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TITLE OF INVENTION

54	RAGE-RELATED METHODS AND COMPOSITIONS FOR TREATING GLOMERULAR INJURY
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57	ABSTRACT (NOT MORE THAT 150 WORDS)	NUMBER OF SHEETS	
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If no classification is finished, Form P.9 should accompany this form.
The figure of the drawing to which the abstract refers is attached.

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ABSTRACT

This invention provides methods, compositions and articles of manufacture for inhibiting the onset of and treating glomerular injury. The instant invention is based on the blockade of RAGE and/or RAGE G82S function.

**RAGE-RELATED METHODS AND COMPOSITIONS FOR TREATING
GLOMERULAR INJURY**

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Throughout this application, various publications are referenced. Full citations for these publications may be found immediately preceding the claims. The disclosures of these publications are hereby
10 incorporated by reference into this application in order to more fully describe the state of the art as of the date of the invention described and claimed herein.

Background of the Invention

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Primary or secondary focal segmental glomerulosclerosis (FSGS) encompasses a range of diseases characterized by glomerular and tubulointerstitial fibrosis that often progress, unhaltingly, to irreversible renal scarring
20 and failure in human subjects (1). Secondary cases of FSGS may emerge in the face of chronic disease (hemodynamic, immunologic or metabolic). However, in both cases of primary and secondary disease, despite many years of study, there is no definitive
25 understanding of the molecular mechanisms that underlie these disorders. As such, insights into means to prevent/treat these disorders have not been elucidated.

Key steps in identifying rational therapeutic targets
30 for these diseases, however, may emerge from animal studies. Development of FSGS by agents that incite pathways linked to glomerular fibrosis and hyperpermeability are useful as a means to track the early, initiating events and the later, amplified
35 consequences of proteinuria and renal scarring. In

this context, multiple studies have employed administration of agents such as puromycin or adriamycin (ADR) to rats, to induce processes analogous to human FSGS in the kidney (2-4). In addition, other
5 studies in rats have included the induction of Passive Heymann Nephritis as a means to induce irreversible glomerular injury (5). Overall, these studies in rats have been frustrated by the inability to precisely link activation of specific cells to the pathogenesis and/or
10 progression of GS upon disease induction.

A paucity of mouse models existed for the study of FSGS-like diseases until the first description of ADR-induced toxicity in mice (6-7). In 2000, Wang and
15 colleagues reported on the impact of ADR up to 42 days (6 weeks) after administration of ADR (9). Male BALB/c mice, 20 to 25 gm, were injected with ADR, 10.5 mg/kg, by IV injection. These investigators carefully followed the time course of events in the ADR-treated
20 mice and observed the following (9).

First, overt proteinuria developed in all mice by day 5. Proteinuria persisted throughout 6 weeks of study. Only 35.7% of mice developed hematuria but 53.6%
25 developed leukocyturia.

Second, levels of serum albumin were consistently lower in ADR-treated mice vs controls beginning one week after ADR treatment.

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Third, creatinine clearance declined with time and was significantly decreased compared to control mice 4 weeks post-ADR.

Fourth, by week 6, tubular atrophy and intratubular cast formation with tubulointerstitial expansion had occurred and was widely seen in the cortex. Extensive FGS and severe interstitial fibrosis and inflammation were present. Global sclerosis was observed in many glomeruli.

Fifth, by EM, effacement of foot processes of podocytes had occurred. At week 1, effacement was segmental, but global by week 6. Control mice failed to demonstrate any epithelial cell abnormalities at any point.

Importantly, in this study, cellular infiltration and inflammation were examined.

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Sixth, at early and later times after ADR, CD4+ and CD8+ T cells, and macrophages were significantly increased in the kidneys of ADR-treated mice. These cell types were found both in the interstitium as well as in the glomeruli after injury. Infiltration of inflammatory cells was noted quite early after ADR, within the first 24 hours, and persisted for up to weeks after ADR. These findings support the premise that inflammation, at least in part, contributes as an early trigger, and/or late progression factor in the molecular pathways leading to sustained glomerular perturbation, fibrosis and albuminuria that converge to cause renal dysfunction.

