Title: INHIBITORS OF MIRNAS IN REGULATION OF ARTERIAL STIFFNESS AND USES THEREOF

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FIG. 1


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(57) Abstract: The present invention provides methods of treating or preventing arterial stiffness in a subject by administering to the subject an inhibitor of miR-137 function or activity and/or an inhibitor of miR-138 function or activity. The present invention also provides methods of treating or preventing one or more conditions associated with arterial stiffness by inhibiting the function or activity of miR-137 and/or miR-138. The present invention further provides methods of modulating the activity or expression of miR-137 and/or miR-138 in cells of a subject.
INHIBITORS OF MIRNAS IN REGULATION OF ARTERIAL STIFFNESS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS
[0001] The present Application claims the benefit of priority to U.S. Provisional Application No. 62/069,681 filed on October 28, 2014, the contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION
[0002] The present invention relates to the treatment or prevention of arterial stiffness by administering agents that modulate the activity or function of one or more microRNAs (miRNAs). In particular, the invention discloses methods for treating or preventing arterial stiffness by inhibiting the function or activity of miR-137 and/or miR-138 in cells of a subject.

BACKGROUND
[0003] Hypertension remains a global health issue, affecting roughly 1 billion people worldwide, with prevalence on the rise (Kearney et al., Lancet, 365(9455): 217-223, 2005). The cost to diagnose, treat and support patients suffering from hypertension is very high. Hypertension is manifested in various different forms with essential (primary) hypertension being the most common form accounting for 90–95% of all cases of hypertension (Carretero and Oparil, Circulation, 101 (3): 329–335, 2000). Essential hypertension has no identifiable cause whereas secondary hypertension results from an identifiable cause. One of the most common forms of secondary hypertension is renovascular hypertension caused by narrowing of the arteries supplying blood to the kidneys. Other forms of hypertension include resistant hypertension and pulmonary arterial hypertension (PAH). Resistant hypertension is defined as blood pressure that remains above goal in spite of the concurrent use of 3 antihypertensive agents of different classes (Calhoun et al., Circulation, 117, 2008:e510-e526). While the exact prevalence of resistant hypertension is unknown, clinical trials suggest that it is not rare, involving perhaps 20% to 30% of study participants (Calhoun et al., Circulation, 117, 2008:e510-e526).
Pulmonary arterial hypertension (PAH) is a disease of the small pulmonary arteries (PAs), characterized by an increase in PA pressure and vascular remodeling leading to a progressive increase in pulmonary vascular resistance (Rich et al., 1987). The consequence of vascular obliteration is right heart failure and high mortality (Jeffery et al., 2002; Voelkel et al., 1997). Various studies have shown that increased arterial stiffness is associated with increased risk of hypertension. For example, it has been shown that arterial stiffness is a predictor of all-cause mortality in patients with hypertension (Laurent et al., Hypertension, 37(5): 1236-1241; 2001). While age and blood pressure have traditionally been recognized as factors contributing to arterial stiffness, a recent review of the Framingham Offspring study demonstrated a temporal relationship between arterial stiffness and development of incident hypertension (Kaess et al., JAMA, 308(9): 875-881, 2012). This study included 1048 patients (average age 60+/-9) over two examination cycles (10 years each) and showed that increased arterial stiffness precedes incident hypertension, suggesting a more causal role for arterial stiffness (Kaess et al., JAMA, 308(9): 875-881, 2012).

Studies indicate that increased arterial stiffness may increase the risk for cardiovascular disease, and other diseases such as stroke. Cardiovascular disease (CVD) and its manifestations, including coronary artery disease, myocardial infarction, heart failure and angina, clearly present a major health risk in the United States today. CVD is a narrowing and hardening of arteries that supply blood and oxygen to the heart. As a result, less blood flows through the arteries and heart muscles can't get the blood or oxygen they need. This can lead to chest pain (angina) or heart attack. Over time, CVD can lead to weakening of heart muscles and result in heart failure. In the United States, half a million individuals are diagnosed with heart failure each year, with a mortality rate approaching 50%.

Myocardial infarction, commonly known as a heart attack, is caused by a sudden and sustained lack of blood flow to the heart tissue, which is usually the result of a narrowing or occlusion of a coronary artery. Without adequate blood supply, the tissue becomes ischemic, leading to the death of cardiomyocytes (e.g. heart muscle cells) and vascular structures. The necrotic tissue resulting from the death of the cardiomyocytes is generally replaced by scar tissue, which is not contractile, fails to contribute to cardiac function, and often plays a
detrimental role in heart function by expanding during cardiac contraction, or by increasing the size and effective radius of the ventricle, for example, becoming hypertrophic.

[0007] Similar to coronary heart disease, blockage of arteries that carry blood to head, limbs, and other organs, may result in a condition known as peripheral artery disease. If the blockage of peripheral arteries is severe or is left untreated, it can lead to gangrene or leg amputation. It is known that patients with diabetes are at an increased risk of developing peripheral artery disease.

[0008] Additionally, arterial stiffening has also been implicated in renal diseases. For example, it has been shown that patients with chronic renal insufficiency demonstrate increased arterial stiffening, and arterial stiffening is a predictor of mortality in this patient population as well (Blacher et al., Circulation, 99(18): 2434-2439, 1999). Moreover, patients with end-stage renal disease demonstrate survival benefit with reduced arterial stiffness, independent of comparable reductions in blood pressure (Guerin et al., Circulation, 103(7): 987-992, 2001).

[0009] The molecular underpinnings of vascular stiffening are diverse, but ultimately culminate in a detrimental ratio of collagen and elastin in the vessel wall that has immediate effects on compliance (Zieman et al., Arterioscler Thromb Vasc Biol, 25(5): 932-943, 2005). Advanced glycation end-products (AGEs) are the most well characterized determinants of vascular stiffening, with direct effects on collagen stabilization as well as purported effects on endothelial biology and nitric oxide (Lee and Cerami, Ann NY Acad Sci, 663: 63-70, 1992; Rojas et al., Circ Res, 86(3): e50-e54, 2000). Neuroendocrine signaling, salt, glucose, and insulin have also all been showed to play a role in vascular stiffening, as well as signaling between endothelial cells and vascular smooth muscle cells (Zieman et al., Arterioscler Thromb Vasc Biol, 25(5): 932-943, 2005). While a number of strategies exist to intervene on vascular stiffening such as lifestyle changes, as well as antioxidants, RAAS inhibitors, Tgf-beta inhibitors and AGE cross-link breakers (Tanaka et al., Circulation, 102(11): 1270-1275, 2000; Avolio et al., Arteriosclerosis, 6(2): 166-169, 1986; Cushman et al., Hypertension, 38(4): 953-957, 2001; Staessen et al., Lancet, 350(9080): 757-764, 1997; Mahmud and Feely, Am J Hypertens, 15(12): 1092-1095, 2002), a clear need exists for additional therapies.
MicroRNAs have recently been implicated in a number of biological processes including regulation of developmental timing, apoptosis, fat metabolism, and hematopoietic cell differentiation among others. MicroRNAs (miRNAs) are small, non-protein coding RNAs of about 18 to about 25 nucleotides in length that are derived from individual miRNA genes, from introns of protein coding genes, or from poly-cistronic transcripts that often encode multiple, closely related miRNAs. See review by Carrington et al. (Science, Vol. 301(5631):336-338, 2003). MiRNAs act as repressors of target mRNAs by promoting their degradation, when their sequences are perfectly complementary, or by inhibiting translation, when their sequences contain mismatches.

