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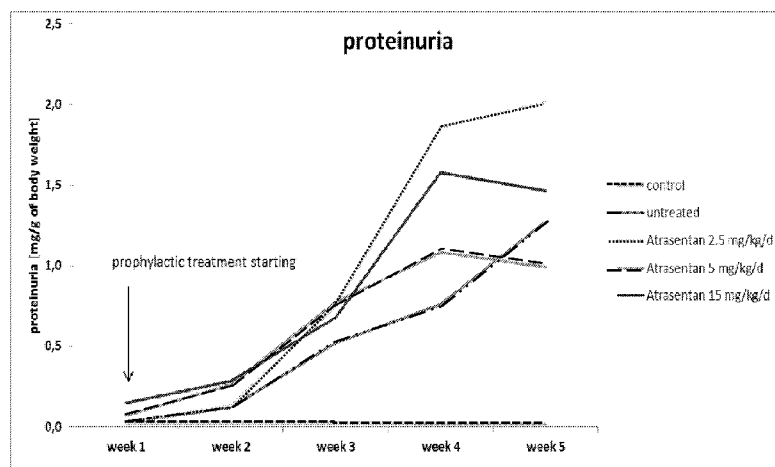
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(54) **Title:** TREATMENT OF MULTIDRUG-RESISTANT NEPHROTIC SYNDROME (MDR-NS) IN CHILDREN

**FIGURE 1A**

(57) **Abstract:** The present disclosure provides compositions and methods for treating multidrug-resistant nephrotic syndrome (MDR-NS) in pediatric subjects comprising atrasentan. Methods of reducing proteinuria in a pediatric patient having MDR-NS are also provided.

## **Treatment of Multidrug-Resistant Nephrotic Syndrome (MDR-NS) in Children**

### **CROSS RELATED APPLICATIONS**

**[0001]** This application claims priority to U.S. Provisional Applications No. 62/193,410 filed July 16, 2015 and No. 62/257,536 filed November 19, 2015, the contents of which are incorporated by reference in their entireties.

### **BACKGROUND OF THE INVENTION**

#### **Kidney Disease in Children**

**[0002]** The high level group term (HLGT) of Nephropathy in the Medical Dictionary for Regulatory Activities (MedDRA) includes the MedDRA Preferred Term (PT): nephrotic syndrome. The reported incidence of childhood nephrotic syndrome (NS) in four studies was 1.15 to 2.3 per 100,000 [ISKDC 1981].

**[0003]** Initial treatment of NS is typically corticosteroids, and the majority of children respond to this treatment with a resolution of proteinuria, indicating complete remission. Overall, 10% to 20% of children with NS will not respond to initial steroid treatment, and other children will develop late resistance. These children will typically be treated with drugs, such as immunosuppressives and RAS inhibitors (ACEi or ARBs). Children who fail to achieve remission with those treatments, or in whom those treatments are judged to be futile due to known genetic or familial variation, are considered to have MDR (multi-drug resistant)-NS. Due to the rarity of this target patient population (MDR-NS), the true incidence and prevalence are currently unknown.

**[0004]** Although the population of patients with MDR-NS is small, no definitive treatments are currently available for them.

**Atrasentan**

**[0005]**     Atrasentan is an endothelin (ET) receptor type A (ETA) antagonist that effectively and selectively inhibits ET binding to the ETA receptor. Atrasentan decreases the binding affinity of ET without affecting the receptor density. The systematic (IUPAC) name for atrasentan is (2*R*,3*R*,4*S*)-4-(1,3-Benzodioxol-5-yl)-1-[2-(dibutylamino)-2-oxoethyl]-2-(4-methoxyphenyl)pyrrolidine-3-carboxylic acid.

**[0006]**     Atrasentan belongs to the "-entan" class of compounds, which block the ETA and/or ETB receptors. ET is a 21-amino acid peptide, which is well known as the most potent endogenous vasoconstrictor. ET is classified into 3 types: ET-1, ET-2, and ET-3 [Kohan 2011]. In kidneys, both ET-1 and ET-3 show widespread tissue distribution. From a physiological perspective, ETs regulate renal blood flow, glomerular filtration, and sodium and water reabsorption. It has been demonstrated that ET-1 mRNA and renal ET-1 clearance are increased in association with proteinuria in kidneys of diabetic rats. ET-1 effects are exerted through two different receptors: ETA receptors localized to vascular smooth muscle cells and fibroblasts and ETB receptors predominantly localized to endothelial cells, and, to a lesser extent, vascular smooth muscle cells. ETB receptors located in the collecting duct of the kidney function to modulate sodium reabsorption and excretion in response to circulating ET-1 levels.

**[0007]**     There is strong evidence that ET-1, a peptide with growth-promoting and vasoconstricting properties, has a central role in the pathogenesis of proteinuria via several different mechanisms (i.e., albuminuria), which is mediated via activation of the ETA receptor. Nonclinical studies have demonstrated that ET receptor antagonists can reverse proteinuric renal disease, and preliminary studies in humans with diabetic as well as nondiabetic renal disease have shown that these drugs have remarkable antiproteinuric effects, which are additive to those

of standard antiproteinuric therapy. The atrasentan relative selectivity for ETA receptor over ETB receptor is greater than 1,800-fold.

**[0008]** Three other ET antagonists, bosentan, macitentan, and ambrisentan, are currently approved by both the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) for the treatment of patients with pulmonary arterial hypertension. These compounds have been shown to be effective in improving exercise capacity and pulmonary symptoms related to interstitial lung scarring.

**[0009]** .

**[0010]** Currently, atrasentan is being developed for treatment of diabetic nephropathy (DN) in the adult patient population. While DN and MDR-NS are both characterized by podocyte dysfunction, DN generally does not exist in the pediatric population because the time course for onset of incipient DN varies from 10 to 15 years after the onset of diabetes.

**[0011]** Needed in the art is an improved method of treating pediatric MDR-NS.

## SUMMARY OF THE INVENTION

**[0012]** In one embodiment, the present invention is the use of atrasentan to treat multidrug-resistant nephrotic syndrome (MDR-NS) in pediatric subjects.

**[0013]** In one embodiment, one would administer to a pediatric subject, preferably 6 months until the age of puberty onset, a sufficient amount of atrasentan or a salt thereof, wherein the administration reduces, treats, improves or ameliorates the symptoms of MDR-NS.

**[0014]** In one embodiment, the present invention will reduce or delay or improve at least one of the following symptoms of nephrotic syndrome to where the symptom is not within the listed levels, which indicate the presence of NS:

- Proteinuria  $> 4 \text{ mg/m}^2/\text{hour}$  (or  $50 \text{ mg/kg/day}$ ) or a urine protein-to-creatinine ratio  $> 2.0 \text{ mg/mg}$

- Hypoalbuminemia  $< 2.5 \text{ g/dL}$
- Presence of edema and hyperlipidemia.

**[0015]** In one embodiment, the treatment is for a period of at least 3 months.

**[0016]** In another embodiment of the invention, the pediatric subject has not responded, or responded poorly, to first-line steroid treatment or second-line drug treatments.

**[0017]** In another embodiment of the invention, the pediatric subject has not responded, or responded poorly, to first-line steroid treatment or is not considered suitable for second-line treatments.

**[0018]** In another embodiment of the invention, the method of administration is selected from the group consisting of oral, rectal, parenteral, intracisternal, intravaginal, intraperitoneal, topical, bucal administration and an oral or nasal spray.

**[0019]** In another embodiment of the invention, the disclosure provides a method of treating MDR-NS, comprising the step of administering to a pediatric subject an effective amount of atrasentan or a salt thereof and an angiotensin-converting-enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARBs), wherein the administration reduces, treats, improves or ameliorates at least one symptom of MDR-NS.

**[0020]** In some embodiments, the administration reduces the level of proteinuria in the patient by at least 10%, and in some embodiments at least 20%. In some embodiments, the method reduces the level of proteinuria below  $4 \text{ mg/m}^2/\text{hour}$  (or  $50 \text{ mg/kg/day}$ ).

## DESCRIPTION OF THE DRAWINGS

**[0021]** The patent or application file contains at least one drawing in color. Copies of this patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

**[0022]** Figure 1A - D are graphs illustrating the effect of atrasentan on proteinuria (Fig. 1A) [mg/g of body weight] and mean arterial blood pressure (Fig. 1C) with prophylactic treatment. Untreated n=17, atrasentan 2.5 mg/kg/d n=3, atrasentan 5 mg/kg/d n=7, atrasentan 15 mg/kg/d n=8. Fig. 1B illustrates the effect of atrasentan on proteinuria with delayed treatment and Fig. 1D is a comparison of relative proteinuria in relation to week 5. Untreated n=21, atrasentan delayed 5 mg/kg/d n=9.

**[0023]** Figure 2 is a graph of mean arterial blood pressure. Healthy n=11, untreated n=20, atrasentan 2.5 mg/kg/d n=3, atrasentan 5 mg/kg/d n=7, atrasentan 15 mg/kg/d n=8, atrasentan delayed 5mg/kg/d n=9.

**[0024]** Figure 3A, 3B, 3C, 3D and 3E are graphs of biochemical parameters (creatinine = Fig 3A, urea = Fig 3B, serum albumin = Fig 3C, cholesterol level = Fig 3D) after atrasentan treatment. Healthy n=8, untreated week 4 n=14, untreated week 8 n=9, atrasentan 2.5 mg/kg/d n=3, atrasentan 5 mg/kg/d n=7, atrasentan 15 mg/kg/d n=8, atrasentan delayed 5 mg/kg/d n=9.

**[0025]** Figure 4 is a graph of endothelin-1 gene expression. Week 2: healthy/untreated n=4/5; week 4: healthy/untreated n=5/5; week 6: healthy/untreated n=4/4; week 8: healthy/untreated n=4/3.

**[0026]** Figure 5 A, B and C are graphs of histopathology measurements. Fig. 5A graphs measurement of tubulointerstitial fibrosis, Fig. 5 B graphs measurement of glomerular sclerosis index, and Fig. 5 C graphs the average number of podocytes per glomerulus.

[0027] Figure 6 is a graph of proteinuria levels over time (mg/g of body weight) for control (n=36-8), untreated (n=55-8), Atrasentan delayed (n=18).

[0028] Figure 7 is a bar graph demonstrating the mean arterial blood pressure for control (n=11), untreated (n=11), Atrasentan wk 1 (n=5), wk2 (n=12), wk3 (n=6), week 4 (n=15), week 5 (n=11), week 6 (n=12), week 7 (n=7), week 8 (n=13), and week 9 (n=10).

[0029] Figure 8 is a set of bar graphs depicting biochemical parameters measured.

[0030] Figures 9A-D are histopathological results. Figure 9A is a set of images of representative immunohistochemical staining. Figure 9B is a graph showing podocyte number per glomerulium. Figure 9C is a graph showing glomerular sclerosis index. Figure 9C is a graph showing tubulointerstitial fibrosis (% effected area).

[0031] Figure 10A-C are bar graphs showing expression levels of Endothelin-1 mRNA (10A), relative ET<sub>A</sub>-R mRNA expression (10B), and relative ET<sub>B</sub>-R mRNA expression.

[0032] Figure 11 depicts Western Blot analysis of mRNA levels of ET-1.

[0033] Figure 12A-C depicts podocin expression in Atrasentan treated animals compared to control and untreated. Figure 12A shows protein expression via Western blot analysis. Figure 12B shows *Nphs2* mRNA expression as determined by qRT-PCR. Figure 12C shows podocin expression as seen by immunofluorescence staining.

#### DESCRIPTION OF THE INVENTION

[0034] The present invention is drawn to the treatment of MDR-NS with atrasentan. The first step of the method is to identify pediatric patients with multidrug-resistant nephrotic syndrome.

[0035] **Diagnosis of MDR-NS and Identification of Patients**

**[0036]** Diagnosis of MDR-NS involves identifying pediatric patients with primary nephrotic syndrome (i.e., not involving treatable systemic disease) who do not respond or respond poorly, to steroid or second line treatments, or are not considered suitable for second line treatments.

**[0037]** Nephrotic syndrome (NS) in children is traditionally classified as primary/idiopathic, which occurs in the absence of an identifiable cause, or secondary, which occurs in the presence of an identifiable causative process. The cardinal feature of nephrotic syndromes is the extensive leakage of plasma proteins into urine. There are five types of primary nephrotic syndromes:

- Congenital nephrotic syndrome (CNS) of the Finnish type (CNF)
- Diffuse mesangial sclerosis
- Minimal change nephrotic syndrome (MCD)
- Focal segmental glomerulosclerosis (FSGS)
- Membranous glomerulopathy

**[0038]** In one embodiment, the pediatric nephrotic syndrome has been defined by:

- Proteinuria  $> 4 \text{ mg/m}^2/\text{hour}$  (or  $50 \text{ mg/kg/day}$ ) or a urine protein-to-creatinine ratio  $> 2.0 \text{ mg/mg}$
- Hypoalbuminemia  $< 2.5 \text{ g/dL}$
- Presence of edema and hyperlipidemia.

**[0039]** Pediatric patients who have been diagnosed with NS will be treated by the present invention if they are multidrug resistant.

**[0040]** There is no known method of curative treatment for MDR-NS. Management of the disease state is directed at treatment to reduce proteinuria and prevent complications caused by NS and/or massive edema. The majority of children with NS will respond to first-line treatment with steroids. However, 10% to 20% of children with NS fail to respond to initial steroid

treatment, and other children develop late resistance. Patients who do not respond to second-line treatments, or in whom second-line treatment is considered futile, are diagnosed with MDR-NS. Second-line treatments are treatments that are administered after a first line treatment does not work. Suitable second line treatments are known in the art and include, but are not limited to, alkylating agents, calcineurin inhibitors, levamisole, mycophenolate mofetil, or rituximab, among others. Second-line treatments are described in Andolino TP, Reid-Adam J. Nephrotic syndrome. *Pediatr Rev* 2015; 36:117-25, incorporated by reference in its entirety.

**[0041]** While not absolute, MDR-NS in many cases is associated with mutations in genes encoding for structural or regulatory proteins of the kidney filtration barrier located in the glomerular capillary wall. Nephrin Gene Mutations (Nephrotic Syndrome Type 1): Congenital nephrotic syndrome of the Finnish type, the most prevalent type of congenital nephrotic syndrome, is a recessively inherited disorder caused by mutations in the nephritic syndrome type 1 (*NPHS1*) gene encoding a major podocyte slit-diaphragm protein, nephrin. The syndrome is characterized by massive proteinuria detectable at birth, marked edema, and histologically characteristic radial dilatations of the proximal tubules. These histologically characteristic lesions are detected more frequently after 3 months of age, although they have also been identified in fetuses. Proteinuria in CNF patients starts *in utero* and can be detected in the first urine sample after birth. Due to the complete absence of nephrin, the resultant phenotype is typically severe with multiple complications and an unstable clinical course. As noted above, although CNF patients are likely to be resistant to steroids and other medical therapies, this patient population is not considered to be the most likely to benefit from atrasentan treatment. This is because of the lack of anticipated clinically meaningful benefit based on the mechanism of action of atrasentan and the underlying pathology.

**[0042]** Mutations in the nephrotic syndrome type 2 (*NPHS2*) gene, encoding for the podocyte protein, podocin, are commonly associated with SRNS in the pediatric population; in addition, these mutations may play an important role in the development of CNS. The *NPHS2* gene mutations were reported to account for half of the CNS cases involving 80 families in Europe, while mutations in the *NPHS1* gene were responsible for only one-third of CNS cases. The *NPHS2* mutations have also been found in the Japanese patients with CNS and those from other regions. These mutations are typically classified as severe, leading to nonfunctional podocin protein, which is often truncated. Because podocin is a podocyte adapter protein involved in proper targeting of nephrin into slit diaphragm, nephrin expression may also be compromised in children with nephrotic syndromes associated with *NPHS2* mutations. *NPHS2* mutations causing renal disorders are highly heterogeneous, but almost all are resistant to corticosteroid therapy. The most common presenting feature of nephrotic syndrome in pediatric patients is persistent proteinuria (> 50 mg/kg/day), usually manifesting in patients younger than 3 years of age.