30 These studies highlighted that even 6 weeks after ADR, progressive renal injury, proteinuria and decreased creatinine clearance were features of the disease. In addition, new insights into proinflammatory mechanisms into the disease process were uncovered by time course

examination of cellular infiltration after ADR. Other studies have, in fact, confirmed inflammatory cell infiltration into the ADR-treated kidney (9). Indeed, the observation that human FSGS is typified by differentiation of podocytes into MP-like cells, along with inflammatory cell infiltration from the periphery (MP and T lymphocytes) in the interstitium, periglomerular regions and glomeruli (1, 10-12) is compatible with the concept put forth in the ADR-induced murine model of FSGS, that is, it is plausible that inflammatory stimuli importantly contribute to the pathogenesis and/or progression of FSGS.

In parallel with progressive renal dysfunction and scarring in primary or secondary FSGS syndromes in human subjects (and murine models), injury and depletion of glomerular podocytes, eventuating in podocyte "insufficiency" and capillary collapse, have been implicated as important steps in the development of FSGS (13, 14). In most cases of nephrotic syndrome, podocyte foot process effacement is considered an early manifestation of injury, and is followed by a continuum of progressive podocyte damage characterized by vacuolization, pseudocyst formation, detachment of podocytes from the GBM; processes that lead to irreversible loss/apoptosis of podocytes (15).

Key evidence that podocytes are not mere bystanders, but rather active participants in molecular pathways of injury, was highlighted by recent studies in TGF- β overexpressing transgenic mice. In those mice, marked upregulation of Smad 7 was observed in damaged podocytes. Both TGF- β and Smad7 were associated with apoptosis in cultured podocytes. In the former case,

activation of p38 MAP kinase and caspase-3 were key intermediary steps in TGF- β -induced apoptosis. In the latter case, suppressed nuclear translocation of the cell survival factor NF- κ B led to Smad7-induced podocyte apoptosis (16). These studies highlight the concept that activation of cell signalling and modulation of gene expression in the podocyte may be early events in the development of FSGS, and thus, may contribute to the pathogenesis of this disease.

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It is important to note that the concept of key roles for podocytes in the pathogenesis/progression of glomerular dysfunction have parallels in diabetes. Diabetes is a highly complex environment in which multiple contributing pathways, such as accumulation/activation of Advanced Glycation Endproducts, activation of PKC, especially the β isoform, as well as hyperglycemia itself are implicated in the pathogenesis of this disorder (17-19). Evidence is accumulating that podocytes are perturbed early in diabetes, and that their products, such as VEGF, may contribute to cellular dysfunction in this disorder (20-25). As in FSGS and FSGS-like disorders, the case for the podocyte as bystander vs contributory agent to the pathogenesis and progression of glomerular injury remains to be rigorously tested.

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Although inhibiting RAGE has been implicated in treating symptoms of diabetes (35), the literature does

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not provide a basis for concluding that inhibiting the binding of RAGE to its ligands could play a role in treating or preventing glomerular injury.

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Summary of the Invention

This invention provides a method for inhibiting the onset of a glomerular injury in a subject comprising
5 administering to the subject a prophylactically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof.

This invention further provides a method for treating a
10 glomerular injury in a subject comprising administering to the subject a therapeutically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof.

15 This invention further provides a method for inhibiting the onset of glomerulosclerosis, proteinuria or albumuria in a subject comprising administering to the subject a prophylactically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and
20 a ligand thereof.

This invention further provides a method for treating glomerulosclerosis, proteinuria or albumuria in a subject comprising administering to the subject a
25 therapeutically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof.

This invention further provides an article of
30 manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label

indicating a use for the agent for inhibiting the onset of glomerular injury in a subject.

This invention further provides an article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for inhibiting the onset of glomerulosclerosis, proteinuria or albuminuria in a subject.

This invention further provides an article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for treating a glomerular injury in a subject.

Finally, this invention provides an article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for treating glomerulosclerosis, proteinuria or albuminuria in a subject.

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Brief Description of the Figures

Figure 1. Administration of ADR to BALB/c mice: effects of sRAGE. BALB/c mice were treated with ADR or control (saline). ADR-treated mice received sRAGE or PBS. At 2 weeks after ADR, kidney wt/body wt ratio and mesangial area & mesangial/glomerular fraction determined. N=5 mice/group. Statistical considerations are indicated in the figures.