MiRNAs are transcribed by RNA polymerase II (pol II) or RNA polymerase III (pol III; see Qi et al. (2006) Cellular & Molecular Immunology, Vol. 3:411-419) and arise from initial transcripts, termed primary miRNA transcripts (pri-miRNAs), that are generally several thousand bases long. Pri-miRNAs are processed in the nucleus by the RNase Drosha into about 70- to about 100-nucleotide hairpin-shaped precursors (pre-miRNAs). Following transport to the cytoplasm, the hairpin pre-miRNA is further processed by Dicer to produce a double-stranded miRNA. The mature miRNA strand is then incorporated into the RNA-induced silencing complex (RISC), where it associates with its target mRNAs by base-pair complementarity. In the relatively rare cases in which a miRNA base pairs perfectly with an mRNA target, it promotes mRNA degradation. More commonly, miRNAs form imperfect heteroduplexes with target mRNAs, affecting either mRNA stability or inhibiting mRNA translation. Until now, there have been no studies directed to the role of miRNAs in arterial stiffening.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that miR-137 and miR-138 are upregulated in rat models of hypertension and arterial stiffness. Inhibiting the activity or function of miR-137 and miR-138 significantly reduced arterial stiffness and blood pressure. Accordingly, the present invention provides compositions and methods for treating or preventing arterial stiffness in a subject in need thereof by modulating the activity or function of miR-137 and/or miR-138 in cells of a subject in need thereof. For instance, in one embodiment, the present invention provides a method of treating or preventing arterial stiffness in a subject in
need thereof comprising administering to the subject an inhibitor of miR-137 and/or an inhibitor of miR-138. In some embodiments, the function or activity of miR-137 and/or miR-138 is reduced in the cells of the subject following administration. In one embodiment, the subject in need of a treatment of arterial stiffness is diagnosed with or is at risk for a condition selected from the group consisting of essential hypertension, secondary hypertension, renovascular hypertension, resistant hypertension, peripheral arterial disease, coronary artery disease, atherosclerosis, arteriosclerosis, aneurysm, angina, hypertensive heart disease, heart failure, ischemia, cor pulmonale, pulmonary hypertension, pulmonary arterial hypertension, diabetic nephropathy, diabetic retinopathy, optic neuropathy, cerebrovascular disease, stroke, hypertensive encephalopathy, myocardial infarction, vascular calcification, hypertensive retinopathy, hypertensive nephropathy, hypertensive nephrosclerosis, restenosis and thrombosis. Accordingly, in one embodiment, the present invention provides methods of treating or preventing the above-mentioned conditions in a subject in need thereof by inhibiting the function or activity of miR-137 and/or miR-138.

[0013] In one embodiment, the present invention provides compositions and methods of restoring or improving arterial elasticity in a subject in need thereof by administering an inhibitor of miR-137 and/or an inhibitor of miR-138. In certain embodiments, the subject in need of restoring or improving arterial elasticity may be diagnosed with or is at risk for one or more conditions disclosed above.

[0014] In one embodiment, the cells of the subject in which the function or activity of miR-137 and/or miR-138 is reduced following administration of an inhibitor of miR-137 and/or miR-138 are selected from the group consisting of cells of aorta, kidney, heart, lung, endothelial cells, and vascular smooth muscle cells.

[0015] In certain embodiments, inhibitors of miR-137 are oligonucleotide inhibitors that comprise a sequence that is at least partially complementary to a miR-137 sequence (e.g. mature, pre-miRNA, or pri-miRNA sequence) and inhibitors of miR-138 are oligonucleotide inhibitors that comprise a sequence that is at least partially complementary to a miR-138 sequence (e.g. mature, pre-miRNA, or pri-miRNA sequence). The oligonucleotide inhibitors used in the
compositions and methods of the invention may have a length of from about 6 to about 22 nucleotides. In some embodiments, the oligonucleotide inhibitors comprise one or more chemical modifications, such as sugar, backbone, and/or base modifications. In other embodiments, inhibitors of miR-137 can be decoy sequences having two or more binding sites which hybridize to the mature miR-137 molecule. In certain other embodiments, inhibitors of miR-138 can be decoy sequences having two or more binding sites which hybridize to the mature miR-138 molecule. In certain additional embodiments, an oligonucleotide inhibitor can comprise a sequence that is at least partially complementary to a miR-137 sequence and a sequence that is at least partially complementary to a miR-138 sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIGURE 1: Expression levels of miR-137 (left panel) and miR-138 (right panel) in the aorta of rats that had undergone unilateral nephrectomy followed by deoxycorticosterone acetate treatment.

[0017] FIGURE 2: Schematic of study design to assess the effect of inhibition of miR-137 or miR-138 on arterial stiffness and hypertension

[0018] FIGURE 3: Effect of treatment with antimiR-137 or antimiR-138 on the pulse-wave velocity (PWV) (left panel) and mean system pressure (right panel) in SHR/L-NAME rats; % p<0.05 vs saline.

[0019] FIGURE 4: Expression levels of two miR-137 targets, Klf2 and Klf4, in kidneys of SHR/L-NAME rats treated with saline, perindopril, or antimiR-137.

[0020] FIGURE 5: Expression levels of various miR-137 targets in aorta, right ventricle, and kidneys of SHR/L-NAME rats treated with saline or antimiR-137; *p<0.05 vs saline; 2-way ANOVA.