**[0043]** Because *NPHS2* nephrotic syndrome typically does not respond to immunosuppressive therapy, it is encompassed by the term steroid-resistance nephrotic syndrome (or SRNS) described above. The severity of proteinuria and other clinical findings in patients with *NPHS2* mutations vary to a greater extent than those in patients with *NPHS1* mutations. The renal histology in patients with *NPHS2* mutations typically reveals focal and segmental glomerular sclerosis or minimal-change disease. These patients usually develop ESRD in early childhood, although in some children the decline in renal function is slower and ESRD develops in adolescence. While SRNS by definition does not respond to steroid therapy, patients with SRNS associated with *NPHS2* mutations have been shown to have a lower rate of

recurrence of disease after renal transplantation. In a mutational analysis of 8 *NPHS2* exons in 190 patients from 165 families with SRNS and 124 patients from 120 families with SSNS, homozygous or compound heterozygous mutations of *NPHS2* were observed in approximately one-quarter of families with SRNS compared with none of the families with SSNS.

[0044] Several research groups have shown that the patients with SRNS who have multiple mutations have a younger age at disease onset than those who have no mutations. In one study of 319 patients with SRNS, the mean age at disease onset of the patients without 2 *NPHS2* mutations was 108.3 months (9.0 years) in those with sporadic SRNS and 69.9 months (5.8 years) in those with autosomal recessive SRNS compared with a mean age at onset of 41.2 months (3.4 years) for those with 2 *NPHS2* mutations ( $P < 0.001$  and  $P = 0.019$ , respectively) [Weber 2004]. In another study of 430 patients with SRNS of whom 267 had no mutation and 78 had multiple mutations, the mean age at onset was 6.4 years for those with no mutation and 2.61 years for those with multiple mutations ( $P < 0.0001$ ).

[0045] Based on available epidemiology data, it is expected that a significant number of the intended target patient population may have mutations in the *NPHS2* gene.

[0046] Mutations in Wilms' tumor suppressor gene (WT1), which plays a key role in the embryonic development of the kidney and genitalia, account for a small percentage of CNS cases. Patients with WT1 mutations may experience moderate proteinuria, while kidney biopsy typically shows diffuse mesangial sclerosis of glomeruli. Mutations in the laminin-beta 2 gene playing a crucial role in the network structure and anchoring of the glomerular basement membrane to podocyte foot processes are also linked to development of CNS.

[0047] Another genetic disorder associated with CNS is Galloway-Mowat syndrome, characterized by nephropathy accompanied by central nervous system anomalies, including

microcephaly, psychomotor retardation, and brain abnormalities. In addition to Galloway-Mowat syndrome, other unique combinations of CNS and extrarenal defects are reported, including mitochondrial cytopathy, nail-patella syndrome, congenital disorder of glycosylation type I, Herlitz junctional epidermolysis bullosa, and mutations in the phospholipase C epsilon gene.

**[0048]** One skilled in the art would understand that the diagnosis of MDR-NS is purely clinical, so patients eligible for the treatment of the present invention are not limited to any of the above-mentioned mutations. In one embodiment, patients for the present invention have at least one mutation in any of these genes. In one embodiment, patients for the present do not have any of the mutations.

**[0049]** Nephrotic syndrome may be the result of other pathologies and may not typically be as suitable for atrasentan treatment, although we envision that in some cases, atrasentan treatment will be useful. Some infections may also cause congenital and infantile nephrotic syndromes, especially in developing countries. These include syphilis, toxoplasmosis, congenital rubella, hepatitis B, cytomegalovirus, and human immunodeficiency virus. First-line therapy for CNS arising from infections is aimed at the infectious organism. For this reason, nephrotic syndromes arising from infectious etiologies will not typically be treated with atrasentan before resolution of the underlying pathology. Other secondary forms of CNS include maternal systemic lupus erythematosus and neonatal alloimmunization against neutral endopeptidase present on podocytes. Treatment of these disease states is directed at the primary disease process, making patients with these conditions typically treated with other drugs before atrasentan treatment.

**[0050]** Pediatric patients with MDR-NS, while having symptom similarities with DN, have a much shorter time course for onset as compared to adults, as the onset of incipient DN varies from 10 to 15 years after the onset of diabetes in adult populations. Thus, pediatric patients with MDR-NS have a different course of symptoms and disease progression than adult patients.

**[0051] Treatment of the present invention**

**[0052]** In one embodiment of the invention, a pediatric patient will receive atrasentan treatment, typically via administration as described in other atrasentan treatment literature or as described below. The treatment is expected to reduce or delay one or more MDR-NS symptoms.

**[0053]** The typical target pediatric patient population for the method of the present invention is MDR-NS patients between 6 months and the onset of puberty who present with persistent or refractory nephrotic syndrome that has failed to respond to steroids and other appropriate therapeutic alternatives, although patients in a wider variety of ages will benefit. This population also encompasses patients with SRNS.

**[0054]** The onset of puberty varies among individuals; however, puberty usually occurs in girls between the ages of 10 and 14, while in boys it generally occurs later, between the ages of 12 and 16. For a particular patient, the timing of his or her onset of puberty can be determined by one skilled in the art using a number of factors known in the art.

**[0055]** In one embodiment, the target pediatric patient population of the method of the present invention is MDR-NS patients 6 months to the onset of puberty, or to 16 years of age, or to 14 years of age, or to 12 years of age, or to 10 years of age, or preferably to 8 years of age. In one specific embodiment, the target pediatric patient for the method of the present invention is between 6 months to 8 years of age.

**[0056]** In one embodiment, a pediatric patient for the treatment of the present invention is 6 months to 16 years of age. In one embodiment, a pediatric patient for the treatment of the present invention is 6 months to 14 years of age. In one embodiment, a pediatric patient for the treatment of the present invention is 6 months to 12 years of age. In one embodiment, a pediatric patient for the treatment of the present invention is 6 months to 10 years of age. In one embodiment, a pediatric patient for the treatment of the present invention is 6 months to 8 years of age.

**[0057]** The treatment regimen of the present invention would typically occur as add-on therapy in patients who have failed first-line or other conventional therapy. In one embodiment of the invention, the pediatric subject has not responded, or responded poorly, to first-line steroid treatment or second-line drug treatments. In one embodiment of the invention, the pediatric subject has not responded, or responded poorly, to first-line steroid treatment or is not considered suitable for second-line treatments.

**[0058]** In one embodiment, the treatment is for a period of at least 3 months.

**[0059]** In one embodiment, the treatment is for a period of at least 6 months.

**[0060]** Albuminuria/proteinuria assessment, preferably by at least 6 months post treatment initiation, is a typical symptom guideline for evaluation of clinical response of the treatment of the present invention. In one embodiment, a treatment will modify the patient's albuminuria/proteinuria profile by at least 10%, in some embodiments by at least 20%, and in some further embodiments by at least 30%. In another embodiment, the modification will be so that symptom levels are below those listed above as indicative of renal disease. For example, the patient's proteinuria measurement will be less than 4 mg/m<sup>2</sup>/hour (or 50 mg/kg/day) or a urine protein-to-creatinine ratio less than 2.0 mg/mg or the patient's hypoalbuminemia will be greater than 2.5

g/dL. In some embodiments, the reduction or delay of one or more symptoms of MDR-NS is demonstrated by a patient having hypoalbuminemia by the increase in the level of albumin in the blood. The level of albumin in the patient may be increased by at least 10%, alternatively at least 20%, alternatively at least 30%. In some embodiments, the reduction or delay of one or more symptoms of MSD-NS is the reduction of edema and/or hyperlipidemia in the patient. Not to be bound by any theory, patients improved in the disease state will have a reduced protein loss, which in turn may improve serum albumin levels.

**[0061]** In some embodiments of the invention, the treatment of a pediatric patient with atrasentan in an effective amount to delay the onset or progression of end-stage renal disease (ESRD) .

**[0062]** In some embodiments, the reduction or delay of one or more symptoms of MSD-NS is demonstrated by the reduction of glomerular hyperfiltration or reducing in tubulointerstitial inflammation.

**[0063]** Our results show that the atrasentan treatment is most successful after the disease state has been established. In one embodiment of the invention, one would evaluate the patient and determine that MDR-NS is fully established. In one embodiment, persistent proteinuria is an indication that MDR-NS is established. In some instances, one of methods that can be used to assess the establishment of MDR-NS is to measure development of FSGS related to the up-regulation of endothelin.

**[0064]** In some embodiments, the treatment with atrasentan will be combined with one or more second-line drug therapy. Suitable second-line drug therapy include, but are not limited to, for example, immunosuppressives and Renin-angiotensin system (RAS) inhibitors (ACEi or ARBs). Immunosuppressives include, but are not limited to, for example, corticosteroids (e.g.,

prednisone, dexamethasone, etc.), calcineurin inhibitor (e.g., cyclosporine, tacrolimus), alkylating agent (e.g., cyclophosphamide, chlorambucil, etc.) mycophenolate mofetil (MMF, T and B-cell proliferation inhibitor), rituximab (monoclonal antibody specific to CD20 found on B cells) and the like.

**[0065]** Suitable RAS inhibitors include, but are not limited to, for example, angiotensin-converting-enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARBs). Suitable ACEi therapies include, but are not limited to, for example, alacepril, benzapril, captopril, ceronapril, cilazapril, delapril, enalapril, enalaprilat, eosinopril, fosinopril, imidapril, lisinopril, moexipril, moveltipril, omapatrilat, perindopril, quinapril, ramipril, sampatrilat, spirapril, temocapril, trandolapril, and combinations thereof, among others. Suitable ARBs include, but are not limited to, for example, candesartan, eprosartan, irbesartan, losartan, olmesartan, tasosartan, telmisartan, valsartan or combinations thereof, among others.

**[0066]** In one embodiment, the present treatment comprises treating with Atrasentan and an ACEi inhibitor in an effective amount to reduce at least one symptom of MDR-NS. In some embodiments, the combination has a synergistic effect, e.g., provides an increased reduction of at least one symptom as compared to either drug alone. In one embodiment, the treatment comprises administering an effective amount of Atrasentan and Candesartan.

### **Pharmaceutical Compositions**

**[0067]** When employed as a pharmaceutical, a compound of the invention is typically administered in the form of an oral pharmaceutical composition. Other suitable forms of administration are discussed below. Atrasentan, solvates thereof, crystalline forms thereof, salts thereof, or any other suitable clinical dose forms thereof, can be made by synthetic chemical processes by one skilled in the art, for example, as described in US Patent Applications

09/714934, 10/266270, 11/063476, 12/037510, 08/458094, 08/457215, 08/457063, 08/457935, 08/457331, 08/457414, 08/457418, 09/572493, 11/502798, 08/293349, 08/334717, 08/442575, 08/497998, 08/600625, 08/794506, 08/905913, 09/048955, 09/634661, 09/653563, 11/229892, 11230043, 11/229894, 11/229922, 14/324603, 14/133297 and 14/594742.

**[0068]** Suitable compositions can be prepared in a manner well known in the pharmaceutical art and comprise a therapeutically effective amount of atrasentan or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier. The phrase “pharmaceutical composition” refers to a composition suitable for administration in medical or veterinary use.

**[0069]** The pharmaceutical compositions that comprise atrasentan, alone or in combination with further therapeutically active ingredient, may be administered to the subjects orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments or drops), buccally or as an oral or nasal spray. The term “parenterally” as used herein, refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

**[0070]** The term “pharmaceutically acceptable carrier” as used herein, means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which may serve as pharmaceutically acceptable carriers are sugars such as, but not limited to, lactose, glucose and sucrose; starches such as, but not limited to, corn starch and potato starch; cellulose and its derivatives such as, but not limited to, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as, but not limited to, cocoa butter and suppository waxes; oils such as, but not limited to, peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil

and soybean oil; glycols; such a propylene glycol; esters such as, but not limited to, ethyl oleate and ethyl laurate; agar; buffering agents such as, but not limited to, magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as, but not limited to, sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants may also be present in the composition, according to the judgment of the formulator.

**[0071]** Pharmaceutical compositions for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous diluents, solvents, or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), vegetable oils (such as olive oil), injectable organic esters (such as ethyl oleate), and suitable mixtures thereof. Proper fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

**[0072]** These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable

pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

**[0073]** In some cases, in order to prolong the effect of the drug, it may be desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form may be accomplished by dissolving or suspending the drug in an oil vehicle.

**[0074]** Injectable depot forms may be made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release may be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

**[0075]** The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

**[0076]** Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In certain embodiments, solid dosage forms may contain from 1% to 95% (w/w) of a compound of atrasentan. In certain embodiments, atrasentan or pharmaceutically acceptable salts thereof, may be present in the solid dosage form in a range of from 5% to 70% (w/w). In

such solid dosage forms, the active compound may be mixed with at least one inert, pharmaceutically acceptable carrier, such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

**[0077]** The pharmaceutical composition may be a unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampules. Also, the unit dosage form may be a capsule, tablet, cachet, or lozenge itself, or it may be the appropriate number of any of these in packaged form. The quantity of active component in a unit dose preparation may be varied or adjusted from 0.1 mg to 1000 mg, from 1 mg to 100 mg, or from 1% to 95% (w/w) of a unit dose, according to the particular application and the potency of the active component. The composition may, if desired, also contain other compatible therapeutic agents.

**[0078]** The dose to be administered to a subject may be determined by the efficacy of the particular compound employed and the condition of the subject, as well as the body weight or

surface area of the subject to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound in a particular subject. In determining the effective amount of the compound to be administered in the treatment or prophylaxis of the disorder being treated, the physician may evaluate factors such as the circulating plasma levels of the compound, compound toxicities, and/or the progression of the disease, etc.

**[0079]** For administration, compounds may be administered at a rate determined by factors that may include, but are not limited to, the LD<sub>50</sub> of the compound, the pharmacokinetic profile of the compound, contraindicated drugs, and the side-effects of the compound at various concentrations, as applied to the mass and overall health of the subject. Administration may be accomplished via single or divided doses.

**[0080]** The compounds utilized in the pharmaceutical method of the invention may be administered at the initial dosage of about 0.001 mg/kg to about 100 mg/kg daily. In certain embodiments, the daily dose range is from about 0.1 mg/kg to about 10 mg/kg. In certain embodiments, the daily dose range is from about 0.1 mg/kg to about 5 mg/kg. Suitable dosages may contain any amounts in-between, for example, but not limited to, about 0.1mg/kg, about 0.25mg/kg, about 0.5 mg/kg, about 0.75mg/kg, about 1.0 mg/kg, about 1.25 mg/kg, about 1.5 mg/kg, about 1.75 mg/kg, about 2 mg/kg, about 5 mg/kg, about 10mg/kg and so forth. For example, suitable dosages may comprise, 0.25mg, 0.5 mg, 0.75 mg, 1.25 mg and 1.75 mg. The dosages, however, may be varied depending upon the requirements of the subject, the severity of the condition being treated, and the compound being employed. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Treatment may be initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the

dosage is increased by small increments until the optimum effect under circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

**[0081]** Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such carriers as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

**[0082]** The solid dosage forms of tablets, dragees, capsules, pills and granules can be prepared with coatings and shells such as enteric coatings and other coatings well-known in the pharmaceutical formulating art. They may optionally contain opacifying agents and may also be of a composition such that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

**[0083]** The active compounds may also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned carriers.

**[0084]** Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan and mixtures thereof.

**[0085]** Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

**[0086]** Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth and mixtures thereof.

**[0087]** Compositions for rectal or vaginal administration are preferably suppositories which may be prepared by mixing the compounds with suitable non-irritating carriers or carriers such as cocoa butter, polyethylene glycol, or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

**[0088]** Compounds may also be administered in the form of liposomes. Liposomes generally may be derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals which are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes may be used. The present compositions in liposome form may contain, in addition to a compound of the invention, stabilizers, preservatives, excipients, and the like. Examples of lipids include, but are not limited to, natural and synthetic phospholipids, and phosphatidyl cholines (lecithins), used separately or together.