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Figure 2. Administration of ADR to BALB/c mice: effects of sRAGE. BALB/c mice were treated with ADR or control (saline). ADR-treated mice received sRAGE or PBS. At 6 weeks after ADR, kidney wt/body wt ratio and mesangial area & mesangial/glomerular fraction determined. N=5 mice/group. Statistical considerations are indicated in the figures.

Figure 3. Blockade of RAGE suppresses albuminuria after administration of ADR. At 2 and 6 weeks after ADR, urine albumin/creatinine ratio was determined. N=5 mice/condition. N=5 mice/condition. Statistical considerations are indicated in the figure.

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Detailed Description of the InventionDefinitions

5 "Agent" shall include, without limitation, an organic compound, a nucleic acid, a polypeptide, a lipid, and a carbohydrate. Agents include, for example, agents which are known with respect to structure and/or function, and those which are not known with respect to
10 structure or function.

"Antibody" shall include, by way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, this term includes polyclonal
15 and monoclonal antibodies, and antigen-binding fragments thereof. Furthermore, this term includes chimeric antibodies and wholly synthetic antibodies, and antigen-binding fragments thereof.

20 As used herein, "inhibit," when used in connection with the binding between RAGE and/or RAGE G82S with a ligand thereof, shall mean to reduce such binding. In one embodiment, "inhibit" shall mean to eliminate such binding.

25 "Inhibiting" the onset of a disorder shall mean either lessening the likelihood of the disorder's onset, or preventing the onset of the disorder entirely. In the preferred embodiment, inhibiting the onset of a
30 disorder means preventing its onset entirely.

"Subject" shall mean any animal, such as a human, non-human primate, mouse, rat, guinea pig or rabbit.

"Treating" a disorder shall mean slowing, stopping or reversing the disorder's progression. In the preferred embodiment, treating a disorder means reversing the disorder's progression, ideally to the point of
5 eliminating the disorder itself. As used herein, ameliorating a disorder and treating a disorder are equivalent.

Embodiments of the Invention

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This invention provides methods for inhibiting the onset of and treating glomerular injury. This invention is based on the surprising discovery of a correlation between suppressing glomerular injury in a
15 non-diabetic subject and blocking RAGE and/or RAGE G82S function.

Specifically, this invention provides a method for inhibiting the onset of a glomerular injury in a
20 subject comprising administering to the subject a prophylactically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof.

25 In one embodiment of the instant method, the glomerular injury is associated with reduced removal of toxins. In another embodiment, the glomerular injury is associated with glomerulosclerosis. In a further embodiment, the glomerular injury is associated with
30 proteinuria. In yet a further embodiment, the glomerular injury is associated with albuminuria.

In the preferred embodiment of the instant method, the subject is human. In one embodiment the subject is

afflicted with diabetes. In another embodiment of the instant method, the subject has been afflicted with diabetes for less than 20 years. In a further embodiment, the subject is not afflicted with diabetes. In yet a further embodiment, the subject is receiving or is about to receive a chemotherapy drug. In yet a further embodiment, the chemotherapy drug is adriamycin. In yet a further embodiment, the chemotherapy drug is selected from the following: 5-fluorouracil; Actinomycin D; Alpha interferon; Bleomycin; Cisplatin; Cyclophosphamide; Dexamethasone; Doxorubicin; Epoetin alfa; Etoposide; Gleevec; Herceptin; Interferon alfa; Interleukin-2; Interleukin-11; Methotrexate; Neupogen; Nitrogen Mustard; Paclitaxel; Prednisolone; Prednisone; PROCRIT; Rituximab; Tamoxifen; Thalidomide; Vinblastine; and Vincristine. Additional chemotherapy drugs are envisioned, and are listed in chemocare.com (<http://www.chemocare.com/bio/default.sps>).

In one embodiment of the instant method, the agent is soluble RAGE. In another embodiment, the agent is soluble RAGE G82S. In a further embodiment, the agent is an antibody directed to RAGE. In yet a further embodiment, the agent is an antibody directed to RAGE G82S.

This invention further provides a method for treating a glomerular injury in a subject comprising administering to the subject a therapeutically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof.