DETAILED DESCRIPTION

[0021] The present invention is based, in part, on the surprising discovery that the expression and activity of miR-137 and miR-138 play an important role in the progression of arterial stiffness.
The inventors have found that miR-137 and miR-138 are significantly up-regulated in response to hypertension and vascular stiffness and that inhibition of miR-137 and/or miR-138 reduces arterial stiffness and blood pressure. Thus, the present invention provides miR-137 and miR-138 as new targets for therapeutic intervention for arterial stiffness and associated conditions.

[0022] In one embodiment, the present invention provides compositions and methods for treating or preventing arterial stiffness by inhibiting the function or activity of miR-137 in cells (e.g. cells of aorta, kidney, heart, lung, endothelial cells, and vascular smooth muscle cells) of a subject. In another embodiment, the present invention provides compositions and methods for treating or preventing arterial stiffness by inhibiting the function or activity of miR-138 in cells (e.g. cells of aorta, kidney, heart, lung, endothelial cells, and vascular smooth muscle cells) of a subject. In yet another embodiment, the present invention provides compositions and methods for treating or preventing arterial stiffness by inhibiting the function or activity of both miR-137 and miR-138 in cells (e.g. cells of aorta, kidney, heart, lung, endothelial cells, and vascular smooth muscle cells) of a subject. In various embodiments, the methods comprise administering an inhibitor of miR-137, an inhibitor of miR-138, or inhibitors of both miR-137 and miR-138 to a subject in need of treatment or prevention of arterial stiffness.

[0023] In certain embodiments, the subject in need of treatment of arterial stiffness is a subject who is diagnosed with or is at risk for one or more conditions selected from the group consisting of essential hypertension, secondary hypertension, renovascular hypertension, resistant hypertension, peripheral arterial disease, coronary artery disease, atherosclerosis, arteriosclerosis, aneurysm, angina, hypertensive heart disease, heart failure, ischemia, cor pulmonale, pulmonary hypertension, pulmonary arterial hypertension, diabetic nephropathy, diabetic retinopathy, optic neuropathy, cerebrovascular disease, stroke, hypertensive encephalopathy, myocardial infarction, vascular calcification, hypertensive retinopathy, hypertensive nephropathy, hypertensive nephrosclerosis, restenosis and thrombosis. Thus, the present invention also provides compositions and methods for treating or preventing the above-mentioned conditions in a subject in need thereof by administering an inhibitor of miR-137, an inhibitor of miR-138, or inhibitors of both miR-137 and miR-138. In some embodiments, the function or activity of miR-137 and/or miR-138 is reduced in the cells of the subject following administration.
[0024] In one embodiment, the present invention is directed to compositions and methods for treating or preventing hypertension in a subject in need thereof by administering an inhibitor of miR-137, an inhibitor of miR-138, or inhibitors of both miR-137 and miR-138. In some embodiments, the function or activity of miR-137 and/or miR-138 is reduced in the cells of the subject following administration. Forms of hypertension that can be treated with the compositions and methods of the invention include, but are not limited to, essential hypertension, secondary hypertension, renovascular hypertension, resistant hypertension, pulmonary hypertension, and pulmonary arterial hypertension.

[0025] miR-137 is located on human chromosome 1 and murine chromosome 3. In humans, the pre-miRNA sequence for miR-137 is processed into a mature sequence. In mice and rats, the pre-miRNA sequence for miR-137 is processed into a mature sequence and a star (i.e. minor) sequence. The star sequence is processed from the other arm of the stem loop structure. The pre-miRNA and mature sequences for human miR-137 and the pre-miRNA, mature, and star sequences for mouse and rat miR-137 are given below:

Human mature miR-137 (SEQ ID NO: 1)
5’ UUAUUGCUUAAGAAUACGCGUAG 3’

Human pre-miR-137 (SEQ ID NO: 2)
5’ GGUCCUCUGACUCUCUUCGGUGACGGGUAAUUCUUGGGUGGAUAAUACGGA UUACGUUGUUAUUGCUGUAAAGAAUACGCGUAGUCGAGGAGAGUACCAGCGGCA3’

Mouse mature miR-137 (SEQ ID NO: 3)
5’ UUAUUGCUUAAGAAUACGCGUAG 3’

Mouse miR-137* (SEQ ID NO: 4)
5’ ACGGGUAAUUCUUGGGUGGAUAAU 3’

Mouse pre-miR-137 (SEQ ID NO: 5)
5' CUUCGGUGACGGGUAAUCUUGGGUGGAUAAUACGGAUUACGUUGUUAAU
UGCUUAAGAAUACGCGUAGUCGAGG 3'

Rat mature miR-137 (SEQ ID NO: 6)
5' UUAUUGCUCUAAGAAUACGCGUAG 3'

Rat miR-137* (SEQ ID NO: 7)
5' ACGGGUAAUCUUGGGUGGAUAA 3'

Rat pre-miR-137 (SEQ ID NO: 8)
5' GGCCCUCCUGACUCUCUCUUCGGUGACGGGUAAUCUUGGGUGGAUAAUACGG
AUUACGUUGUUAUUGCUUAAGAAUACGCGUAGUCGAGGAGAGUACCAGCG
GCA 3'

[0026] miR-138 is a family of miRNAs containing miR-138-1 and miR-138-2. The mature
sequences for miR-138-1 and miR-138-2 are identical whereas the pre-miRNA sequences for the
two forms are different. miR-138-1 is located on human chromosome 3 and murine
chromosome 9. miR-138-2 is located on human chromosome 16 and murine chromosome 8.
The pre-miRNA sequences for miR-138-1 and miR-138-2 are processed into a mature sequence
and a star (i.e. minor) sequence. The pre-miRNA, mature, and star sequences for the human,
mouse, and rat miR-138-1 and miR-138-2 are given below:

Human mature miR-138-1 (SEQ ID NO: 9)
5' AGCUGGUGUUGGAUCAAGCCG 3'

Human miR-138-1* (SEQ ID NO: 10)
5' GCUACUUCAACACCAACCAGGCC 3'

Human pre-miR-138-1 (SEQ ID NO: 11)
5' CCCUGGCAUGGUGUGGGGCCCAGCUGGUGUGUGGAUCAAGCCGCUUGCC
AAUCAGAGAACGGCUACUAACACACCAGGCCACACACCACUCACACGG 3'
Human mature miR-138-2 (SEQ ID NO: 12)
5’ AGCUGGUGUUGUGAAUCAGGCCG 3’

Human miR-138-2* (SEQ ID NO: 13)
5’ GCUAUUUUCACGACACCAGGGUU 3’