**[0089]** Methods to form liposomes have been described, see example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

**[0090]** Dosage forms for topical administration of a compound described herein include powders, sprays, ointments, and inhalants. The active compound may be mixed under sterile

conditions with a pharmaceutically acceptable carrier and any needed preservatives, buffers or propellants which may be required. Ophthalmic formulations, eye ointments, powders and solutions are also contemplated as being within the scope of this invention.

[0091] A compound of the invention may also be administered in sustained release forms or from sustained release drug delivery systems.

[0092] In one version of the present invention, one would combine the atrasentan treatment with a dose of at least one of an angiotensin converting enzyme inhibitor or an angiotensin II receptor blocker.

## EXAMPLES

### Example 1

[0093] We have analyzed results from studies in a transgenic mouse model where the mice were treated with atrasentan. Using the Cre recombinase technology, we generated a novel inducible mouse model of podocin-related nephrotic syndrome. Cross-breeding of animals carrying one floxed podocin construct, one podocin allele with the R140Q mutation, and tamoxifen-sensitive inducible Cre enabled us to induce hemizygoty for the R140Q mutant. In this work, we were able to examine the human missense mutation R138Q of *NPHS2* gene identified in pediatric patients with nephrotic syndrome *in vivo* in an inducible mouse model of R140Q mutation (murine analogue to the human mutation). The induced animals with *Nphs2*<sup>R140Q/-</sup> genotype developed proteinuria within the first week after the induction with tamoxifen, which reached its maximum at week 4 and 5. Podocytes injury in our model resulted in progressive renal disease, demonstrating phenotype of nephrotic syndrome in human, such as proteinuria, hyperlipidemia, hypertension and kidney failure. *Nphs2*<sup>R140Q/-</sup> mice displayed clear

difference in plasma level of cholesterol and albumin already two weeks after the induction. Furthermore, from the end of the week four of observation the values of plasma urea and creatinine started to increase slightly and the creatinine clearance appeared to reduce, pointing to the beginning of renal failure.

[0094] Seven animals were treated with 5 mg/kg/d atrasentan and another three animals were treated with the lower dose of 2.5 mg/kg/d atrasentan via food intake for four weeks. Furthermore, we started a new group of 8 animals with a higher dose treatment of 15 mg/kg/d atrasentan.

[0095] We found that there is a late upregulation of endothelin-1 mRNA expression (Fig. 4) in the course of disease, raising the possibility that atrasentan is only effective when FSGS (focal segmental glomerulosclerosis) is fully established. Therefore, we started a therapeutic treatment of 5 mg/kg/d with a four-week delay from when animals were shown to establish proteinuria (n=9).

[0096] Proteinuria (Fig. 1A, Fig. 1B, and Fig. 1D), weight gain and blood pressure (Fig. 1C) were monitored once weekly. All animals were sacrificed at the end of the observation period at week 5 (or week 9 with delayed treatment) and the biochemical parameters (Fig. 3A-E) were determined. All results were compared to two control groups of healthy and sick untreated animals.

[0097] Referring to Fig. 1A-D, no beneficial effect in attenuating proteinuria was seen in animals with immediate or prophylactic treatment of atrasentan, regardless of the dose administered.

[0098] Referring to Fig. 1C, animals treated with 2.5 mg/kg/d and 5 mg/kg/d atrasentan demonstrated a higher blood pressure comparing to untreated and healthy animals. Animals

treated with the higher dose of 15 mg/kg/d display a lower blood pressure compared to untreated animals.

**[00099]** Referring to Fig. 1B and D, when we started atrasentan 5 mg/kg/d four weeks after induction, when proteinuria was fully established, an effective attenuation of proteinuria was observed. The experiment reported in Fig. 1B records n=21 untreated and n=9 atrasentan treated animals (delayed 5 mg/kg/d).

**[00100]** Referring to Fig. 2, delayed atrasentan treatment also lowered blood pressure compared to untreated animals.

**[00101]** Figs. 3A-E record biochemical parameters of the treated animals.

**[00102]** Fig. 4 indicates that real-time rtPCR showed a late upregulation of endothelin-1 expression in the course of disease, reaching significance only by week 4.

**[00103]** Fig. 5A shows that tubulointerstitial fibrosis determined by Sirius Red staining. Results demonstrate a reduced area affected by fibrosis in treated animals with dose of 2.5 and 5 mg/kg/d Atrasentan ( $*p < 0.05$  compared to untreated group). Animals treated with 15 mg/kg/d showed an increase of tubulointerstitial fibrosis.

**[00104]** Fig. 5B demonstrates sclerosis within the glomerular tuft examined by PAS staining of 3µm paraffin sections. Quantification was done with Image ProPremier (glomerular sclerosis index – GSI, 50 glomeruli/animal). No improvement was seen in GSI in all treatment groups.

**[00105]** Fig. 5C tabulates podocyte numbers per glomerulus WT1-via immunostaining of glomerular cross-sections (4 µm and 10 µm). Quantification was done with ImageJ according to Animal Models of Diabetic Complications Consortium protocol. 50 glomeruli/animal. We found no effect on the number of podocytes per glomerulus in treated animals.

[00106] In summary, we saw no antiproteinuric effect of atrasentan in the first 4 weeks after induction of the podocin defect, regardless of the dose applied – much different from ACE/ARBs, which are strongly antiproteinuric.

[00107] We then checked ET1 expression in the kidney over time and found that the ET1 system is gradually up-regulated only after a few weeks of active disease.

[00108] We then proceeded to a second series, starting atrasentan at 4 weeks when FSGS is fully established and ET1 expression is up. (See Fig. 1B) With this design, we were excited to see an antiproteinuric effect attributable to atrasentan treatment.

[00109] Example 2

[00110] **Delayed treatment**

[00111] Using the same mouse model as Example 1, we found that there is a late upregulation of endothelin-1 mRNA expression (figure 4) in the course of disease, raising the possibility that Atrasentan is only effective when FSGS is fully established. Therefore, we started the treatment after 4 weeks of disease state (5 mg/kg/d) when animals are showing high proteinuria, performing 4-weeks treatment duration (n=14) and 8 weeks treatment duration (n=4), respectively.

[00112] Proteinuria (Fig. 6), weight gain and blood pressure (Fig. 7) were monitored at least every 2 weeks. All animals were sacrificed at the end of the observation period and biochemical parameters (Fig. 8) as well as histological changes (Fig. 9) were determined consequently. All results were compared to control groups of healthy and sick untreated animals.

[00113] Fig. 6 demonstrates that after drug administration, proteinuria reaches a peak and declines afterwards progressively. Fig. 7 demonstrates that animals treated with four-week delay

with doses of 5 mg/kg/d Atrasentan showed a significant decrease of blood pressure compared to untreated animals at week 5 and 7 ( $***p=0.001$ ,  $*p=0.047$ )

**[00114]** Fig. 8 shows results of biochemical parameters with control (healthy mice,  $n=8$ ), untreated week 8 ( $n=9$ ), Atrasentan delayed 4 weeks ( $n=14$ ), untreated week 12 ( $n=6$ ) and Atrasentan delayed treatment 8 weeks ( $n=3$ ) (Atrasentan delayed are mice who begin Atrasentan treatment after four weeks of disease state). Fig. 9A are exemplary images of healthy, untreated and Atrasentan treated kidney samples. Fig. 9B-D shows the results from histological examination from 9 out of 14 delayed treated animals (4 weeks of treatment). Fig. 9B shows podocyte number per glomerium, Fig. 9C shows glomerular sclerosis index per animal, and Fig. 9D shows tubulointerstitial fibrosis (% affected area). Histopathological examinations demonstrated that glomerulosclerosis and tubulointerstitial fibrosis are mitigated in the Atrasentan treated animals as compared to sick untreated animals of the same age. No notable effects were seen on podocyte population.

**[00115]** Real time investigations of the expression of Endothelin-1,  $ET_A$ R and  $ET_B$ R by mRNA expression were measured at week 1, 2, 4, 6, and 8 weeks in untreated mice (Fig. 10) revealed that there is a late upregulation of endothelin-1,  $ET_A$ R and  $ET_B$ R in the course of disease, reaching significant from week 5 (ET-1). In Atrasentan treated animals, lower expression of endothelin-1,  $ET_A$ R and  $ET_B$ R was observed, suggesting a negative cellular feedback.

**[00116]** Western blot analysis was performed to confirm the observation of increased mRNA level in the late course of disease (Fig. 11). Atrasentan treated animals displayed decreased protein expression of endothelin-1. Specifically, Atrasentan was administered at the time of maximal proteinuria and strong ET-1 expression, proteinuria decreased progressively and MAP

was lowered significantly (wk7: 80.4 (D) v 99.5 (U) mmHg,  $p=0.001$ ). Histological evaluation (n=9) demonstrates attenuation in glomerulosclerosis (GSI: 1.69 (D) v. 2.27 (U wk 8),  $p=0.0008$ ) and tubulointerstitial fibrosis (TIF & of total area 4.18 (D) v. 7.56 (U wk 8),  $p=0.008$ ; podocyte numbers tend to be better preserved (podocytes per glom: 54% (D) vs. 32% (U wk 8) of healthy controls, n.s.). Of note, three of the nine animals displayed endothelin-1 expression, suggesting that there may be some individuals Atrasentan may be less effective in, and we observed that these mice had higher levels of proteinuria compared to others from this group (results not shown).

**[00117]** Further podocin expression analysis was carried out. Podocin protein abundance and mRNA expression was partly preserved in the treated animals. Podocin expression analysis included protein expression analysis by Western blot (Fig. 12A), mRNA expression assessed by qRT-PCR (Fig. 12B) and histological staining using immunofluorescence (Fig. 12C), demonstrating podocin expression was partly preserved in Atrasentan treated animals.

**[00118]** In order to investigate the course of proteinuria and the impact on survival we are filling up the group with treatment period of 8 weeks and are keeping animals for open end observation (max. 35 weeks).

**[00119]** Combinational Studies

**[00120]** In our previous studies, we found out, that RAS blockade may provide effective nephroprotection in hereditary nephropathy, delays renal failure and prolongs survival. We are performing a combined treatment with ERA and ARB (Atrasentan+Cansesartan). Not to be bound by any theory, but we believe that the combination of atrasentan and an (ACEi) or (ARBs) will provide a synergistic effect, e.g., they will provide a reduction in at least one symptom of MDS-NS greater than either treatment alone.

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## CLAIMS

We claim

1. A method of treating MDR-NS comprising the step of administering to a pediatric subject a sufficient amount of atrasentan or a salt thereof, wherein the administration reduces, treats, improves or ameliorates one or more symptoms of MDR-NS.
2. The method of claim 1, wherein the treatment is for a period of at least 3 months.
3. The method of claim 1, wherein the treatment is for a period of at least 6 months.
4. The method of any one of claims 1-3, wherein the treatment is combined with a dose of at least one of an angiotensin converting enzyme inhibitor or an angiotensin II receptor blocker.
5. The method of any one of claims 1-4, wherein the pediatric subject is 6 months to onset of puberty of the subject.
6. The method of claim 5, wherein the pediatric subject is 6 months to 16 years of age.
7. The method of claim 5, wherein the pediatric subject is 6 months to 14 years of age.
8. The method of claim 5, wherein the pediatric subject is 6 months to 12 years of age.
9. The method of claim 5, wherein the pediatric subject is 6 months to 10 years of age.

10. The method of claim 5, wherein the pediatric subject is 6 months to 8 years of age..

11. The method of any one of claims 1-10, wherein the pediatric subject does not respond, or responds poorly, to first-line steroid treatment or second-line drug treatments.

12. The method of any one of claims 1-10, wherein the pediatric subject does not respond, or responds poorly, to first-line steroid treatment or is not considered suitable for second-line drug treatment.

13. The method of any one of claims 1-10, wherein the method of administration is selected from the group consisting of oral, rectal, parenteral, intracisternal, intravaginal, intraperitoneal, topical, bucal administration and an oral or nasal spray.

14. The method of any one of claims 1-10, wherein a dosage is between about 0.001 mg/kg and about 100 mg/kg daily.

15. The method of claim 14, wherein the dosage is between 0.001 mg/kg and 10 mg/kg daily.

16. The method of claim 15, wherein the dosage is between 0.01 mg/kg and 5 mg/kg daily.

17. The method of any one of the preceding claims, wherein the treating of the pediatric patient in an effective amount to delay the onset or progression of end-stage renal disease (ESRD) .

18. The method of any one of the preceding claims, wherein reduction or delay of one or more symptoms of MSD-NS reduces glomerular hyperfiltration or reduces tubulointerstitial inflammation.
19. A method of treating MDR-NS comprising the step of administering to a pediatric subject an effective amount of atrasentan or a salt thereof and an angiotensin-converting-enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARBs), wherein the administration reduces, treats, improves or ameliorates at least one symptom of MDR-NS.
20. The method of claim 19, wherein the ARB is candesartan, eprosartan, irbesartan, losartan, olmesartan, tasosartan, telmisartan, valsartan or combinations thereof.
21. The method of claim 19, wherein the ACEi is alacepril, benzapril, captopril, ceronapril, cilazapril, delapril, enalapril, enalaprilat, eosinopril, fosinopril, imidapril, lisinopril, moexipril, moveltipril, omapatrilat, perindopril, quinapril, ramipril, sampatrilat, spirapril, temocapril, trandolapril, or combinations thereof.
22. The method of claim 19, wherein the atrasentan or a salt thereof and the angiotensin-converting-enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARBs) have a synergistic effect in reducing, treating, improving or ameliorating at least one symptom of MDR-NS.

23. A method of reducing proteinuria in a pediatric subject suffering from MDR-NS, comprising the step of administering to the pediatric subject an effective amount of atrasentan or a salt thereof, wherein the administration reduces the level of proteinuria in the pediatric subject.

24. The method of claim 23, wherein the administration reduces the level of proteinuria in the patient by at least 10%.

25. The method of claim 24, wherein the administration reduces the level of proteinuria in the patient by at least 20%.

26. The method of any one of claims 23-25, wherein the administration reduces the level of proteinuria below  $4 \text{ mg/m}^2/\text{hour}$  (or  $50 \text{ mg/kg/day}$ ).

27. The method of any one of claims 23-25, wherein proteinuria is measured by calculating a urine protein-to-creatinine, and wherein the urine protein-to-creatinine level is reduced at least 10%.