In one embodiment of the instant method, the glomerular injury is associated with reduced removal of toxins. In another embodiment, the glomerular injury is associated with glomerulosclerosis. In a further
5 embodiment, the glomerular injury is associated with proteinuria. In yet a further embodiment, the glomerular injury is associated with albuminuria.

In the preferred embodiment of the instant method, the
10 subject is human. In one embodiment, the subject is not afflicted with diabetes. In another embodiment, the subject is receiving or is about to receive a chemotherapy drug. In a further embodiment, the chemotherapy drug is adriamycin. In yet a further
15 embodiment, the chemotherapy drug is selected from the following: 5-fluorouracil; Actinomycin D; Alpha interferon; Bleomycin; Cisplatin; Cyclophosphamide; Dexamethasone; Doxorubicin; Epoetin alfa; Etoposide; Gleevec; Herceptin; Interferon alfa; Interleukin-2;
20 Interleukin-11; Methotrexate; Neupogen; Nitrogen Mustard; Paclitaxel; Prednisolone; Prednisone; PROCIT; Rituximab; Tamoxifen; Thalidomide; Vinblastine; and Vincristine. Additional chemotherapy drugs are envisioned, and are listed in chemocare.com
25 (<http://www.chemocare.com/bio/default.sps>).

In one embodiment of the instant method, the agent is soluble RAGE. In another embodiment, the agent is soluble RAGE G82S. In a further embodiment, the agent
30 is an antibody directed to RAGE. In yet a further embodiment, the agent is an antibody directed to RAGE G82S.

This invention further provides a method for inhibiting the onset of glomerulosclerosis, proteinuria or albumuria in a subject comprising administering to the subject a prophylactically effective amount of an agent
5 that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof.

In the preferred embodiment of the instant method, the subject is human. In one embodiment the subject is
10 afflicted with diabetes. In another embodiment of the instant method, the subject has been afflicted with diabetes for less than 20 years. In a further embodiment, the subject is not afflicted with diabetes. In yet a further embodiment, the subject is receiving
15 or is about to receive a chemotherapy drug. In yet a further embodiment, the chemotherapy drug is adriamycin. In yet a further embodiment, the chemotherapy drug is selected from the following: 5-fluorouracil; Actinomycin D; Alpha interferon;
20 Bleomycin; Cisplatin; Cyclophosphamide; Dexamethasone; Doxorubicin; Epoetin alfa; Etoposide; Gleevec; Herceptin; Interferon alfa; Interleukin-2; Interleukin-11; Methotrexate; Neupogen; Nitrogen Mustard; Paclitaxel; Prednisolone; Prednisone; PROCRIT;
25 Rituximab; Tamoxifen; Thalidomide; Vinblastine; and Vincristine. Additional chemotherapy drugs are envisioned, and are listed in chemocare.com (<http://www.chemocare.com/bio/default.sps>).

30 In one embodiment of the instant method, the agent is soluble RAGE. In another embodiment, the agent is soluble RAGE G82S. In a further embodiment, the agent is an antibody directed to RAGE. In yet a further

embodiment, the agent is an antibody directed to RAGE G82S.

This invention further provides a method for treating
5 glomerulosclerosis, proteinuria or albumuria in a
subject comprising administering to the subject a
therapeutically effective amount of an agent that
inhibits binding between RAGE and/or RAGE G82S and a
ligand thereof.

10

In the preferred embodiment of the instant method, the
subject is human. In one embodiment, the subject is
not afflicted with diabetes. In another embodiment,
the subject is receiving or is about to receive a
15 chemotherapy drug. In a further embodiment, the
chemotherapy drug is adriamycin. In yet a further
embodiment, the chemotherapy drug is selected from the
following: 5-fluorouracil; Actinomycin D; Alpha
interferon; Bleomycin; Cisplatin; Cyclophosphamide;
20 Dexamethasone; Doxorubicin; Epoetin alfa; Etoposide;
Gleevec; Herceptin; Interferon alfa; Interleukin-2;
Interleukin-11; Methotrexate; Neupogen; Nitrogen
Mustard; Paclitaxel; Prednisolone; Prednisone; PROCRIT;
Rituximab; Tamoxifen; Thalidomide; Vinblastine; and
25 Vincristine. Additional chemotherapy drugs are
envisioned, and are listed in chemocare.com
(<http://www.chemocare.com/bio/default.sps>).