Human pre-miR-138-2 (SEQ ID NO: 14)
5’ CGUUGCUGCAGCUGGUGUUGUGAAUCAGGCCGACGACGCGCAUCCUCU
UACCCGCUAUUUUCACGACACCAGGGGUUGCAUCA 3’

Mouse mature miR-138-1 (SEQ ID NO: 15)
5’ AGCUGGUGUUGUGAAUCAGGCCG 3’

Mouse miR-138-1* (SEQ ID NO: 16)
5’ CGGCUCACUAACAACACCAGGG 3’

Mouse pre-miR-138-1 (SEQ ID NO: 17)
5’ CUCUAGCAUGGUGUUGUGGGACACGCUUGGUGUUGUGAAUCAGCCGCUUGCC
AAUCAGAGAAGGCUCUACUUCACAACACCAGGGCCACACUGCUGCAAG 3’

Mouse mature miR-138-2 (SEQ ID NO: 18)
5’ AGCUGGUGUUGUGAAUCAGGCCG 3’

Mouse miR-138-2* (SEQ ID NO: 19)
5’ GCUAUUUUCACGACACCAGGGU 3’

Mouse pre-miR-138-2 (SEQ ID NO: 20)
5’ CAGCUGGUGUUGUGAAUCAGCGCCGACGAGCAGCGCAUCCUCUACCC
GCUAUUUUCACGACACCAGGGGUUG 3’

Rat mature miR-138-1 (SEQ ID NO: 21)
5’ AGCUGGUGUUGUGAAUCAGGCCG 3’
Rat miR-138-1* (SEQ ID NO: 22)
5' CGGCUACUUCACAACACCAGGG 3' 

Rat pre-miR-138-1 (SEQ ID NO: 23)
5' CUCUGGCAUGGUUGUUGGGGACAGCUGGUGUUGGAAUCAGGCGGUUG 
CCAAUCAGAGAAGCGCUACUUCACAACACCAGGUCUCACUGCACUCAGG3'

Rat mature miR-138-2 (SEQ ID NO: 24)
5' AGCUGGUGUUGGAUACAGGCCG 3' 

Rat miR-138-2* (SEQ ID NO: 25)
5' GCUAUUUCACGACACCAGGGU 3' 

Rat pre-miR-138-2 (SEQ ID NO: 26)
5' GUUGCUGCACGAGCCGUACUUCACAACACCAGGCCGAUCACUGCACUCUUGCACC3'

[0027] It is understood that all ribonucleic acid sequences disclosed herein can be converted to deoxyribonucleic acid sequences by substituting a thymidine base for a uridine base in the sequence. Likewise, all deoxyribonucleic acid sequences disclosed herein can be converted to ribonucleic acid sequences by substituting a uridine base for a thymidine base in the sequence. Deoxyribonucleic acid sequences, ribonucleic acid sequences, and sequences containing mixtures of deoxyribonucleotides and ribonucleotides of all sequences disclosed herein are included in the invention.

[0028] In one embodiment, the present invention provides a method of ameliorating or preventing arterial stiffness in a subject in need thereof comprising administering to the subject an inhibitor of miR-137, an inhibitor of miR-138, or inhibitors of miR-137 and miR-138 together. Arterial stiffness, also called vascular stiffness, is assessed by measurement of pulse-wave velocity (PWV). The PWV is higher in stiffer arteries (Blacher et al., Circulation, 99(18): 2434-2439, 1999). In various embodiments of the invention, administration of an inhibitor of
miR-137, an inhibitor of miR-138, or inhibitors of both miR-137 and miR-138 reduces arterial stiffness and accordingly, the subject shows decreased PWV following administration.

[0029] In various embodiments, the present invention provides compositions and methods of treating hypertension in a subject in need thereof comprising administering to the subject an inhibitor of miR-137, an inhibitor of miR-138, or inhibitors of miR-137 and miR-138 together. Hypertension can be assessed by measuring blood pressure (systolic and diastolic) of a subject. Arterial stiffness leads to higher systolic blood pressure (SBP) and lower diastolic blood pressure (DBP) (Blacher et al., Circulation, 99(18): 2434-2439, 1999). According to the present invention, administration of an inhibitor of miR-137, an inhibitor of miR-138, or inhibitors of miR-137 and miR-138 together reduces hypertension and accordingly, the subject shows lower SBP and improved DBP values following administration. In certain embodiments, blood pressure may be expressed as mean system pressure, in which case the administration of a miR-137 inhibitor, a miR-138 inhibitor, or inhibitors of both miR-137 and miR-138 in a subject results in decreased mean system pressure compared to the mean system pressure before administration.

[0030] In some embodiments, the subject upon administration of a miR-137 inhibitor, a miR-138 inhibitor, or inhibitors of both miR-137 and miR-138 shows decreased PWV, lower SBP and improved DBP values following administration. In some other embodiments, the subject upon administration of a miR-137 inhibitor, a miR-138 inhibitor, or inhibitors of both miR-137 and miR-138 shows decreased PWV and decreased mean system pressure following administration.

[0031] In one embodiment, the activity or function of miR-137 and/or miR-138 is reduced in various organs or tissues such as aorta, kidney, heart, and/or lungs of the subject following administration of the inhibitors of miR-137 and/or miR-138. In one embodiment, the activity or function of miR-137 and/or miR-138 is reduced in one or more cells such as aortic cells, kidney cells, heart cells, lung cells, endothelial cells, and/or vascular smooth muscle cells of the subject following administration of an inhibitor of miR-137 and/or an inhibitor of miR-138. “Heart cells,” as used herein, include cardiomyocytes, cardiac fibroblasts, endothelial cells, and vascular smooth muscle cells of the heart. “Kidney cells,” as used herein include glomerulus parietal cell, glomerulus podocytes, cells of proximal tubule, cells of loop of Henle, cells of thick ascending
limb, distal tubule cells, collecting duct cells, Interstitial kidney cells, stromal cells, and cells of renal arteries. “Lung cells,” as used herein, include alveolar cells (also called pneumocytes) and cells of pulmonary arteries. “Cells of aorta” as used herein include endothelial cells and smooth muscle cells.