28. The method of claim 27, wherein the urine protein-to-creatinine ratio is less than  $2.0 \text{ mg/mg}$ .

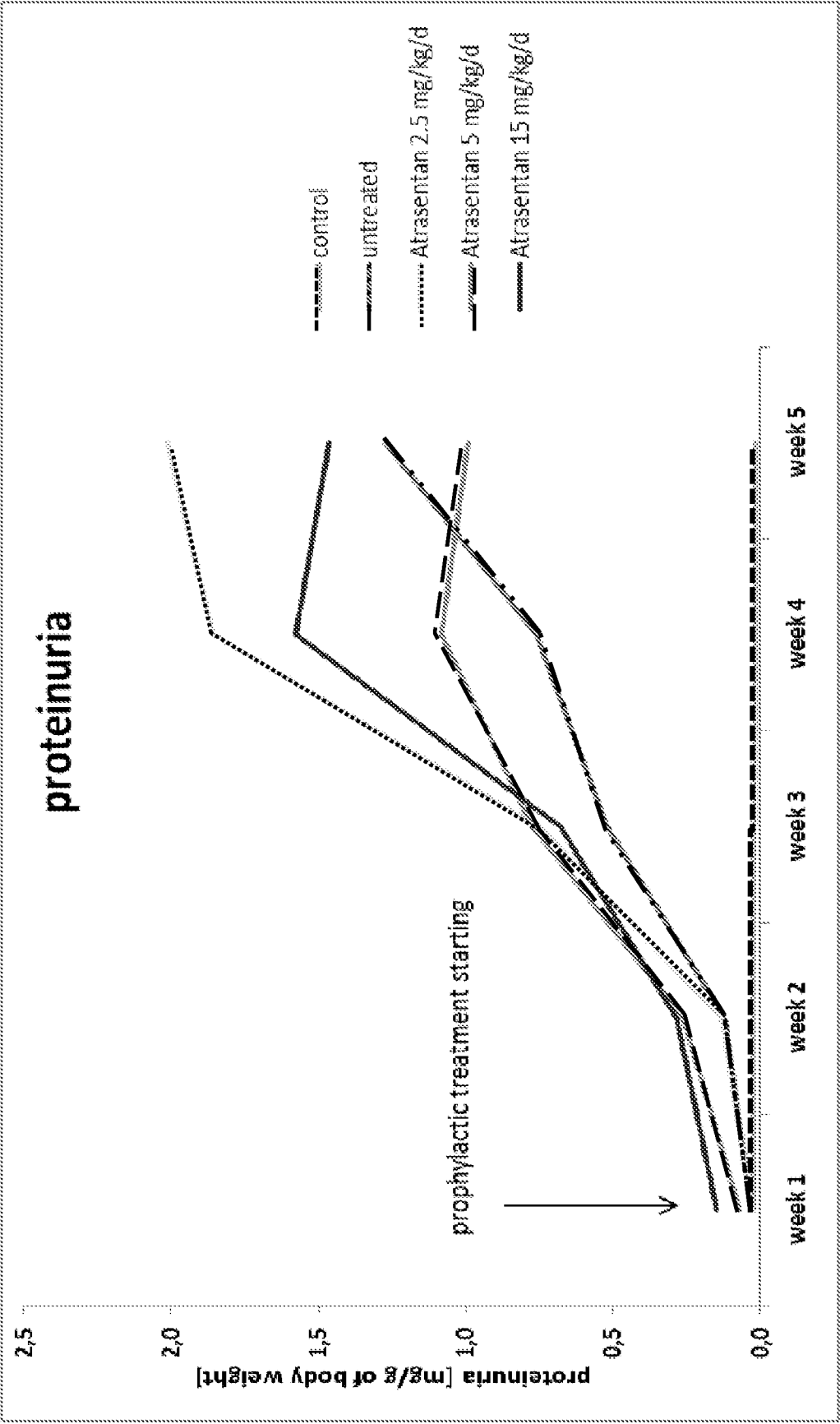
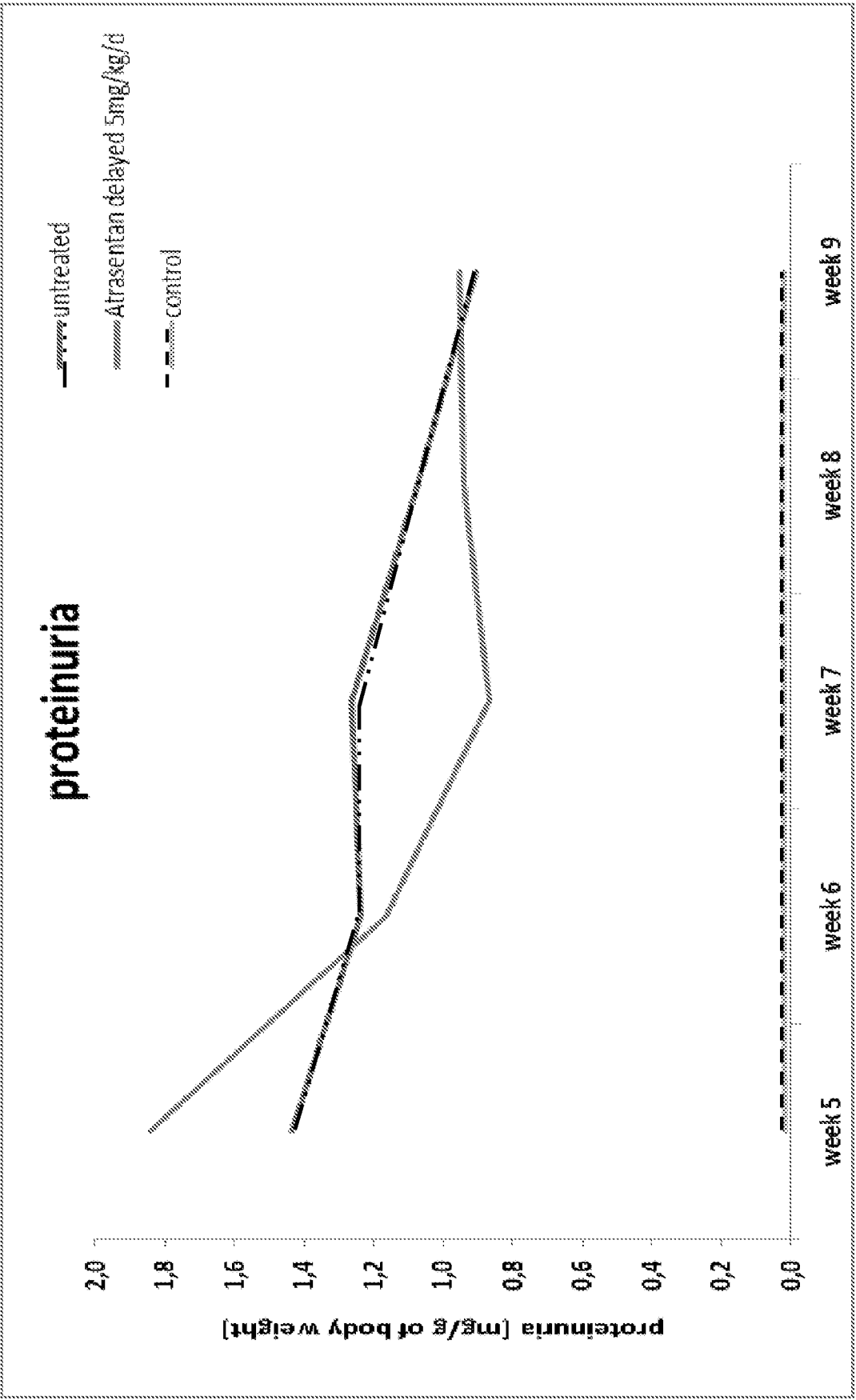


FIGURE 1A



**FIGURE 1B**

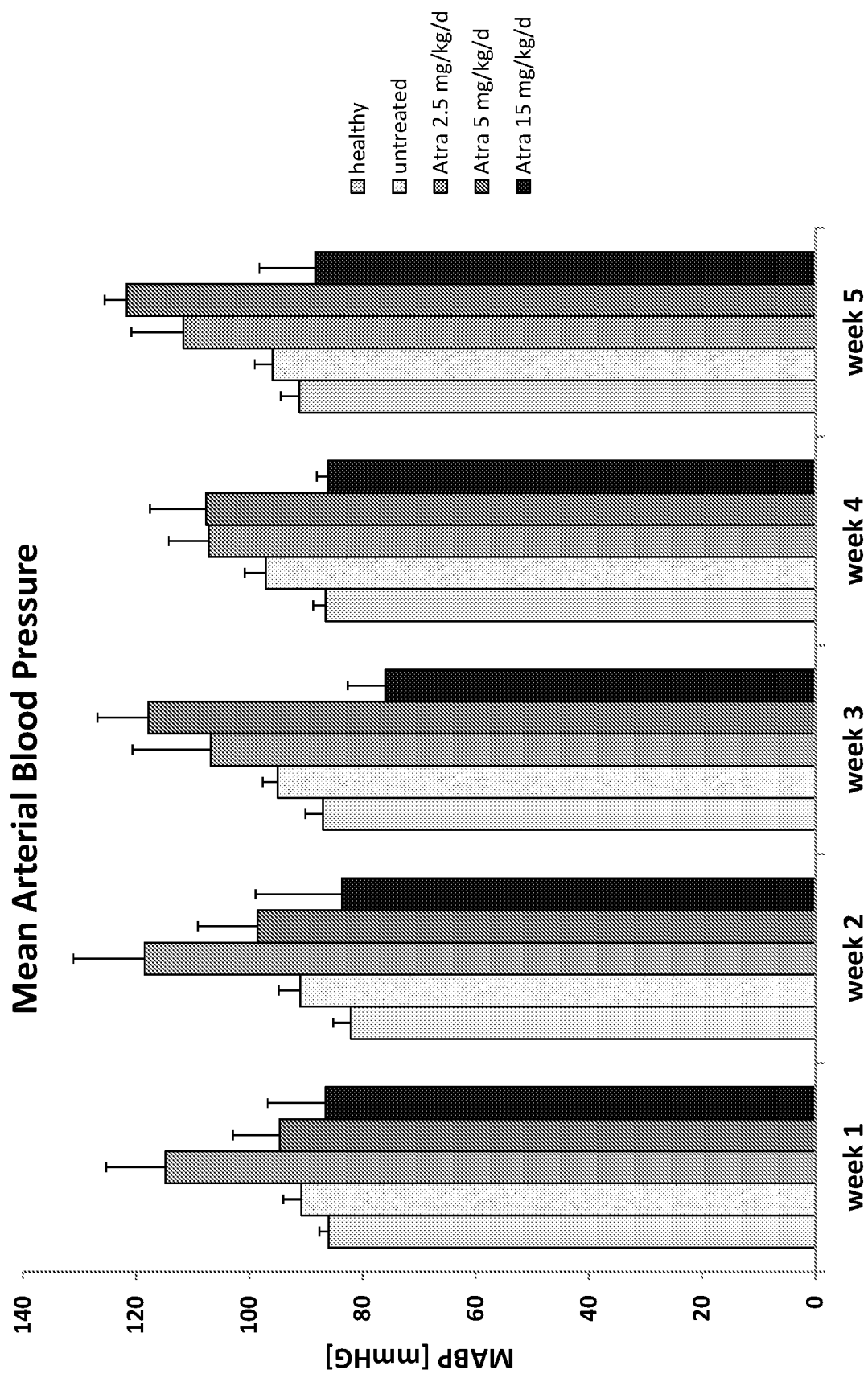


FIGURE 1C

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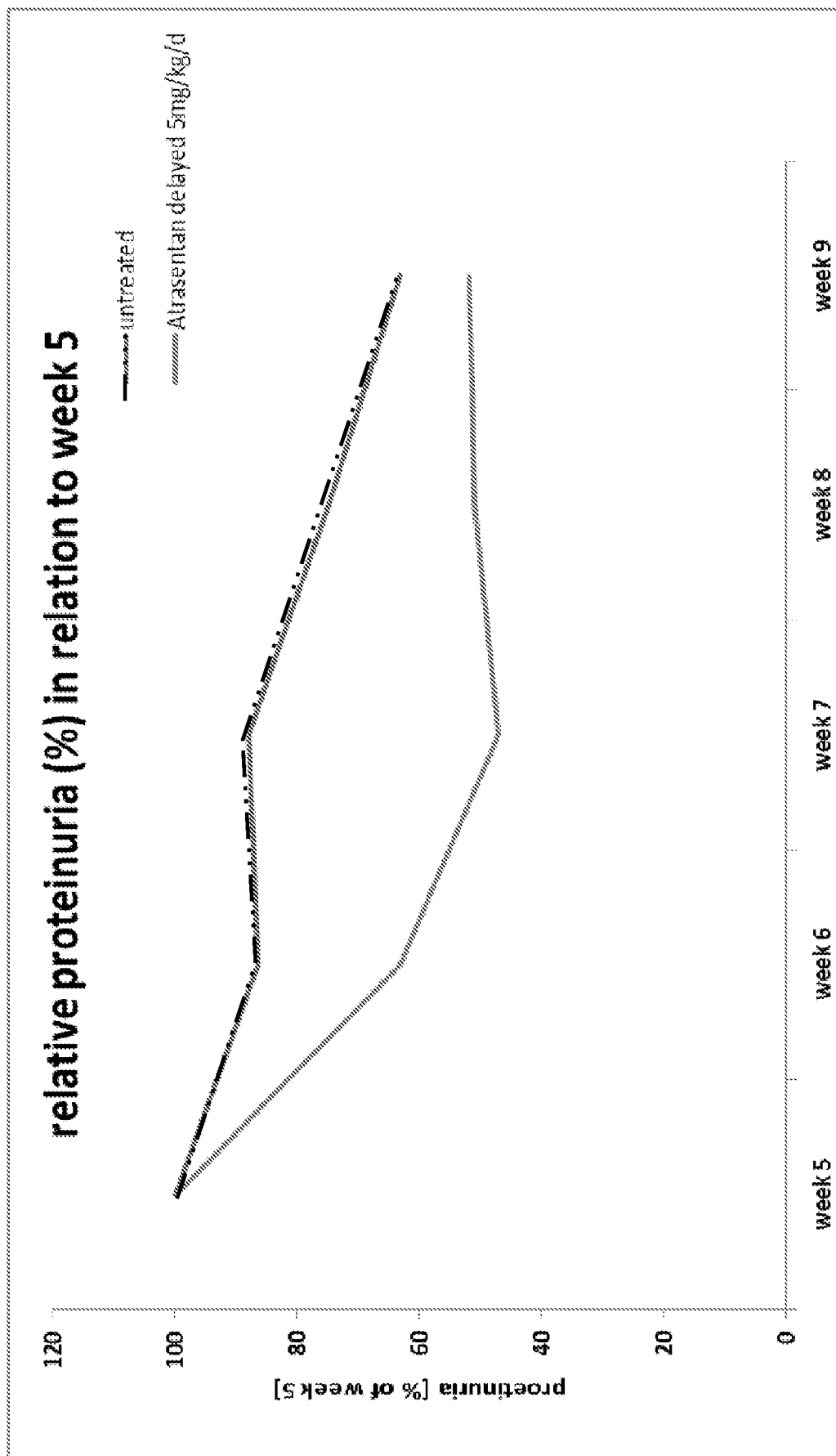
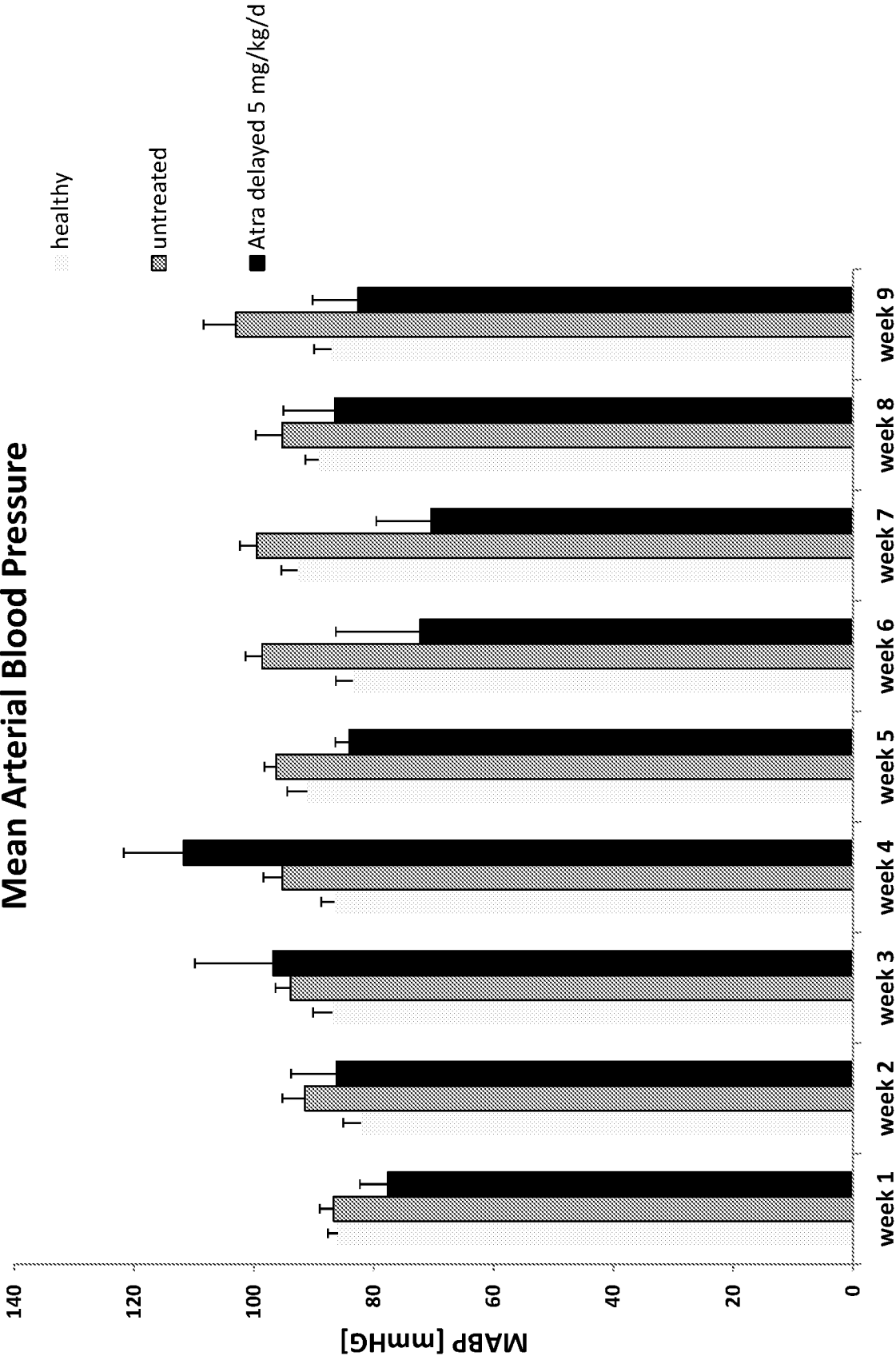