In one embodiment of the instant method, the agent is
30 soluble RAGE. In another embodiment, the agent is
soluble RAGE G82S. In a further embodiment, the agent
is an antibody directed to RAGE. In yet a further
embodiment, the agent is an antibody directed to RAGE
G82S.

Determining a therapeutically or prophylactically effective amount of agent can be done based on animal data using routine computational methods. In one
5 embodiment, the therapeutically or prophylactically effective amount contains between about 1ng and about 1g of protein, as applicable. In another embodiment, the effective amount contains between about 10ng and about 100mg of protein, as applicable. In a further
10 embodiment, the effective amount contains between about 100ng and about 10mg of the protein, as applicable. In a yet a further embodiment, the effective amount contains between about 1 μ g and about 1mg of the protein, as applicable. In a yet a further embodiment,
15 the effective amount contains between about 10 μ g and about 100 μ g of the protein, as applicable. In a yet a further embodiment, the effective amount contains between about 100 μ g and about 10mg of the protein, as applicable. In yet a further embodiment, the effective
20 amount of agent, wherein the agent is soluble RAGE, is administered to the subject at a rate from about 2 μ g/kg/hr to about 100 μ g/kg/hr (e.g. about 5, 10, 25, 50 or 75 μ g/kg/hr).

25 In this invention, administering agents can be effected or performed using any of the various methods and delivery systems known to those skilled in the art. The administering can be performed, for example, intravenously, orally, via implant, transmucosally,
30 transdermally, intramuscularly, and subcutaneously. The following delivery systems, which employ a number of routinely used pharmaceutical carriers, are only representative of the many embodiments envisioned for administering the instant compositions.

Injectable drug delivery systems include solutions, suspensions, gels, microspheres and polymeric injectables, and can comprise excipients such as
5 solubility-altering agents (e.g., ethanol, propylene glycol and sucrose) and polymers (e.g., polycaprylactones and PLGA's). Implantable systems include rods and discs, and can contain excipients such as PLGA and polycaprylactone.

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Oral delivery systems include tablets and capsules. These can contain excipients such as binders (e.g., hydroxypropylmethylcellulose, polyvinyl pyrrolidone, other cellulosic materials and starch), diluents (e.g.,
15 lactose and other sugars, starch, dicalcium phosphate and cellulosic materials), disintegrating agents (e.g., starch polymers and cellulosic materials) and lubricating agents (e.g., stearates and talc).

20 Transmucosal delivery systems include patches, tablets, suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty
25 acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

Dermal delivery systems include, for example, aqueous
30 and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids,

fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a
5 transdermal enhancer.

Solutions, suspensions and powders for reconstitutable delivery systems include vehicles such as suspending agents (e.g., gums, zanthans, cellulose and sugars),
10 humectants (e.g., sorbitol), solubilizers (e.g., ethanol, water, PEG and propylene glycol), surfactants (e.g., sodium lauryl sulfate, Spans, Tweens, and cetyl pyridine), preservatives and antioxidants (e.g., parabens, vitamins E and C, and ascorbic acid), anti-
15 caking agents, coating agents, and chelating agents (e.g., EDTA).

In one embodiment of this invention, the delivery system used comprises more than water alone, or more
20 than buffer alone.

This invention further provides an article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE
25 and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for inhibiting the onset of glomerular injury in a subject. This invention further provides an article of manufacture comprising a
30 packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent

for inhibiting the onset of glomerulosclerosis, proteinuria or albuminuria in a subject.

In the preferred embodiment of the instant articles of manufacture, the subject is human. In one embodiment the subject is afflicted with diabetes. In another embodiment of the instant methods, the subject has been afflicted with diabetes for less than 20 years. In a further embodiment, the subject is not afflicted with diabetes. In yet a further embodiment, the subject is receiving or is about to receive a chemotherapy drug. In yet a further embodiment, the chemotherapy drug is adriamycin. In yet a further embodiment, the chemotherapy drug is selected from the following: 5-fluorouracil; Actinomycin D; Alpha interferon; Bleomycin; Cisplatin; Cyclophosphamide; Dexamethasone; Doxorubicin; Epoetin alfa; Etoposide; Gleevec; Herceptin; Interferon alfa; Interleukin-2; Interleukin-11; Methotrexate; Neupogen; Nitrogen Mustard; Paclitaxel; Prednisolone; Prednisone; PROCRIT; Rituximab; Tamoxifen; Thalidomide; Vinblastine; and Vincristine. Additional chemotherapy drugs are envisioned, and are listed in chemocare.com (<http://www.chemocare.com/bio/default.sps>).