[0032] In certain embodiments, the subject in need of treatment of arterial stiffness may be diagnosed with or at risk for one or more conditions selected from the group consisting of essential hypertension, secondary hypertension, renovascular hypertension, resistant hypertension, peripheral arterial disease, coronary artery disease, atherosclerosis, arteriosclerosis, aneurysm, angina, hypertensive heart disease, heart failure, ischemia, cor pulmonale, pulmonary hypertension, pulmonary arterial hypertension, diabetic nephropathy, diabetic retinopathy, optic neuropathy, cerebrovascular disease, stroke, hypertensive encephalopathy, myocardial infarction, vascular calcification, hypertensive retinopathy, hypertensive nephropathy, hypertensive nephrosclerosis, restenosis and thrombosis. The subject at risk may be diagnosed as having a genetic predisposition to the above-mentioned conditions or may have a familial history of these conditions. The present invention is also directed to compositions and methods for treating or preventing the above-mentioned conditions in a subject in need thereof by administering a miR-137 inhibitor, a miR-138 inhibitor, or inhibitors of miR-137 and miR-138 together, wherein the function or activity of miR-137 and/or miR-138 is reduced in the cells of the subject following administration.

[0033] In one embodiment, the subject may be a diabetic patient who may be at risk for developing other disorders such as, diabetic retinopathy, diabetic neuropathy, or peripheral artery disease.

[0034] Preferably, administration of a miR-137 inhibitor and/or a miR-138 inhibitor to the subject results in the improvement of one or more symptoms of the above-mentioned conditions. For instance, in one embodiment, administration of a miR-137 inhibitor and/or a miR-138 inhibitor results in the improvement of one or more symptoms of hypertension, coronary heart disease, heart failure, or myocardial infarction in the subject. In another embodiment, administration of an inhibitor of a miR-137 inhibitor and/or a miR-138 inhibitor to the subject
results in the improvement of one or more symptoms of disorders related to diabetes, such as, diabetic retinopathy, peripheral artery disease and the like. The one or more improved symptoms may be, for example, increased blood flow to heart, lungs and peripheral organs, increased exercise capacity, increased cardiac ejection volume, decreased systolic blood pressure, decreased pulmonary capillary wedge pressure, increased cardiac output, increased cardiac index, lowered pulmonary artery pressures, decreased left ventricular end systolic and diastolic dimensions, decreased cardiac fibrosis, decreased collagen deposition in cardiac muscle, decreased left and right ventricular wall stress, decreased wall tension, increased quality of life, and decreased disease-related morbidity or mortality.

[0035] In some embodiments, administration of a miR-137 inhibitor and/or a miR-138 inhibitor to the subject prevents or slows the development and/or progression of the above-mentioned conditions. For instance, in one embodiment, administration of a miR-137 inhibitor and/or a miR-138 inhibitor prevents or slows the development and/or progression of hypertension in the subject.

[0036] In one embodiment, the present invention provides compositions and methods of treating or preventing hypertension in a subject in need thereof comprising administering a miR-137 inhibitor and/or a miR-138 inhibitor to the subject. In one embodiment, administration of an inhibitor of miR-137 and/or miR-138 in the subject improves one or more symptoms of hypertension. The one or more improved symptoms may be, for example, decreased or normal systolic pressure, increased or normal diastolic pressure, decreased mean system pressure, improved arterial elasticity increased quality of life, and decreased disease-related morbidity or mortality. In some embodiments, administration of an inhibitor of miR-137 and/or miR-138 prevents the development or onset of hypertension in the subject. In some other embodiments, administration of an inhibitor of miR-137 and/or miR-138 prevents or slows the progression of hypertension in the subject.

[0037] The present invention also provides methods for ameliorating or restoring arterial elasticity in a subject in need thereof by administering to the subject an inhibitor of miR-137 and/or an inhibitor of miR-138. Two proteins, collagen and elastin, in the vascular walls play an
important role in maintaining the elasticity and resilience of the arteries (Zieman et al., Arterioscler Thromb Vasc Biol, 25(5): 932-943, 2005). In one embodiment, the administration of an inhibitor of miR-137 and/or an inhibitor of miR-138 restores arterial elasticity by modulating the synthesis or degradation of collagen and/or elastin. In certain embodiments, the administration of an inhibitor of miR-137 and/or an inhibitor of miR-138 may restore arterial elasticity by promoting the synthesis of collagen and/or elastin. In other embodiments, the administration of an inhibitor of miR-137 and/or an inhibitor of miR-138 may restore arterial elasticity by preventing or inhibiting the damage or degradation of collagen and/or elastin.

[0038] In one embodiment, the methods of the invention comprise administering a composition comprising an inhibitor of miR-137 as an active agent. In another embodiment, the methods of the invention comprise administering a composition comprising an inhibitor of miR-138 as an active agent. In yet another embodiment, the methods of the invention comprise administering inhibitors of both miR-137 and miR-138 as active agents. In other embodiments, the methods of the invention comprise administering an additional therapeutic agent in addition to an inhibitor of miR-137 and/or an inhibitor of miR-138. Additional therapeutic agents include drugs used for treating one or more conditions disclosed herein. In one embodiment, the additional therapeutic agent is a drug used to treat hypertension. In another embodiment, the additional therapeutic agent is selected from the group consisting of diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and calcium channel blockers (CCBs). For instance, in one embodiment, the additional therapeutic agent may be a thiazide-type diuretic. In another embodiment, the additional therapeutic agent may be an ACE inhibitor such as perindopril, enalapril, ramipril, quinapril, lisinopril, benazepril, imidapril, trandolapril, cilazapril, captopril or zofenopril. In other embodiments, the additional therapeutic agent may be an ARB such as, valsartan, telmisartan, losartan, irbesartan, azilsartan, or olmesartan. In some other embodiments, the additional therapeutic agent may be a CCB, such as, amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, nisoldipine, or verapam. In one embodiment, the additional therapeutic agent is an ACE inhibitor, perindopril.

[0039] In the embodiments where inhibitors of both miR-137 and miR-138 are administered to the subject, it is contemplated that inhibitors of miR-137 and miR-138 may be given
simultaneously in the same formulation. Alternatively, the miR-137 inhibitor and the miR-138 inhibitor are administered in separate formulations either sequentially or concurrently, with concurrently referring to agents given within short period, for example, about 30 minutes of each other. Additional therapeutic agents may be given simultaneously in the same formulation along with a miR-137 inhibitor and/or a miR-138 inhibitor. Alternatively, a miR-137 inhibitor and/or a miR-138 inhibitor and the additional therapeutic agents are administered in separate formulations but concurrently, with concurrently referring to agents given within short duration, for example, about 30 minutes of each other.