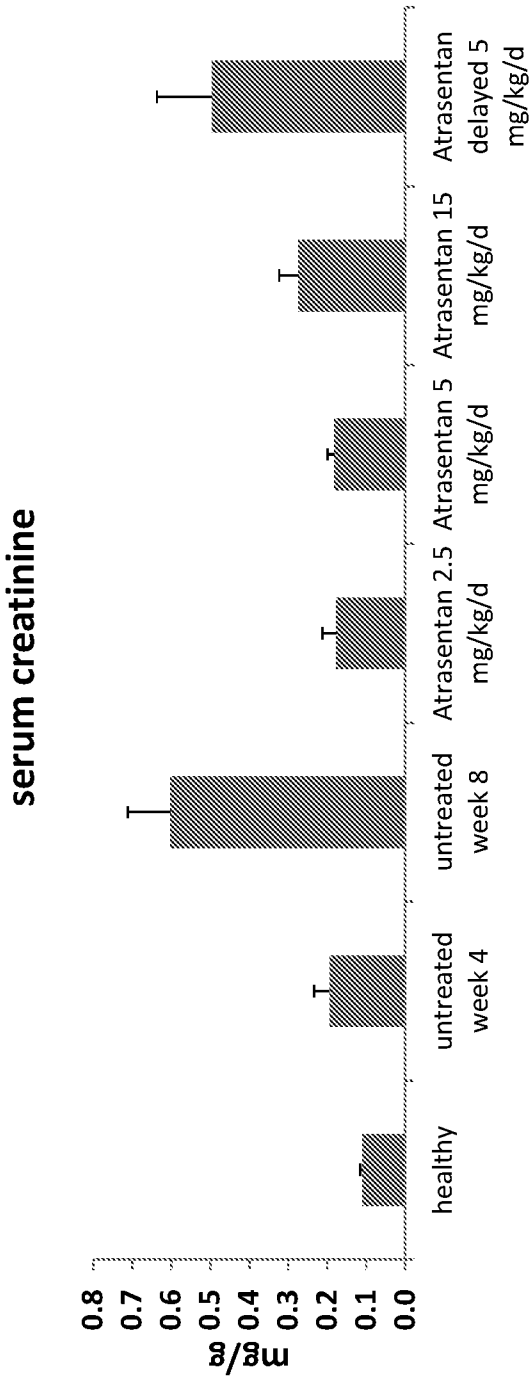
FIGURE 1D

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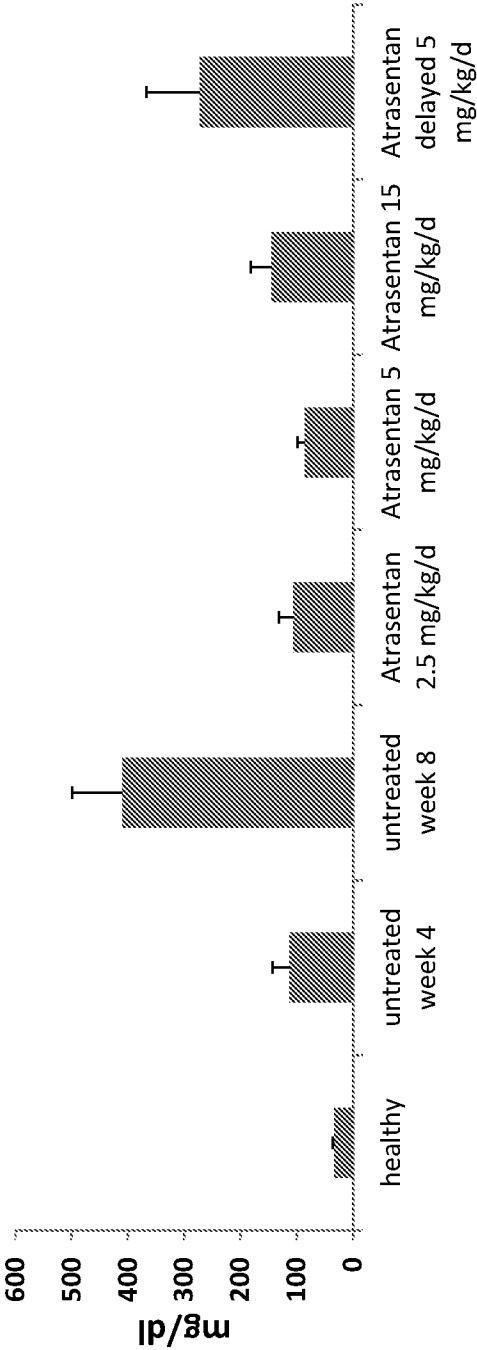
**FIGURE 2**