In one embodiment of the instant articles of manufacture, the agent is soluble RAGE. In another embodiment, the agent is soluble RAGE G82S. In a further embodiment, the agent is an antibody directed to RAGE. In yet a further embodiment, the agent is an antibody directed to RAGE G82S.

This invention further provides an article of manufacture comprising a packaging material having

therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for treating a glomerular injury in a subject. Finally, this invention provides an article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for treating glomerulosclerosis, proteinuria or albuminuria in a subject.

In the preferred embodiment of the instant articles of manufacture, the subject is human. In one embodiment, the subject is not afflicted with diabetes. In another embodiment, the subject is receiving or is about to receive a chemotherapy drug. In a further embodiment, the chemotherapy drug is adriamycin. In yet a further embodiment, the chemotherapy drug is selected from the following: 5-fluorouracil; Actinomycin D; Alpha interferon; Bleomycin; Cisplatin; Cyclophosphamide; Dexamethasone; Doxorubicin; Epoetin alfa; Etoposide; Gleevec; Herceptin; Interferon alfa; Interleukin-2; Interleukin-11; Methotrexate; Neupogen; Nitrogen Mustard; Paclitaxel; Prednisolone; Prednisone; PROCRIT; Rituximab; Tamoxifen; Thalidomide; Vinblastine; and Vincristine. Additional chemotherapy drugs are envisioned, and are listed in chemocare.com (<http://www.chemocare.com/bio/default.sps>).

In one embodiment of the instant articles of manufacture, the agent is soluble RAGE. In another embodiment, the agent is soluble RAGE G82S. In a

further embodiment, the agent is an antibody directed to RAGE. In yet a further embodiment, the agent is an antibody directed to RAGE G82S.

5 This invention is illustrated in the Experimental Details section which follows. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to, limit in any way the invention set forth in the claims
10 which follow.

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Experimental Details

Methods

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Animal studies

Male BALB/c mice at the age of six weeks received one intravenous dose of adriamycin (ADR), 10.5 mg/kg.

10 Immediately after injection of ADR, mice received once daily administration of murine soluble RAGE, the extracellular ligand binding domain of RAGE, 100 µg per day, beginning immediately at the time of ADR treatment, and continued until the day of sacrifice.

15

Morphologic studies

Dissected kidneys were fixed in buffered formalin (10%) overnight and then routinely processed for light
20 microscopy. Fixed paraffin-embedded tissues were cut (3 µm thick) and mounted on slides coated with 3-aminopropyltriethoxy silane (Sigma) followed by incubation at 37°C overnight. Light microscopic views after staining with periodic acid Schiff (PAS) were
25 scanned into a computer and the quantification of areas of mesangial matrix and glomerulus was performed using a Zeiss microscope and image analysis system (MediaCybernetics). To calculate mesangial area, only nuclei-free regions were included. Forty glomeruli from
30 each animal were selected at random on the stained sections (20 from the outer region and 20 from the inner region). Morphometry was performed by investigators blinded to the experimental protocol.

Functional studies

Twenty-four hour urine collection was obtained from
5 each animal using metabolic cages. Urine albumin and
creatinine were determined using Albuwell M and
creatinine assays from Exocell (Philadelphia, PA)
according to the manufacturer's instructions.

10 *Statistical analysis*

The mean \pm standard error (SE) of the mean is reported.
Statistical significance (defined as $p < 0.05$) was
determined by ANOVA. Where indicated, post-hoc
15 analysis was employed using Dunnett's t-test using
StatView 4.0 (Abacus Concepts, Inc., Berkeley, CA).