[0040] In other embodiments, a miR-137 inhibitor is administered prior to administration of a miR-138 inhibitor or vice versa. In some other embodiments, a miR-137 inhibitor and/or a miR-138 inhibitor is administered prior to or after administration of additional therapeutic agents. Prior administration includes, for instance, administration of the first agent within the range of about one week to up to 30 minutes prior to administration of the second agent. Prior administration may also include, for instance, administration of the first agent within the range of about 2 weeks to up to 30 minutes prior to administration of the second agent. After or later administration includes, for instance, administration of the second agent within the range of about one week to up to 30 minutes after administration of the first agent. After or later administration may also include, for instance, administration of the second agent within the range of about 2 weeks to up to 30 minutes after administration of the first agent.

[0041] The present invention provides oligonucleotide inhibitors that reduce or inhibit the function of human miR-137 or human miR-138. In the context of the present invention, the term “oligonucleotide inhibitor”, “antimiR” (e.g., antimiR-137 or antimiR-138), “antagonist”, “antisense oligonucleotide or ASO”, “oligomer”, “anti-microrna oligonucleotide, anti-miRNA oligonucleotide, or AMO”, or “mixmer” is used broadly and encompasses an oligomer comprising ribonucleotides, deoxyribonucleotides, modified ribonucleotides, modified deoxyribonucleotides or a combination thereof, that inhibits the activity or function of the target microRNA (e.g., miR-137 or miR-138) by fully or partially hybridizing to the miRNA thereby repressing the function or activity of the target miRNA.
[0042] The term “miR-137” as used herein includes pri-miR-137, pre-miR-137, miR-137-5p, and hsa-miR-137-5p. Likewise, the term “miR-138” as used herein includes pri-miR-138, pre-miR-138, miR-138-5p, and hsa-miR-138-5p.

[0043] As described herein, in some embodiments, inhibitors of miR-137 and miR-138 are oligonucleotide inhibitors. The oligonucleotide inhibitor can include ribonucleotides or deoxyribonucleotides or a combination thereof.

[0044] In one embodiment, the oligonucleotide inhibitor of miR-137 and/or miR-138 contains at least one backbone modification, such as at least one phosphorothioate, morpholino, or phosphonocarboxylate internucleotide linkage (see, for example, U.S. Patent Nos. 6,693,187 and 7,067,641, which are herein incorporated by reference in their entireties). In certain embodiments, the oligonucleotide inhibitor of miR-137 and/or miR-138 is fully phosphorothioate-linked.

[0045] In one embodiment, the oligonucleotide inhibitor of miR-137 and/or miR-138 has at least one modified nucleotide such as a locked nucleotide or a nucleotide containing other sugar or base modifications. The terms “locked nucleotide,” “locked nucleic acid unit,” “locked nucleic acid residue,” or “LNA unit” may be used interchangeably throughout the disclosure and refer to a bicyclic nucleoside analogue. For instance, suitable oligonucleotide inhibitors can be comprised of one or more “conformationally constrained” or bicyclic sugar nucleoside modifications (BSN) that confer enhanced thermal stability to complexes formed between the oligonucleotide containing BSN and their complementary target strand. In one embodiment, the oligonucleotide inhibitors contain locked nucleotides or LNAs containing the 2’-O, 4’-C-methylene ribonucleoside (structure A) wherein the ribose sugar moiety is in a “locked” conformation. In another embodiment, the oligonucleotide inhibitors contain at least one 2’, 4’-C-bridged 2’ deoxyribonucleoside (CDNA, structure B). See, e.g., U.S. Patent No. 6,403,566 and Wang et al. (1999) Bioorganic and Medicinal Chemistry Letters, Vol. 9: 1147-1150, both of which are herein incorporated by reference in their entireties. In yet another embodiment, the oligonucleotide inhibitors contain at least one modified nucleoside having the structure shown in structure C. The oligonucleotide inhibitors targeting miR-137 and/or miR-138 can contain
combinations of BSN (LNA, CDNA and the like) or other modified nucleotides, and ribonucleotides or deoxyribonucleotides.

![Diagram A](image)

![Diagram B](image)

![Diagram C](image)

[0046] When referring to substituting a DNA or RNA nucleotide by its corresponding locked nucleotide in the context of the present invention, the term “corresponding locked nucleotide” is intended to mean that the DNA/RNA nucleotide has been replaced by a locked nucleotide containing the same naturally-occurring nitrogenous base as the DNA/RNA nucleotide that it has replaced or the same nitrogenous base that is chemically modified. For example, the corresponding locked nucleotide of a DNA nucleotide containing the nitrogenous base C may contain the same nitrogenous base C or the same nitrogenous base C that is chemically modified, such as 5-methylcytosine.

[0047] The term “non-locked nucleotide” refers to a nucleotide different from a locked-nucleotide, i.e., the term “non-locked nucleotide” includes a DNA nucleotide, an RNA nucleotide as well as a modified nucleotide where a base and/or sugar is modified except that the modification is not a locked modification.

[0048] Oligonucleotide inhibitors of the present invention may include modified nucleotides that have a base modification or substitution. The natural or unmodified bases in RNA are the purine bases adenine (A) and guanine (G), and the pyrimidine bases cytosine (C) and uracil (U) (DNA has thymine (T)). Modified bases, also referred to as heterocyclic base moieties, include other...
synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-alkyl, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (including 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines), 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino- adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. In certain embodiments, oligonucleotide inhibitors targeting miR-137 and/or miR-138 comprise one or more BSN modifications in combination with a base modification (e.g. 5-methyl cytidine).

[0049] Oligonucleotide inhibitors of the present invention may include nucleotides with modified sugar moieties. Representative modified sugars include carbocyclic or acyclic sugars, sugars having substituent groups at one or more of their 2’, 3’ or 4’ positions and sugars having substituents in place of one or more hydrogen atoms of the sugar. In certain embodiments, the sugar is modified by having a substituent group at the 2’ position. In additional embodiments, the sugar is modified by having a substituent group at the 3’ position. In other embodiments, the sugar is modified by having a substituent group at the 4’ position. It is also contemplated that a sugar may have a modification at more than one of those positions, or that an oligonucleotide inhibitor may have one or more nucleotides with a sugar modification at one position and also one or more nucleotides with a sugar modification at a different position.