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**FIGURE 3A**

**urea**



**FIGURE 3B**

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serum albumin

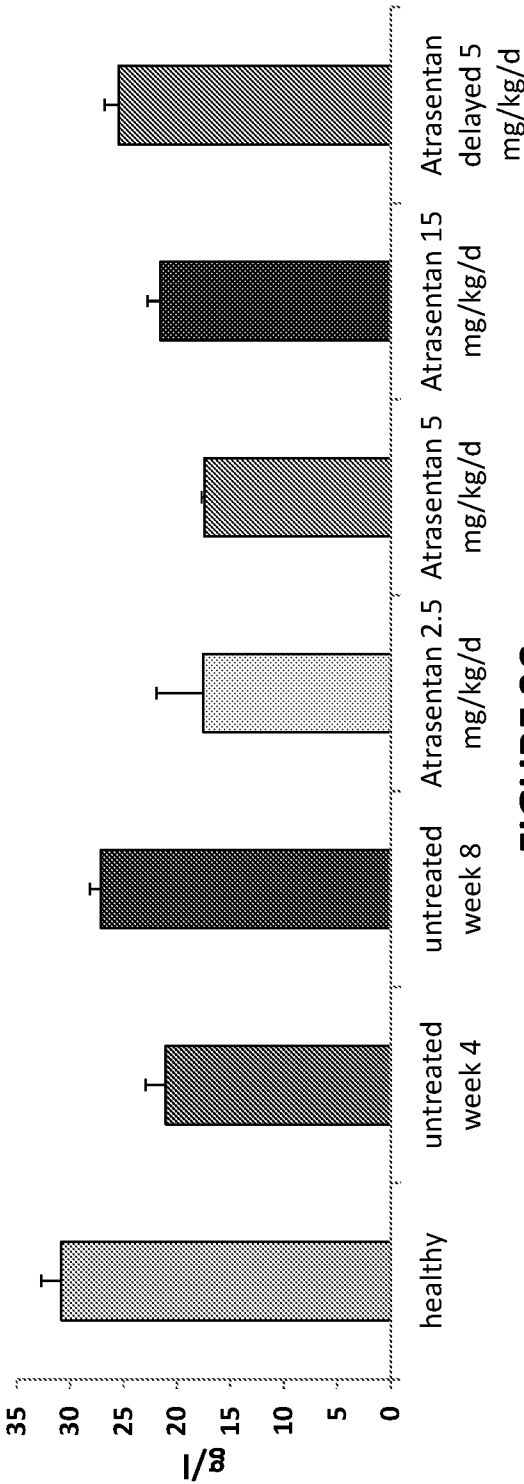


FIGURE 3C

cholesterol level

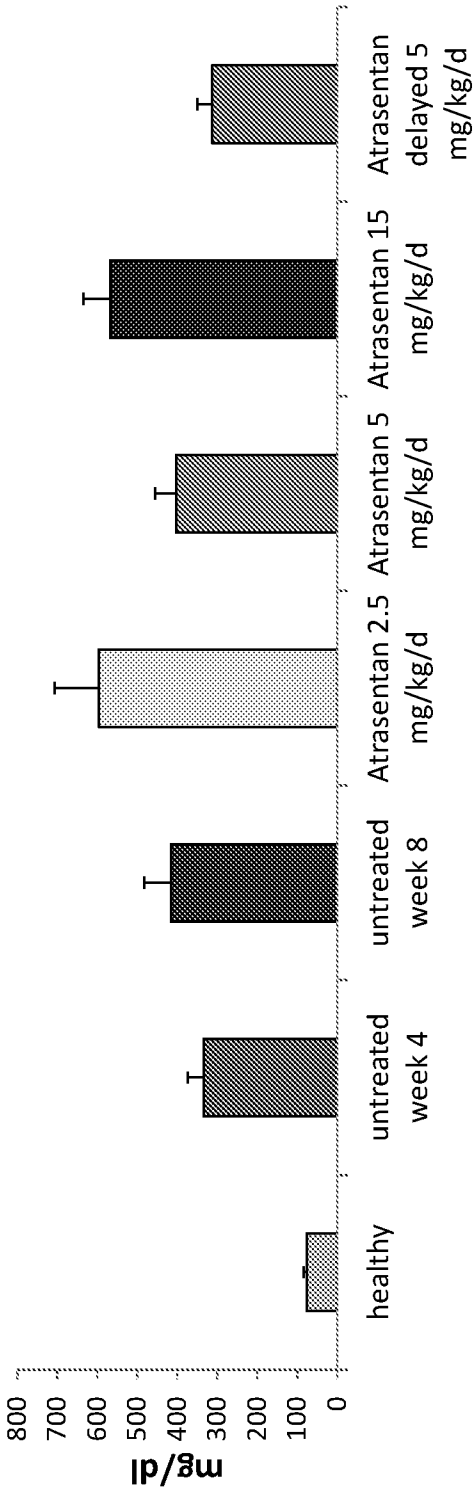


FIGURE 3D

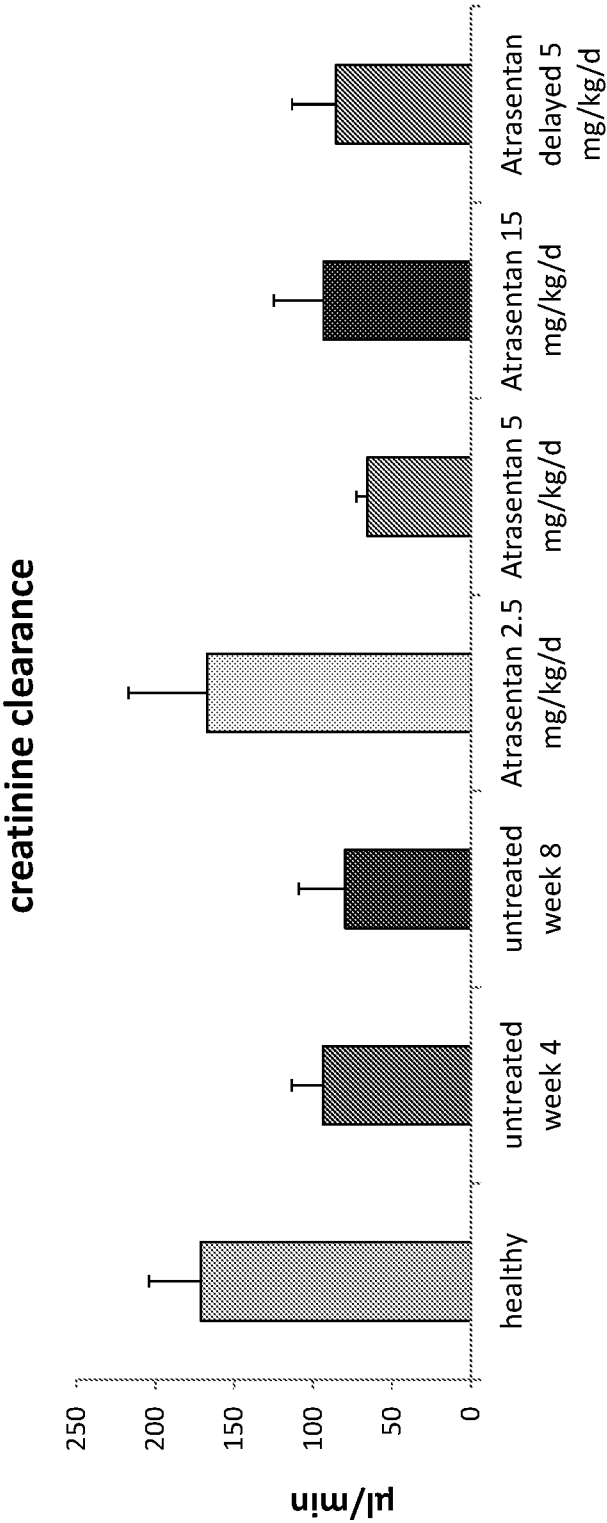


FIGURE 3E

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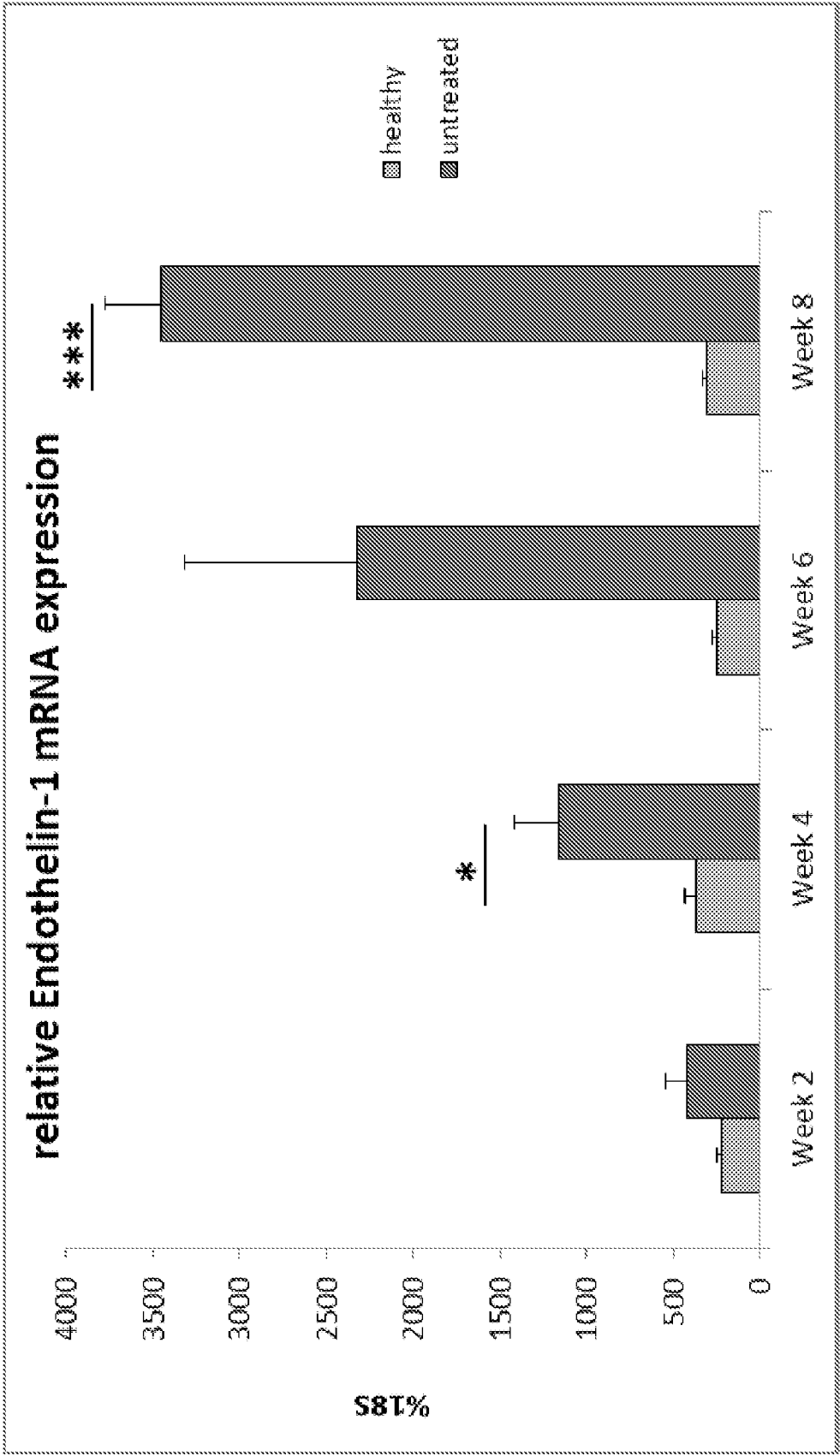
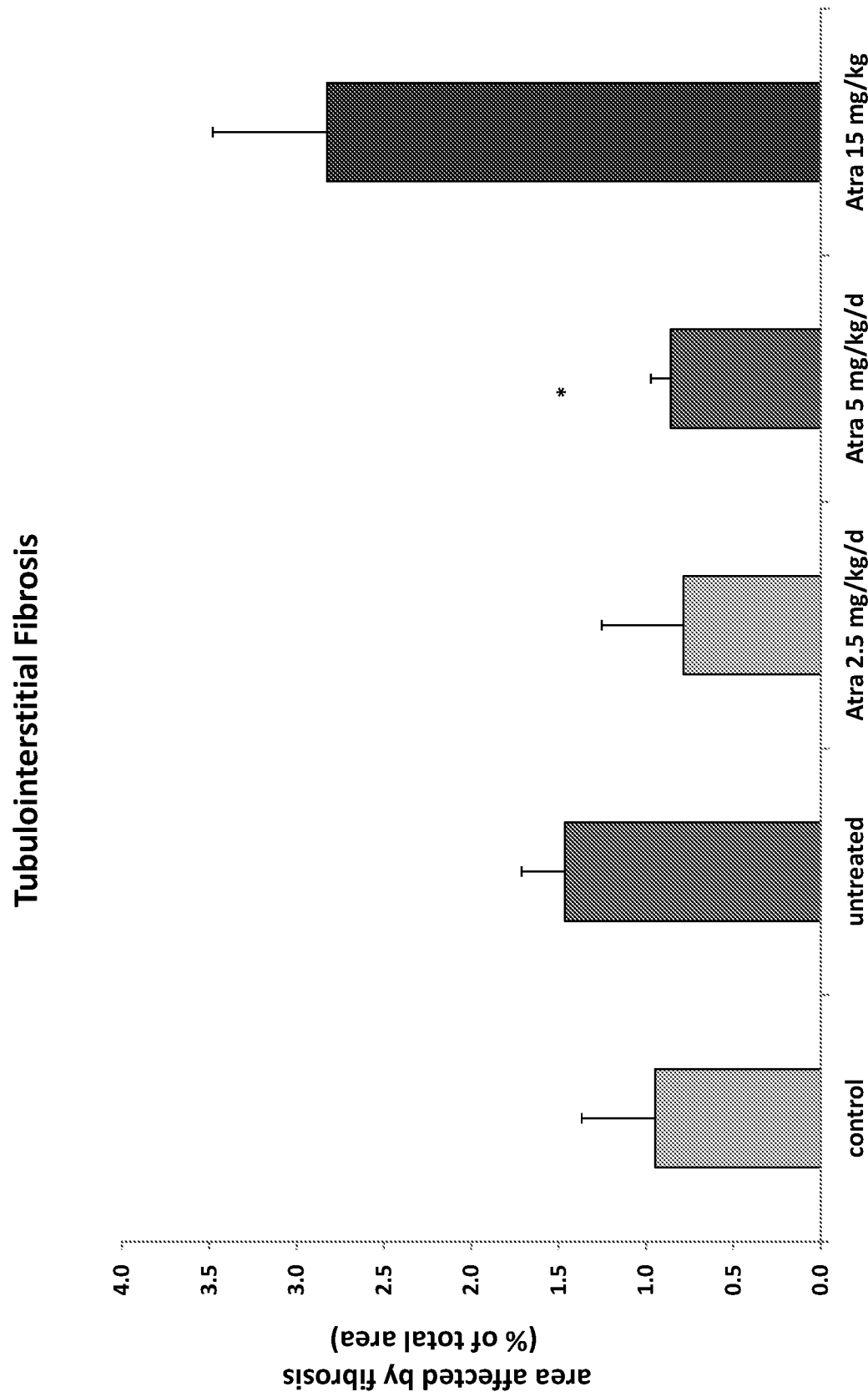


FIGURE 4

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**FIGURE 5A**

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Glomerular Sclerosis Index

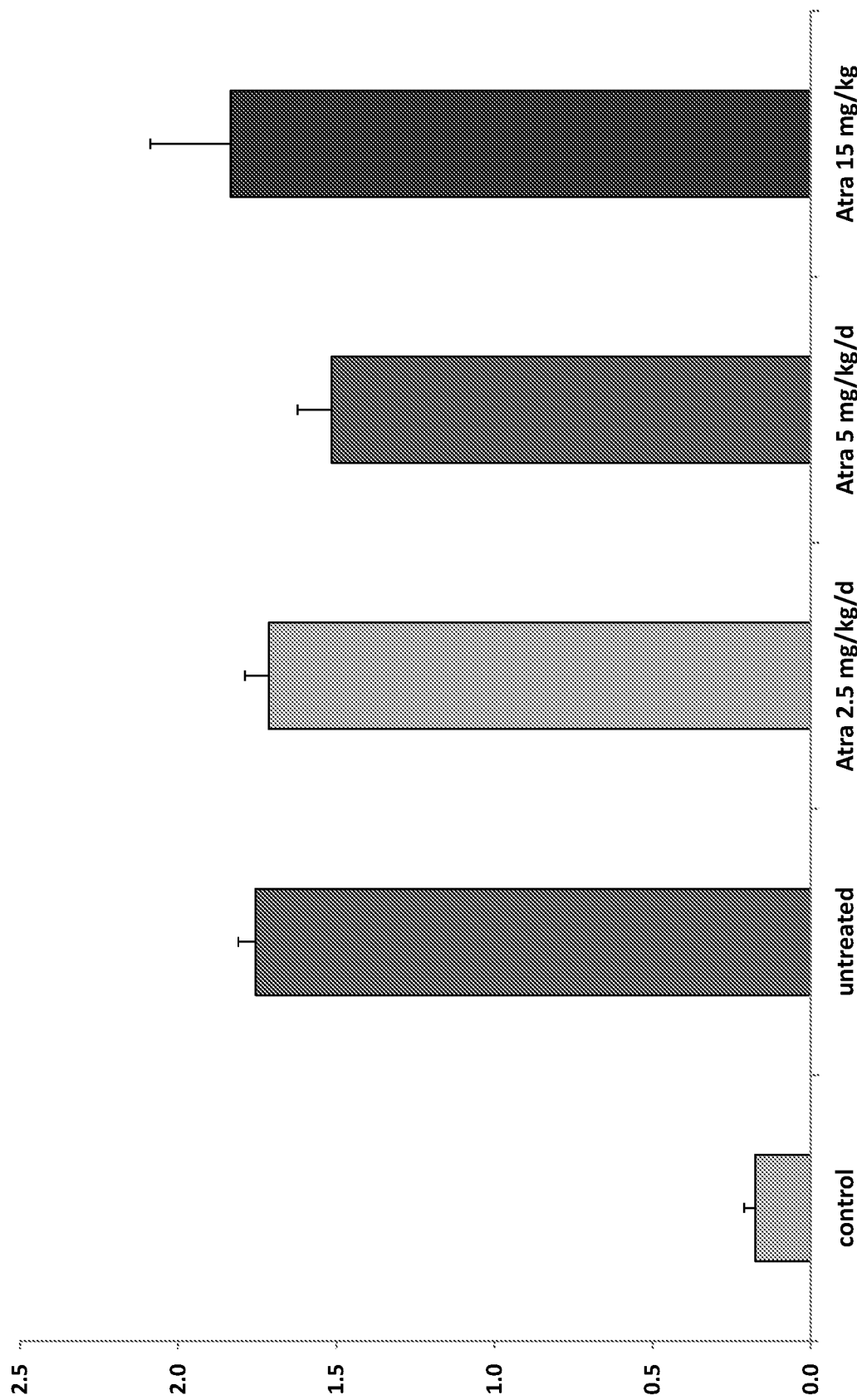


FIGURE 5B

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Average Number of Podocytes per Glomerulus