Results

20 *RAGE and cellular activation*

It was in the context of roles for inflammatory cells
and podocytes in the pathogenesis of FSGS that a role
for Receptor for AGE (RAGE) was first speculated. RAGE
25 is a multiligand member of the immunoglobulin
superfamily of cell surface molecules (26-27) that
engages distinct molecules; ligand-RAGE interaction
activates cell signalling pathways (such as NF- κ B;
p44/p42, p38 and SAPK/JNK MAP kinases; cdc42/rac; and
30 JAK/STAT, for example) (28-33) that are required for
RAGE-mediated effects. Importantly, deletion of the
cytosolic tail of RAGE imparts a dominant negative
effect in cultured cells and *in vivo*.

RAGE is principally expressed in the podocyte in the glomerulus

The findings have demonstrated that the principal site
5 of RAGE expression in the glomerulus is the podocyte,
at low levels in homeostasis (34); podocyte RAGE
expression is upregulated in human and murine diabetes
(34).

10 To address the concept that RAGE may be involved in the
pathogenesis of ADR-mediated FSGS, a single injection
of ADR, 10.5 mg/kg, to male BALB/c mice at age 6 weeks
was administered. ADR-treated mice received once daily
administration of murine soluble RAGE, the
15 extracellular ligand binding domain of RAGE, 100 µg per
day, beginning immediately at the time of ADR
treatment, and continued until the day of sacrifice.
Other ADR-treated mice received vehicle, PBS. At 2 and
6 weeks after ADR, kidney weight/body weight ratios
20 were significantly decreased in sRAGE-treated vs. PBS-
treated mice. Examination of mesangial area at 2 and
6 weeks after ADR revealed that in a time-dependent
manner, ADR administration was associated with
increased mesangial area, and increased mesangial
25 matrix/glomerular area fraction by PAS staining (Fig.
1&2, respectively). At 2 and 6 weeks, administration of
sRAGE resulted in significantly decreased mesangial
area and mesangial/glomerular area compared with PBS
treatment (Fig. 1&2, respectively).

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The key test of these concepts was the degree to which
blockade of RAGE would suppress the development of
albuminuria. Mice were placed in metabolic cages and
24 hr urine collected. Urine levels of albumin and

creatinine were determined; results are reported as μg albumin/ μg creatinine. At 2 weeks after ADR, PBS-treated mice displayed an ≈ 10 -fold increase in urine albumin/creatinine compared to saline-treated mice not receiving ADR (809.55 ± 365.85 vs. 85.78 ± 17.56 albumin/creatinine; $p < 0.01$) (Fig. 3). In mice receiving ADR and sRAGE, levels of albumin/creatinine were markedly reduced (191.08 ± 49.93 ; $p < 0.05$ vs. PBS-treated mice receiving ADR) (Fig. 3). At six weeks, the results were similarly striking. PBS-treated mice receiving ADR displayed urine albumin/creatinine of $1,362.96 \pm 987.97$ vs 84.47 ± 49.93 in control mice not receiving ADR; $p < 0.01$ (Fig. 3). In the presence of sRAGE, ADR-mediated albuminuria was significantly reduced, to 249.76 ± 283.19 μg albumin/creatinine; $p < 0.01$ vs PBS/ADR (Fig. 3).

Taken together, these findings strongly support the hypothesis that RAGE activation importantly contributes to mechanisms linked to glomerular injury. Administration of soluble RAGE afforded significant protection against the morphologic and functional indices of glomerular injury upon administration of glomerulosclerosis-inducing agents. RAGE blockade is proposed as a new means to prevent glomerular injury in this class of diseases.

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What is claimed is:

1. Use of a prophylactically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof for the manufacture of a medicament for inhibiting the onset of a glomerular injury in a subject.
5
2. Use of a therapeutically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof for the manufacture of a medicament for treating a glomerular injury in a subject.
10
3. Use of a prophylactically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof for the manufacture of a medicament for inhibiting the onset of glomerulosclerosis, proteinuria or albumuria in a subject.
15
4. Use of a therapeutically effective amount of an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof for the manufacture of a medicament to treat glomerulosclerosis, proteinuria or albumuria in a subject.
20
5. An article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for inhibiting the onset of glomerular injury in a subject.
25

