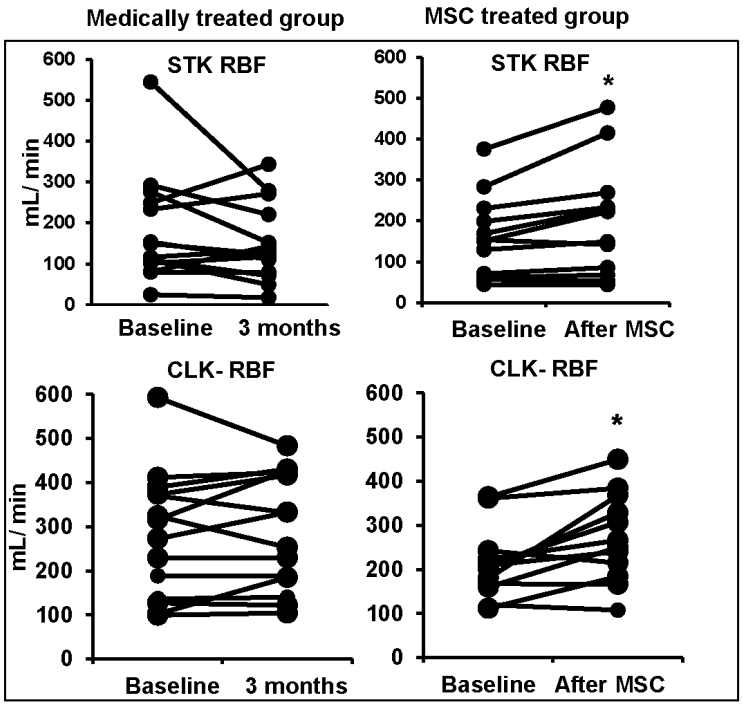
slowly over 5 minutes. Follow-up angiography was obtained once the infusion was complete, and the catheter removed. Patients remained hospitalized for 24 hours after MSC administration for observation. The following day, day 7, and monthly until the 3-month return visit peripheral blood samples were obtained for complete blood count, C-reactive protein (CRP), urine cytology, lactate dehydrogenase (LDH) and markers of renal injury (e.g. neutrophil-gelatinase associated lipocalin [NGAL]). Renal vein blood samples for vascular endothelial growth factor (VEGF-C), angiopoietin and inflammatory cytokine levels were obtained from the STK and CLK renal vein of all patients, as previously described[4, 20]. Samples were stored at −80°C until measurement. Collected samples were centrifuged, and the supernatant was stored. NGAL (ng/mL) was tested by ELISA according to the manufacturer's protocol (BioPorto Diagnostics, Cat no. KIT 036). Levels of VEGF-C, angiopoietin, interleukin-6 (IL-6), interferon-γ, tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) were measured by luminex (Millipore, cat no: MPXHCYTO-60K). Signals were read by the Bio-plex 200 systems (BIO-RAD).

Statistical analysis:

Results were expressed using mean values and standard deviation (SD) or median values (interquartile range), as appropriate. Qualitative variables were expressed as number (percentage). Comparisons between the two groups of RVD patients were performed using two-sample t-tests with unequal variance (or the Wilcoxon rank-sum test for skewed data) for continuous variables, and a Chi-squared test or Fisher's exact test for categorical variables as appropriate. Comparisons between two kidneys within the same individuals and repeated measurements for specific kidneys within individuals

before and after treatment were performed using paired-t tests. Percent (%) change in STK-GFR was calculated as: [((3 month STK-GFR − baseline STK-GFR) / baseline STK-GFR) × 100%]. Statistical significance was accepted for $P \leq 0.05$. Statistical analysis was performed using JMP software package version 8.0 (SAS Institute Inc., Cary, NC). This trial is registered with ClinicalTrials.gov, number: NCT02266394.

Supplemental figure 1:



* P<0.05 vs baseline (Wilcoxon signed-rank test)- Data shown as median (IQR) and "whiskers" above and below the box show the minimum and maximum range. RBF: renal blood flow, STK: stenotic kidney and CLK: contralateral kidney.

Supplemental table 1: Tissue oxygenation levels defined both by R2* values and fractional hypoxia

		Medically treated		Medically treated plus MSC	
Stenotic kidneys STK		**Baseline**	**3 months**	**Baseline**	**3 months**
Cortical R2*- STK	Pre-Furosemide	20.3 ± 4.3	21.7 ± 5.8	19.7 ± 2.7	19.3 ± 2.1
	Post-furosemide	18.5 ± 3.2#	20.6 ± 5.01	18.8 ± 2.9	19 ± 2.4
Fractional-hypoxia %> 30 - STK	Pre-Furosemide	10 (6.3, 25)	9.6 (2.4, 37.7)	12.1 (3.3, 17.8)	6.8 (1.8, 12.9)*
	Post-furosemide	5.2 (2.5, 16.6)#	7.6 (3.4, 26.2)#	8.1 (2.6, 12.3)#	5.1 (1.2, 10.7)
Contra-Lateral kidneys CLK		**Baseline**	**3 months**	**Baseline**	**3 months**
Cortical R2*- CLK	Pre-Furosemide	18.2 ± 3.2	19.3 ± 1.9	18.1 ± 1.9	18.4 ± 2.3
	Post-furosemide	16.9 ± 2.3#	18.9 ± 3	17.4 ± 1.7	17.3 ± 2.1#
Fractional hypoxia %>30 - CLK	Pre-Furosemide	6.8 (3.9, 9.6)	9.3 (5.6, 11.8)	4.4 (2.6, 8.2)	3.4 (0.6, 7.8)
	Post-furosemide	1.9 (0.8, 5.8)#	5 (2.5, 11)#	3.7 (2, 5.5)#	3 (0.5, 5.8)#

* Vs baseline, # Vs pre-furosemide P value < 0.05 (Both paired-T. Test and Wilcoxon signed-rank test)