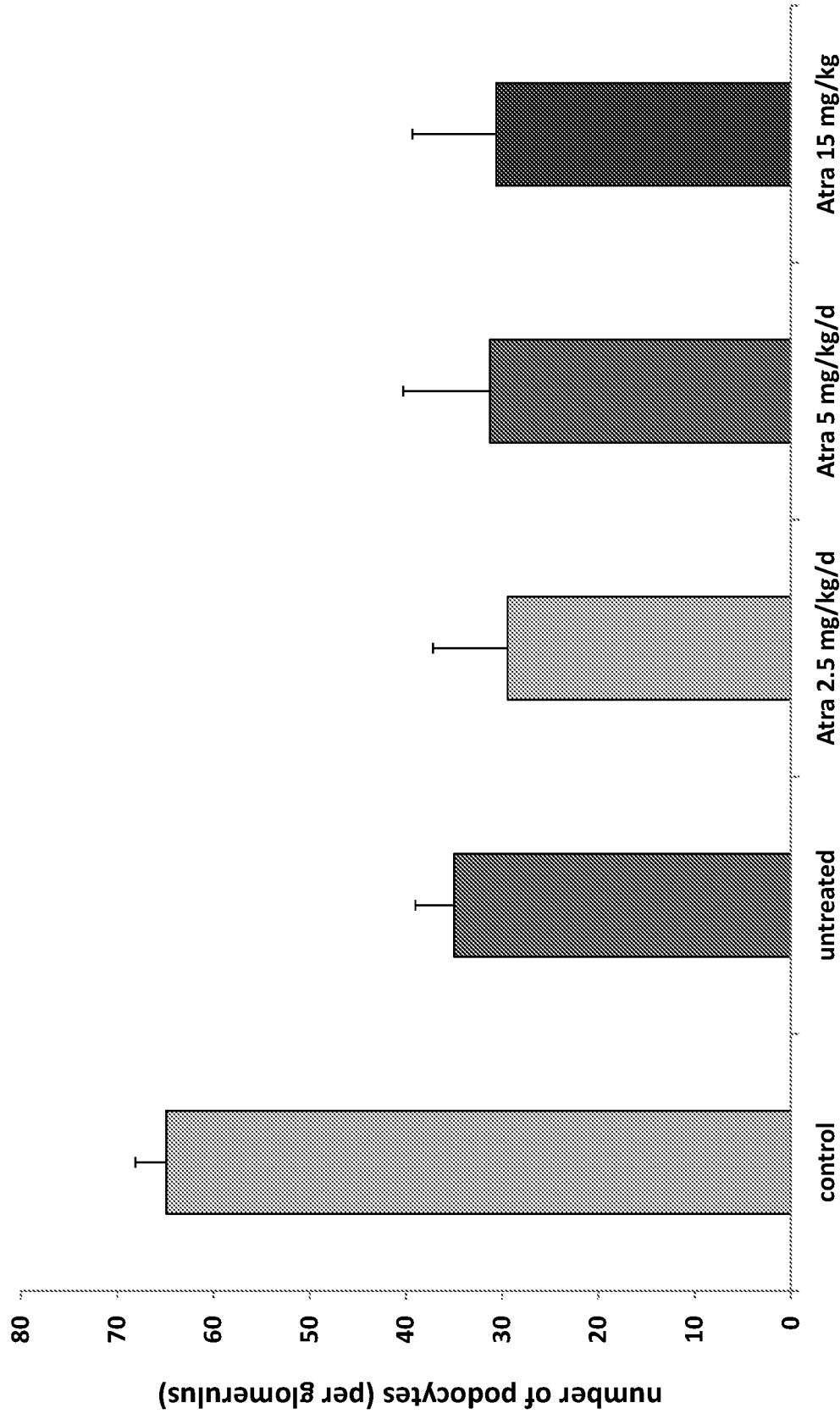


FIGURE 5C

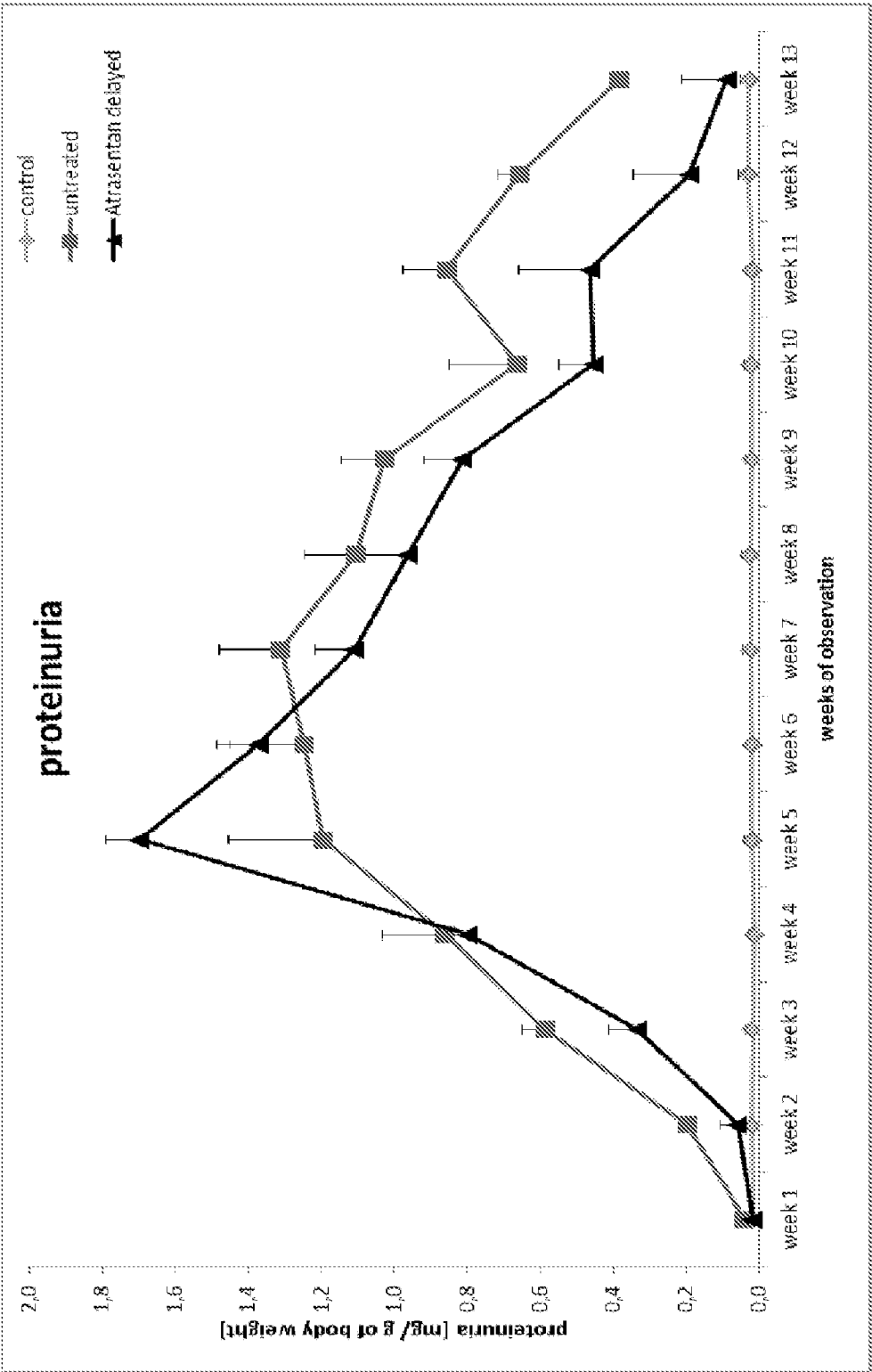
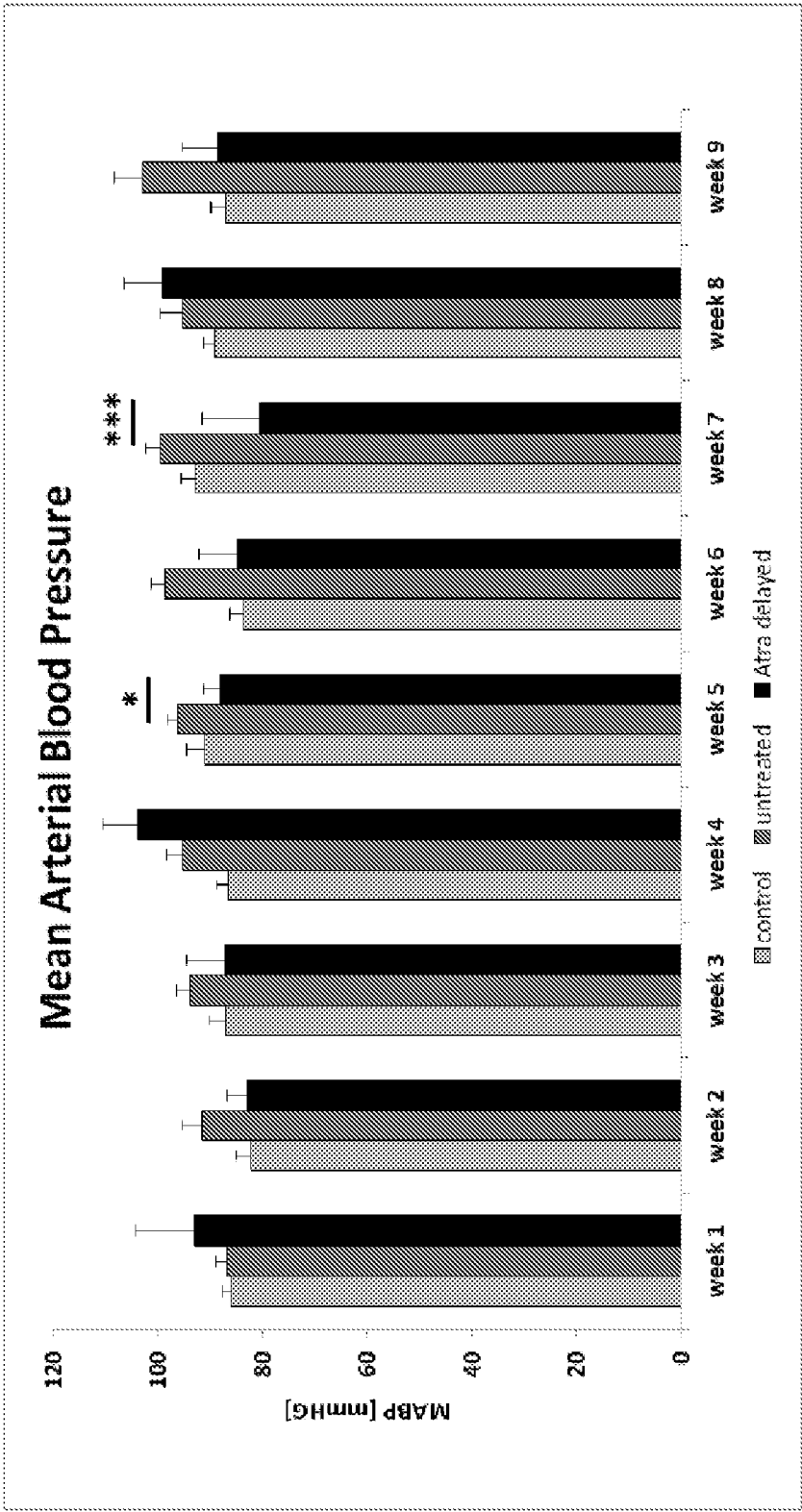


FIGURE 6



**FIGURE 7**

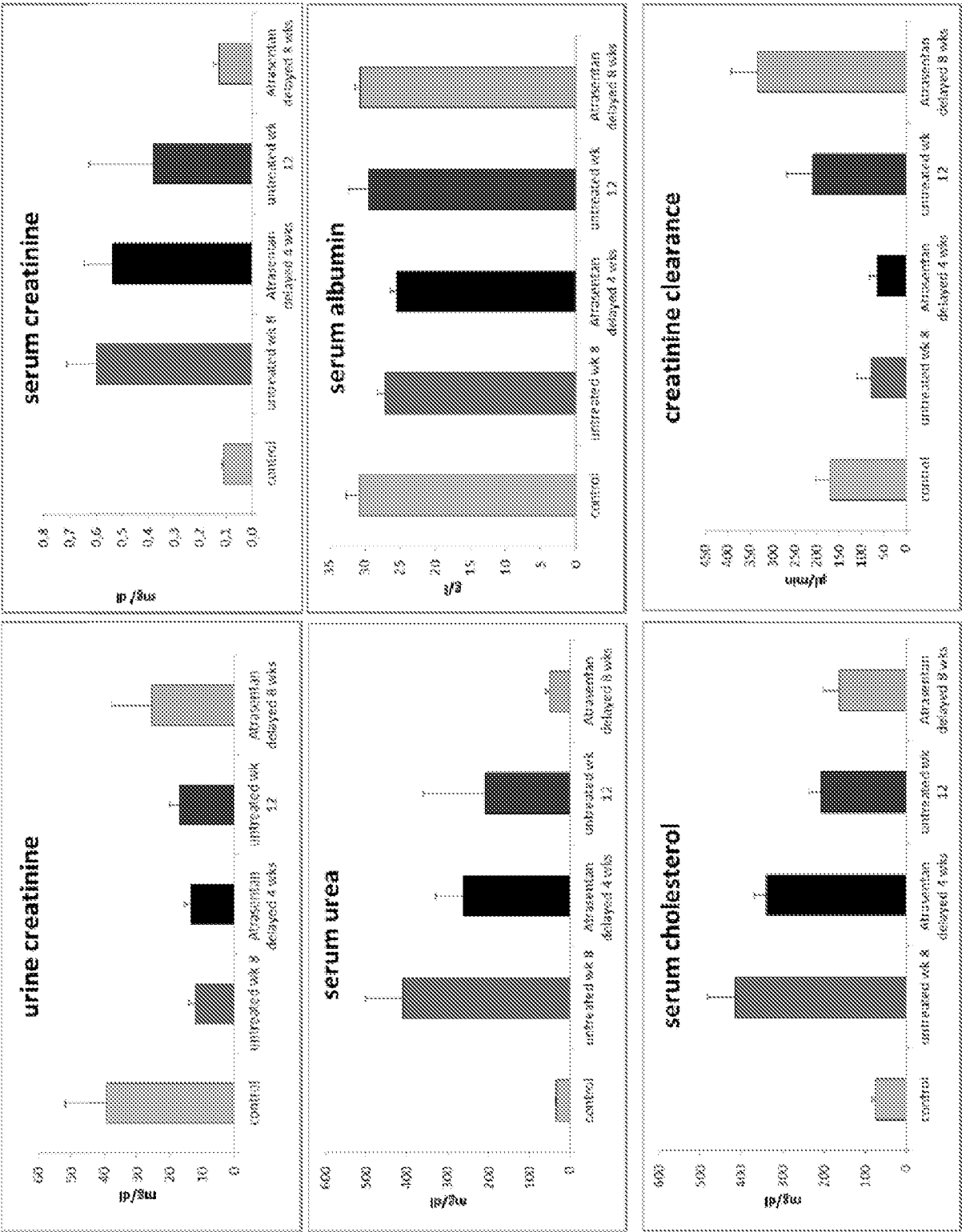
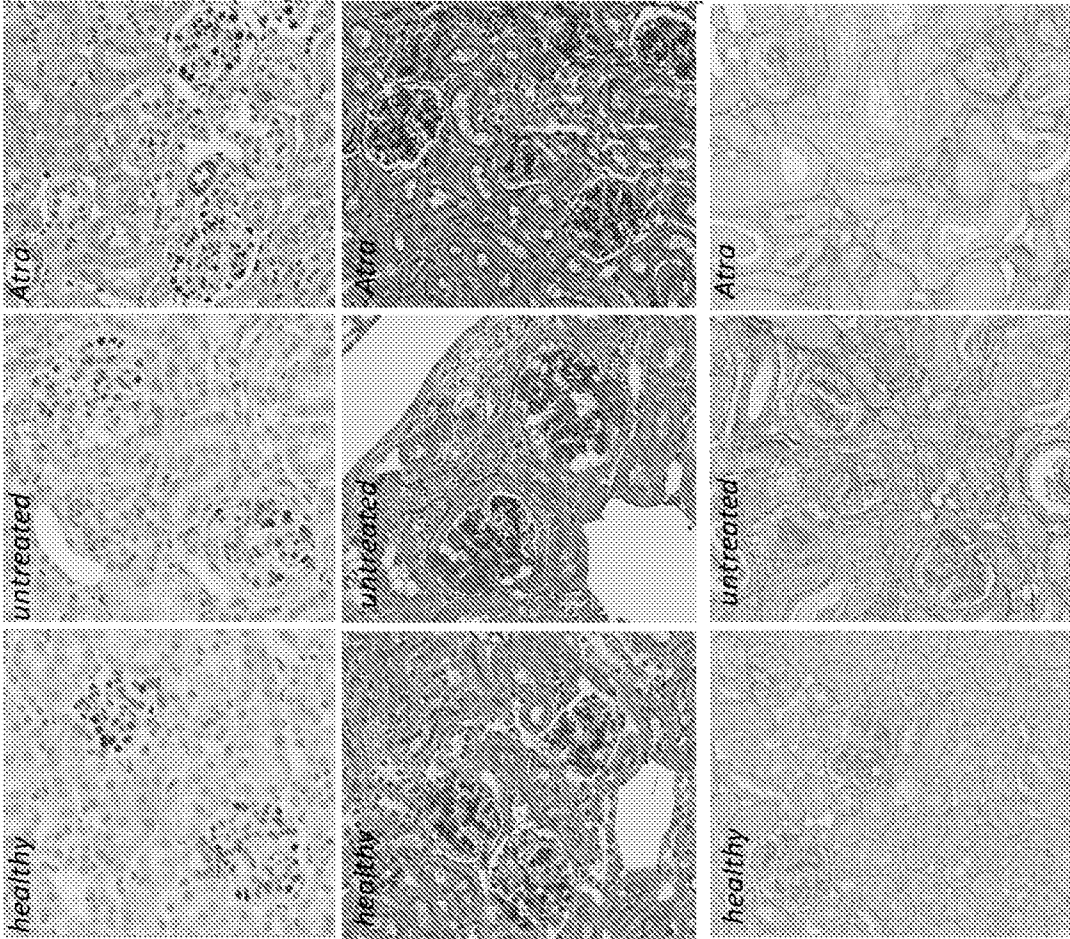