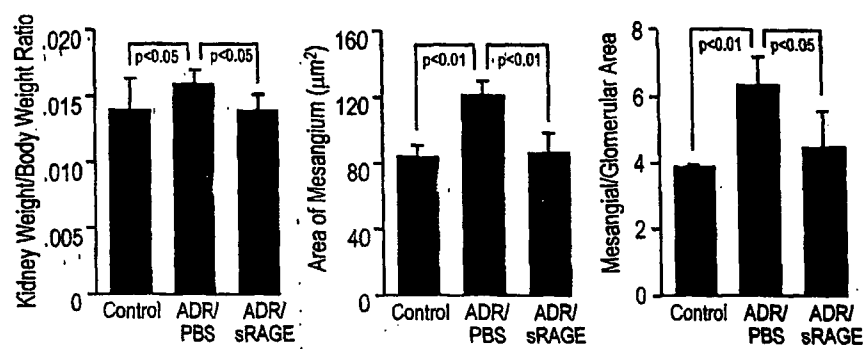
6. An article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for inhibiting the onset of glomerulosclerosis, proteinuria or albuminuria in a subject.
7. An article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for treating a glomerular injury in a subject.
8. An article of manufacture comprising a packaging material having therein an agent that inhibits binding between RAGE and/or RAGE G82S and a ligand thereof, wherein the packaging material has affixed thereto a label indicating a use for the agent for treating glomerulosclerosis, proteinuria or albuminuria in a subject.
9. The use of claim 1 or 2 wherein the glomerular injury is associated with reduced removal of toxins from the subject.
10. The use of claim 1 or 2 wherein the glomerular injury is associated with glomerulosclerosis.
11. The use of claim 1 or 2 wherein the glomerular injury is associated with proteinuria.
12. The use of claim 1 or 2 wherein the glomerular injury is associated with albuminuria.

13. The use of claim 1, 2, 3, or 4 wherein the subject is human.
14. The article of claim 5, 6, 7, or 8 wherein the subject is human.
- 5 15. The use of claim 13 wherein the subject is afflicted with diabetes
16. The article of claim 14 wherein the subject is afflicted with diabetes.
- 10 17. The use of claim 15 as it depends on claim 1 or 3 wherein the subject has been afflicted with diabetes for less than 20 years.
18. The article of claim 16 as it depends on claim 5 or 6 wherein the subject has been afflicted with diabetes for less than 20 years.
- 15 19. The use of claim 13, wherein the subject is not afflicted with diabetes.
20. The article of claim 14, wherein the subject is not afflicted with diabetes.
- 20 21. The use of claim 13, wherein the subject is receiving or is about to receive a chemotherapy drug.
22. The article of claim 14, wherein the subject is receiving or is about to receive a chemotherapy drug.
- 25 23. The use of claim 21 wherein the chemotherapy drug is adriamycin.

24. The article of claim 22, wherein the chemotherapy drug is adriamycin.
25. The use of claim 1, 2, 3, or 4 wherein the agent is soluble RAGE.
- 5 26. The article of claim 5, 6, 7, or 8 wherein the agent is soluble RAGE.
27. The use of claim 1, 2, 3, or 4 wherein the agent is an antibody directed to RAGE.
28. The article of claim 5, 6, 7, or 8 wherein the agent
10 is an antibody directed to RAGE.
29. The use of claim 1, 2, 3, or 4 wherein the agent is an antibody directed to RAGE G82S.
30. The article of claim 5, 6, 7, or 8 wherein the agent is an antibody directed to RAGE G82S.

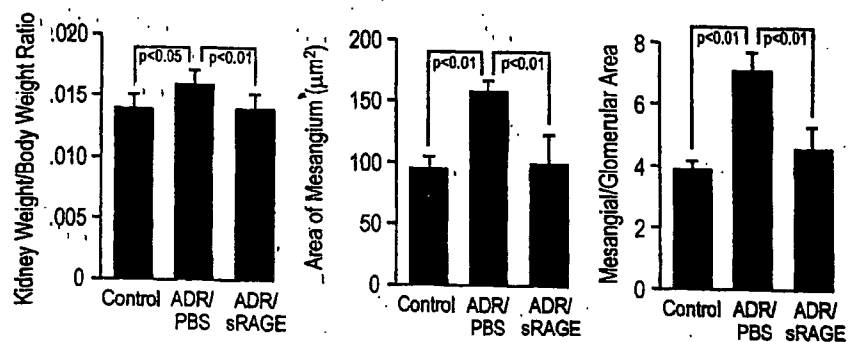
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Figure 1



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Figure 2



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Figure 3.