[0050] Sugar modifications contemplated in the oligonucleotide inhibitors of the present invention include, but are not limited to, a substituent group selected from: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted with C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. In one embodiment, the modification includes 2’-methoxyethoxy (2’-O-CH₂CH₂OCH₃, which is also known as 2’-O-(2-methoxyethyl) or 2’-MOE), that is, an alkoxyalkoxy group. Another modification includes 2’-dimethylaminoxyethoxy, that is, a O(CH₃)₂ON(CH₃)₂ group,
also known as 2’-DMAOE and 2’-dimethylaminoethoxyethoxy (also known in the art as 2’-O-dimethyl-amino-ethoxy-ethyl or 2’-DMAEOE), that is, 2’-O-CH₂-O-CH₂-N(CH₃)₂.

**[0051]** Additional sugar substituent groups include allyl (-CH₂-CH=CH₂), -O-allyl, methoxy (-O-CH₃), aminopropoxy (-OCH₂CH₂CH₂NH₂), and fluoro (F). Sugar substituent groups on the 2’ position (2’-) may be in the arabino (up) position or ribo (down) position. One 2’-arabino modification is 2’-F. Other similar modifications may also be made at other positions on the sugar moiety, particularly the 3’ position of the sugar on the 3’ terminal nucleoside or in 2’-5’ linked oligonucleotides and the 5’ position of 5’ terminal nucleotide. In certain embodiments, the sugar modification is a 2’-O-alkyl (e.g., 2’-O-methyl, 2’-O-methoxyethyl), 2’-halo (e.g., 2’-fluoro, 2’-chloro, 2’-bromo), and 4’ thio modifications.

**[0052]** Other modifications of oligonucleotide inhibitors to enhance stability and improve efficacy, such as those described in U.S. Patent No. 6,838,283, which is herein incorporated by reference in its entirety, are known in the art and are suitable for use in the methods of the invention. For instance, to facilitate *in vivo* delivery and stability, the oligonucleotide inhibitor can be linked to a steroid, such as cholesterol moiety, a vitamin, a fatty acid, a carbohydrate or glycoside, a peptide, or other small molecule ligand at its 3’ end.

**[0053]** In one embodiment, oligonucleotide inhibitors targeting miR-137 and/or miR-138 may contain one or more combinations of modifications described herein.

**[0054]** Preferable oligonucleotide inhibitors useful for inhibiting the activity of miRNAs are about 5 to about 25 nucleotides in length, about 6 to about 22 nucleotides in length, about 10 to about 30 nucleotides in length, or about 20 to about 25 nucleotides in length. In certain embodiments, oligonucleotide inhibitors targeting miR-137 or miR-138 are about 8 to about 18 nucleotides in length, and in other embodiments about 12 to about 16 nucleotides in length. In some embodiments, oligonucleotide inhibitors targeting miR-137 or miR-138 are about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, or 25 nucleotides in length. Any 6-mer or longer oligonucleotide complementary to target miRNA (miR-137 or miR-138) may be used, *i.e.*, any antimiR complementary to the 5’ end of the target miRNA and progressing across the
full complementary sequence of the miRNA. In one embodiment, the oligonucleotide inhibitor of miR-137 comprises a sequence of 5'-IGs.dTs.dAs.dTs.lTs.1Gs.1As.1Gs.dCs.1As.dAs.lTs.lA-3' (SEQ ID NO: 27). In another embodiment, the oligonucleotide inhibitor of miR-137 comprises a sequence of 5'-GTATTCTTAAGCAATA-3' (SEQ ID NO: 28). In one embodiment, the oligonucleotide inhibitor of miR-138 comprises a sequence of 5'-IGs.dAs.dTs.dTs.lTs.lCs.dTs.lTs.dAs.lAs.lGs.dCs.lAs.dAs.lCs.lAs.dAs.lGs.lC-3' (SEQ ID NO: 29). In another embodiment, the oligonucleotide of miR-138 comprises a sequence of 5'-GATTCACAACACCAGC-3' (SEQ ID NO: 30). As used herein, an “l” preceding a base notation (e.g. A, U, G, C) indicates a locked nucleic acid modification, a “d” preceding a base notation indicates a deoxynucleotide, and an “s” indicates a phosphorothioate linkage. Unless otherwise indicated, the nucleotides in the oligonucleotide inhibitors disclosed herein are linked by phosphodiester linkages.

[0055] Oligonucleotide inhibitors targeting miR-137 comprise a sequence that is sufficiently complementary to a miR-137 sequence to hybridize to miR-137 under physiological conditions and inhibit the function or activity of miR-137 in the cells of a subject. Similarly, oligonucleotide inhibitors targeting miR-138 comprise a sequence that is sufficiently complementary to a miR-138 sequence to hybridize to miR-138 under physiological conditions and inhibit the function or activity of miR-138 in the cells of a subject. For instance, in some embodiments, oligonucleotide inhibitors comprise a sequence that is at least partially complementary to a mature miR-137 or miR-138 sequence, e.g. at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% complementary to a mature miR-137 or miR-138 sequence. In some embodiments, the oligonucleotide inhibitors can be substantially complementary to a mature miR-137 or miR-138 sequence, that is at least about 90%, 95%, 96%, 97%, 98%, or 99% complementary to a target polynucleotide sequence. In one embodiment, the oligonucleotide inhibitor comprises a sequence that is 100% or completely complementary to a mature miR-137 or miR-138 sequence. In certain embodiments, the oligonucleotide inhibitor targeting miR-137 is at least partially complementary or completely complementary to SEQ ID NO: 1 and the oligonucleotide inhibitor targeting miR-138 is at least partially complementary or completely complementary to SEQ ID NO: 9 or SEQ ID NO: 12.
In some embodiments, the oligonucleotide inhibitors are antagomirs. “Antagomirs” are single-stranded, chemically-modified ribonucleotides that are at least partially complementary to a miR-137 or miR-138 sequence. Antagomirs may comprise one or more modified nucleotides, such as 2’-O-methyl-sugar modifications. In some embodiments, antagomirs comprise only modified nucleotides. Antagomirs can also comprise one or more phosphorothioate linkages resulting in a partial or full phosphorothioate backbone. To facilitate *in vivo* delivery and stability, the antagomir can be linked to cholesterol or other moiety at its 3’ end. Antagomirs suitable for inhibiting miR-137 or miR-138 can be about 15 to about 50 nucleotides in length, more preferably about 18 to about 30 nucleotides in length, and most preferably about 20 to about 25 nucleotides in length. The antagomirs can be at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% complementary to a mature miR-137 or miR-138 sequence. In some embodiments, the antagomir may be substantially complementary to a mature miR-137 or miR-138 sequence, that is at least about 95%, 96%, 97%, 98%, or 99% complementary to a target polynucleotide sequence. In other embodiments, the antagomirs are 100% complementary to a mature miR-137 or miR-138 sequence.