FIGURE 8

FIGURE 9A



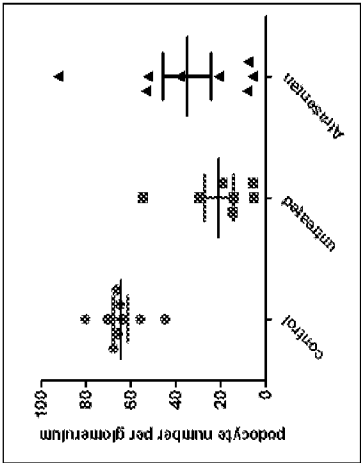


FIGURE 9B

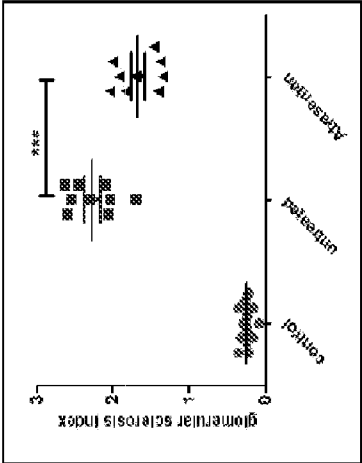


FIGURE 9C

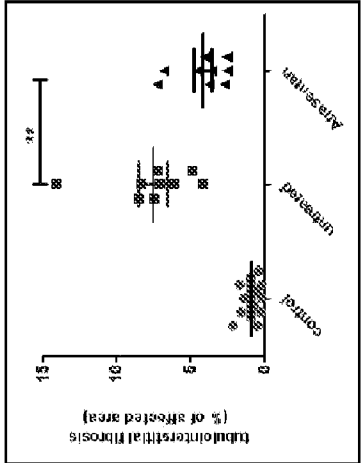


FIGURE 9D

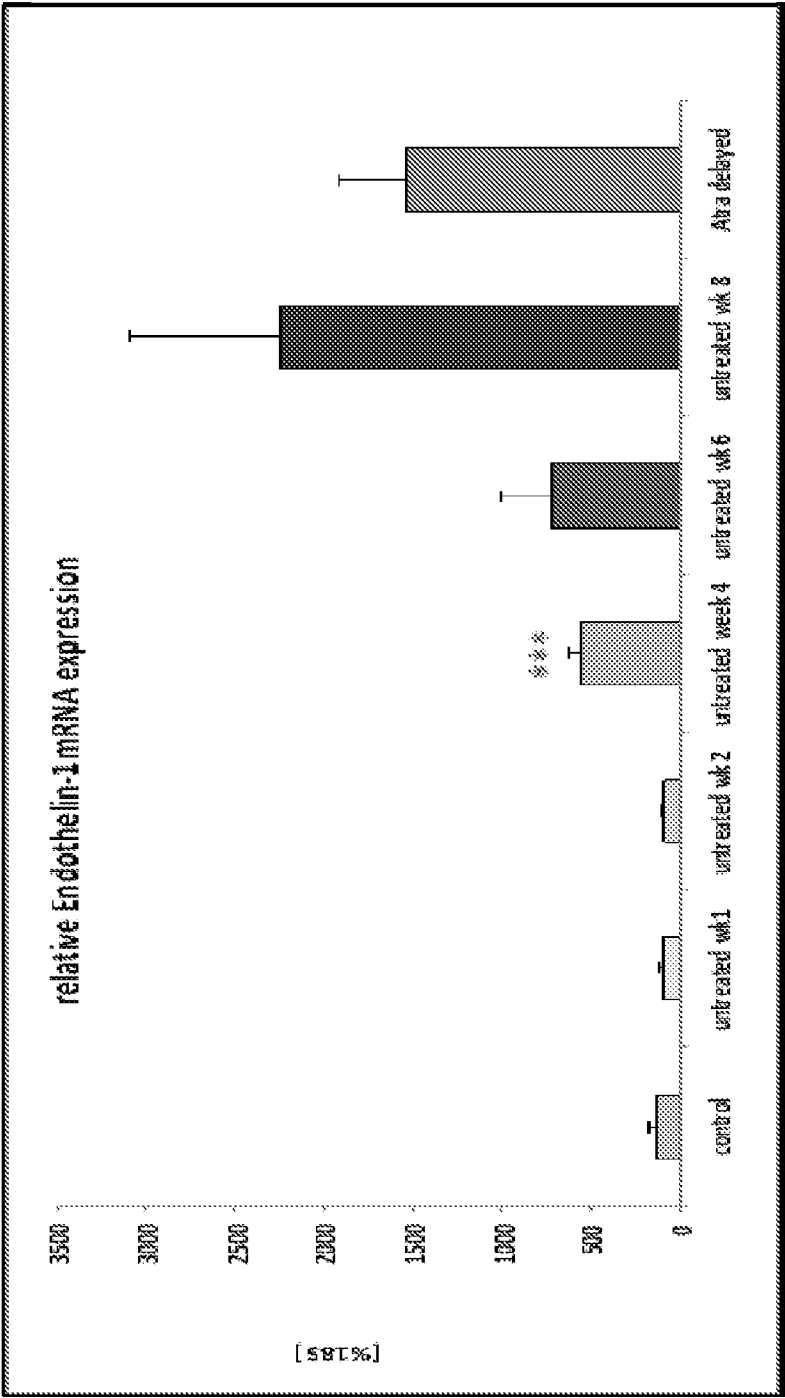


Figure 10A

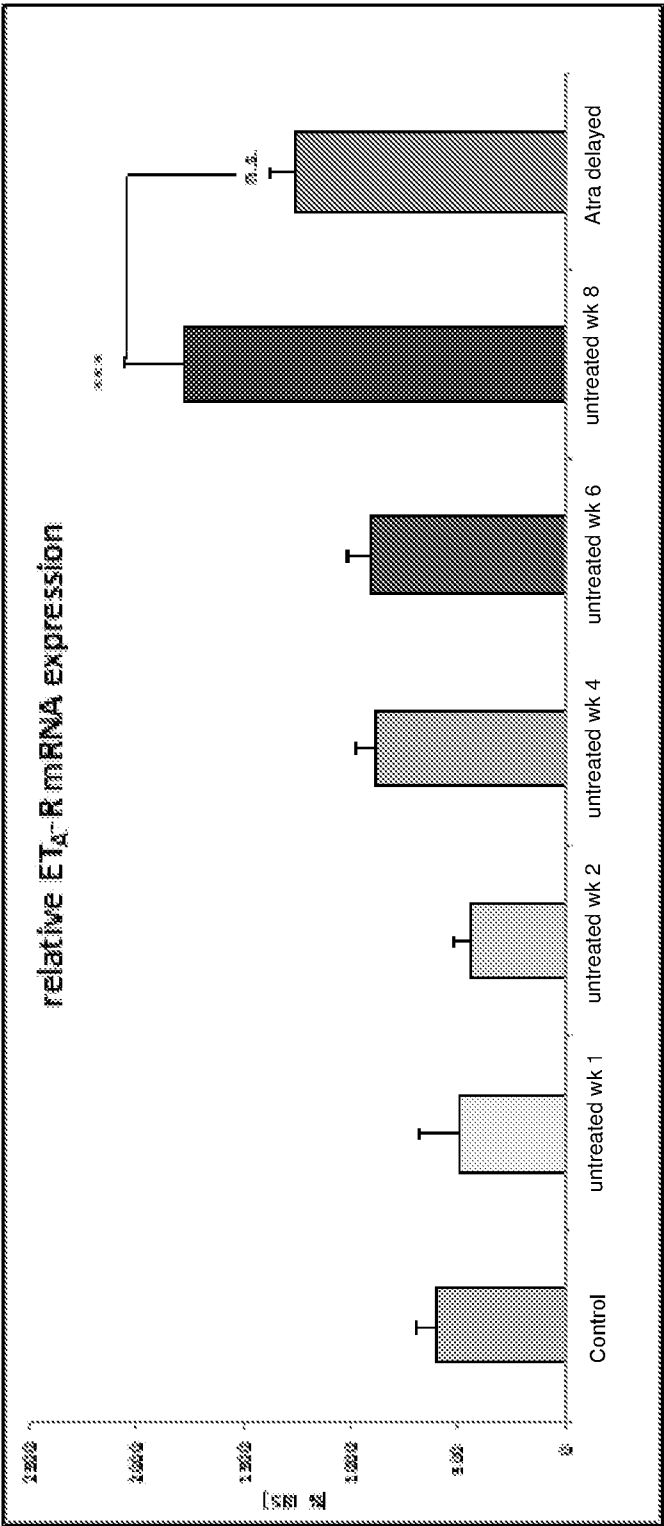


Figure 10B

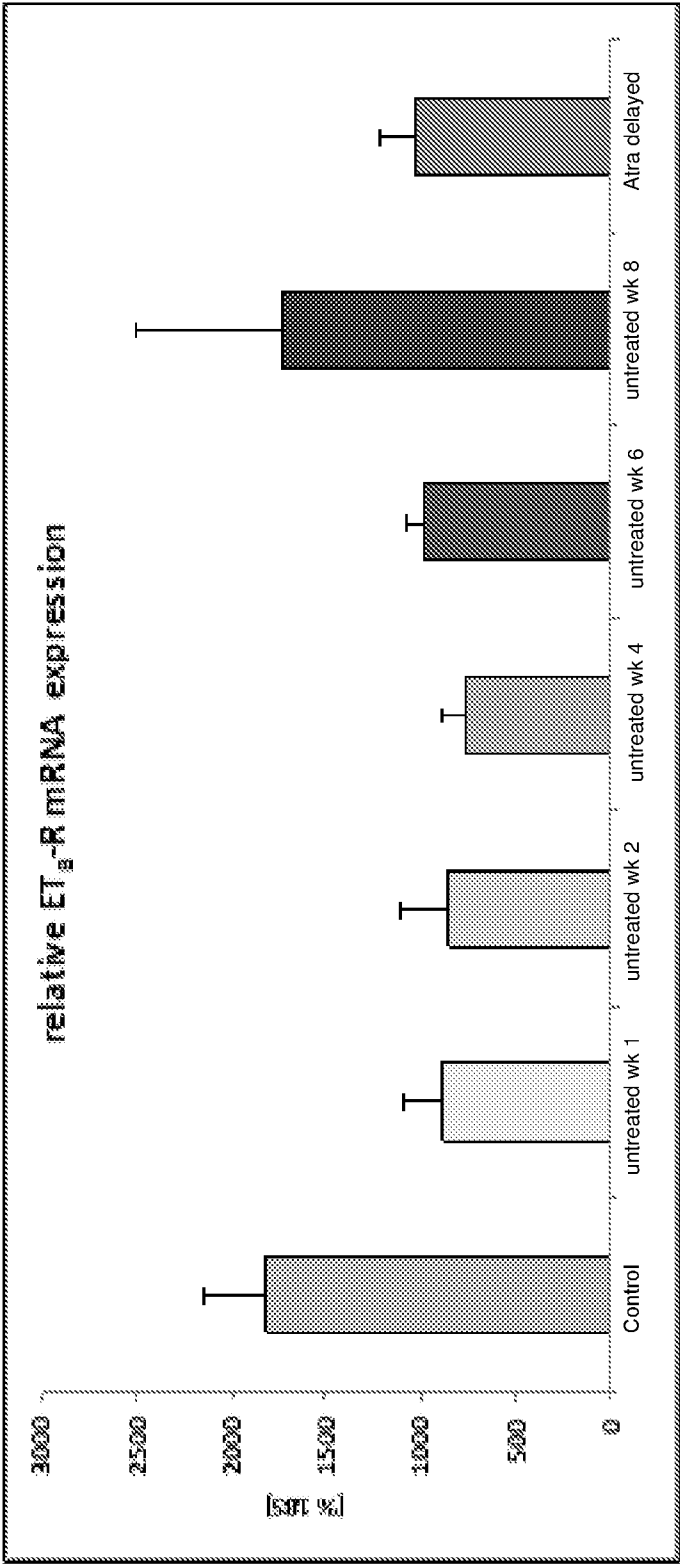
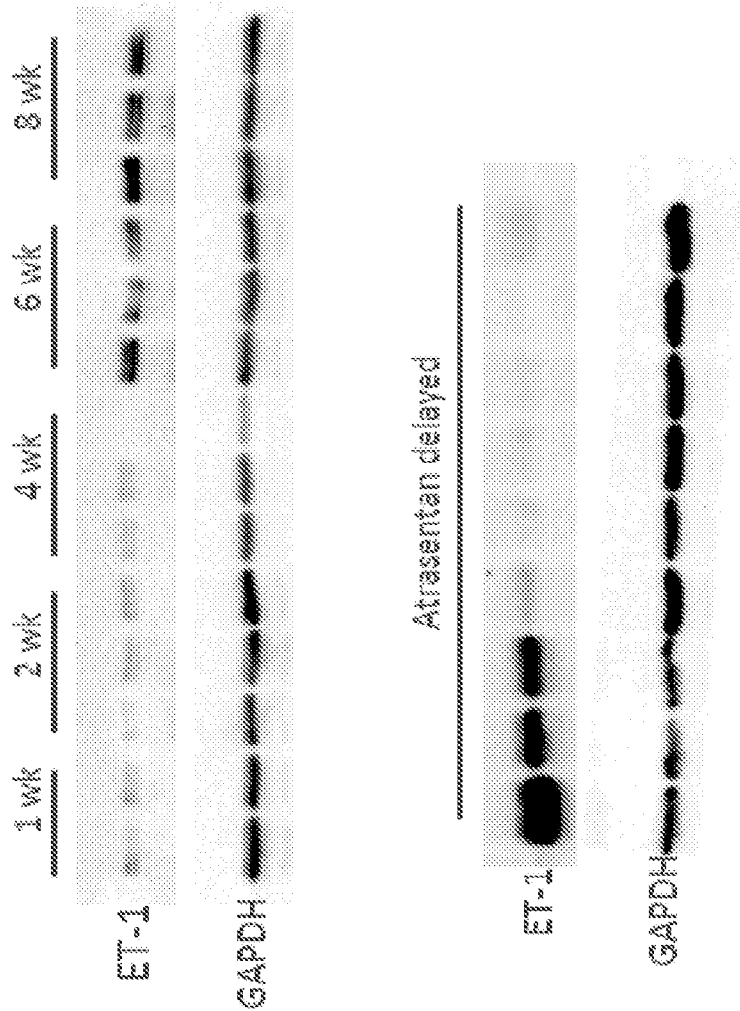


Figure 10C

FIGURE 11



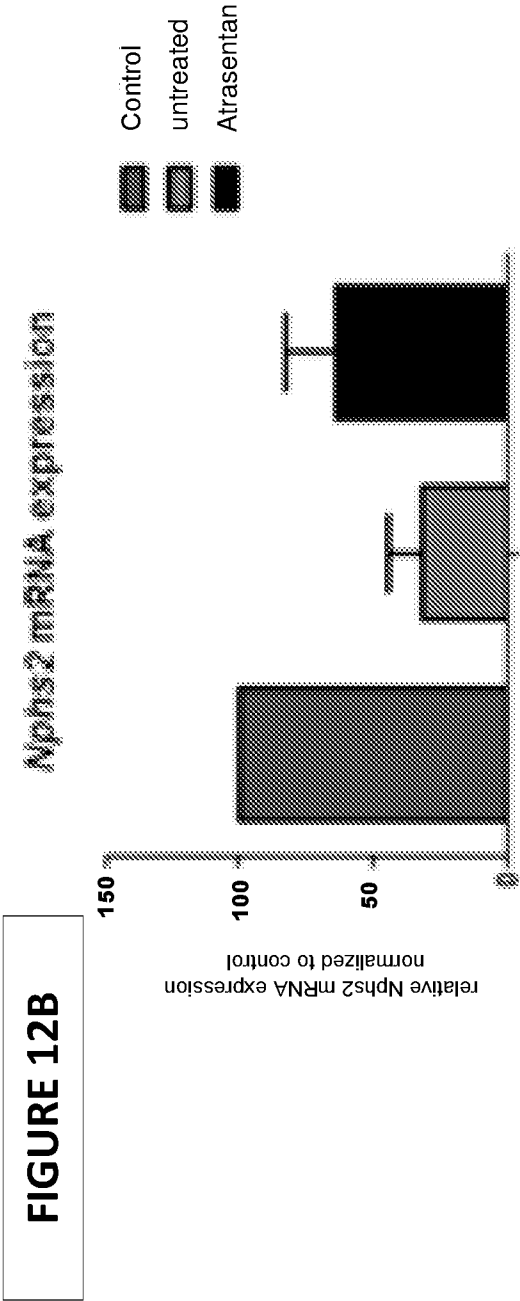
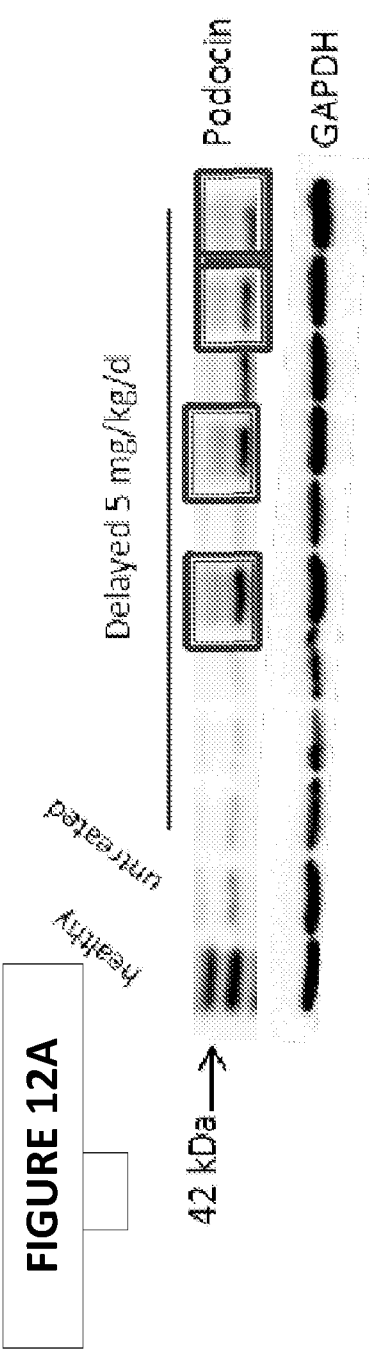


FIGURE 12C



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/42567

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC(8) - A61P 13/12; A61K 31/573 (2016.01)<br>CPC - A61K 31/573, 31/165<br>According to International Patent Classification (IPC) or to both national classification and IPC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                             |                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC(8): A61P 13/12; A61K 31/573 (2016.01)<br>CPC: A61K 31/573, 31/165<br>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched<br>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO; PubMed; Google/Google Scholar; pediatric, nephrotic, syndrome, drug, resistant, MDR, MDR-NS, proteinuria, atrasentan, angiotensin, converting, enzyme, inhibitor, blocker, losartan, enalapril                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                             |                                                                                           |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                             |                                                                                           |
| Category*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                          | Relevant to claim No.                                                                     |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | WO 2009/145831 A1 (GEISINGER CLINIC) 03 December 2009; abstract; paragraphs [0006], [0021], [0037]-[0056]                                                                                                                                   | 1-3, 4/1-3, 19-25, 26/23-25, 27/23-25, 28/27/23-25                                        |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | (de ZEEUW, D et al.) The Endothelin Antagonist Atrasentan Lowers Residual Albuminuria in Patients with Type 2 Diabetic Nephropathy. May 2014, Journal of the American Society of Nephrology; Volume 25; Number 5, pages 1083-1093; abstract | 1-3, 4/1-3, 19-25, 26/23-25, 27/23-25, 28/27/23-25                                        |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | US 2008/0161321 A1 (FELDMAN, DL et al.) 03 July 2008; abstract; paragraphs [0003], [0018], [0025], [0085], [0130], [0135], [0145], [0159]                                                                                                   | 4/1-3, 19-22                                                                              |
| Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | (LINDSKOG, A et al.) Melanocortin 1 Receptor Agonists Reduce Proteinuria. 01 August 2010, Journal of the American Society of Nephrology; Volume 21; Number 8, pages 1290-1298; page 1291, column 1, paragraph [3]                           | 26/23-25                                                                                  |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                             |                                                                                           |
| * Special categories of cited documents:<br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier application or patent but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed<br>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art<br>"&" document member of the same patent family |                                                                                                                                                                                                                                             |                                                                                           |
| Date of the actual completion of the international search<br>01 September 2016 (01.09.2016)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                             | Date of mailing of the international search report<br><b>07 OCT 2016</b>                  |
| Name and mailing address of the ISA/<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                             | Authorized officer<br>Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/42567

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☒ Claims Nos.: 5-18  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.