The inhibitory nucleotide molecules described herein preferably target the mature sequence of miR-137 or miR-138 (SEQ ID NO: 1 or SEQ ID NO: 9). In some embodiments, inhibitors of miR-137 or miR-138 are antagomirs comprising a sequence that is perfectly complementary to the mature miR-137 or miR-138 sequence. In one embodiment, an inhibitor of miR-137 is an antagomir having a sequence that is partially or perfectly complementary to SEQ ID NO: 1. In some embodiments, an inhibitor of miR-138 is an antagomir having a sequence that is partially or perfectly complementary to SEQ ID NO: 9 or SEQ ID NO: 12. In some embodiments, inhibitors of miR-137 and/or miR-138 are chemically-modified antisense oligonucleotides. In one embodiment, an inhibitor of miR-137 is a chemically-modified antisense oligonucleotide comprising a sequence substantially complementary to SEQ ID NO: 1. In some embodiments, an inhibitor of miR-138 is a chemically-modified antisense oligonucleotide comprising a sequence substantially complementary to SEQ ID NO: 9 or SEQ ID NO: 12. As used herein “substantially complementary” refers to a sequence that is at least
about 95%, 96%, 97%, 98%, 99%, or 100% complementary to a target polynucleotide sequence (e.g. mature, minor, or precursor miRNA sequence).

[0058] Oligonucleotide inhibitors or antagomirs may comprise a sequence that is substantially complementary to a precursor miRNA sequence (pre-miRNA) or primary miRNA sequence (pri-miRNA) for miR-137 or miR-138. In one embodiment, an inhibitor of miR-137 is an antagomir having a sequence that is partially or perfectly complementary to SEQ ID NO: 2. In some embodiments, the oligonucleotide inhibitor comprises a sequence that is substantially complementary to a sequence located outside the stem-loop region of the pre-miR-137 sequence. In one embodiment, an inhibitor of miR-138 is an antagomir having a sequence that is partially or perfectly complementary to SEQ ID NO: 11 or SEQ ID NO: 14. In some embodiments, the oligonucleotide inhibitor comprises a sequence that is substantially complementary to a sequence located outside the stem-loop region of the pre-miR-138 sequence.

[0059] In another embodiment of the invention, an inhibitor of miR-137 or miR-138 may be a decoy miR-137 or a decoy miR-138. Decoy sequences can be nucleic acid sequences comprising multiple (e.g. two or more) binding sites that each hybridize to the target miRNA sequence. In one embodiment, the binding sites comprise a sequence that is at least partially complementary to a miR-137 or miR-138 sequence thereby sequestering endogenous miR-137 or miR-138, respectively. For example, a decoy sequence targeting miR-137 may comprise sequences from the 3’UTR of one or more targets of miR-137, such as Klf2, Klf4, Sik1, Hmgn3, Lsd1, Cdc42, and Asph. Similarly, a decoy sequence targeting miR-138 may comprise sequences from the 3’UTR of one or more targets of miR-138, such as Sirt1. In certain embodiments, the decoy sequence may be expressed from a vector. The decoy nucleic acid sequences comprising two or more miR-137 or miR-138 binding sites may be from about 15 to about 500 nucleotides in length, about 25 to about 400 nucleotides in length, about 30 to about 300 nucleotides in length, about 40 to about 200 nucleotides in length, about 50 to about 100 nucleotides in length, or about 60 to about 80 nucleotides in length. For example, the nucleic acid may be 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 350, 400, 500 nucleotides in length. The decoy nucleic acid may contain 2, 3, 4, 5, 6, 7, 8, 9,
10, 12, 15, or 20 miR-137 or miR-138 binding sites. The multiple target binding sites may be adjacent or may be separated by spacers of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20 or more nucleotides.

[0060] As used herein, the term “subject” or “patient” refers to any vertebrate including, without limitation, humans and other primates (e.g., chimpanzees and other apes and monkey species), farm animals (e.g., cattle, sheep, pigs, goats and horses), domestic mammals (e.g., dogs and cats), laboratory animals (e.g., rodents such as mice, rats, and guinea pigs), and birds (e.g., domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like). In some embodiments, the subject is a mammal. In other embodiments, the subject is a human.

[0061] Any of the oligonucleotide inhibitors of miR-137 or miR-138 described herein can be delivered to the target cell (e.g. cells of aorta, kidney, heart, lung, endothelial cells, and vascular smooth muscle cells) by delivering to the cell an expression vector encoding the miR-137 and/or miR-138 inhibitors. A "vector" is a composition of matter which can be used to deliver a nucleic acid of interest to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, and the like. In one embodiment, the viral vector is a lentiviral vector or an adeno viral vector. An expression construct can be replicated in a living cell, or it can be made synthetically. For purposes of this application, the terms “expression construct,” “expression vector,” and “vector,” are used interchangeably to demonstrate the application of the invention in a general, illustrative sense, and are not intended to limit the invention.

[0062] In one embodiment, an expression vector for expressing an oligonucleotide inhibitor of miR-137 and/or an oligonucleotide inhibitor of miR-138 comprises a promoter operably linked to a polynucleotide encoding the oligonucleotide inhibitor, wherein the sequence of the oligonucleotide inhibitor directed to miR-137 is partially or perfectly complementary to a mature
sequence of miR-137 (e.g., SEQ ID NO: 1) and the sequence of the oligonucleotide inhibitor directed to miR-138 is partially or perfectly complementary to a mature sequence of miR-138 (e.g., SEQ ID NO: 9 or 12). In one embodiment, an expression vector for expressing an oligonucleotide inhibitor of miR-137 and/or an oligonucleotide inhibitor of miR-138 comprises a promoter operably linked to a polynucleotide encoding a decoy miR-137 sequence and/or a promoter operably linked to a polynucleotide encoding a decoy miR-138 sequence. The phrase "operably linked" or "under transcriptional control" as used herein means that the promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.


[0064] In certain embodiments, the promoter operably linked to a polynucleotide encoding a miR-137 or miR-138 inhibitor can be an inducible promoter. Inducible promoters are known in
the art and include, but are not limited to, tetracycline promoter, metallothionein IIA promoter, heat shock promoter, steroid/thyroid hormone/retinoic acid response elements, the adenovirus late promoter, and the inducible mouse mammary tumor virus LTR.

[0065] Methods of delivering expression constructs and nucleic acids to cells are known in the art and can include, for example, calcium phosphate co-precipitation, electroporation, microinjection, DEAE-dextran, lipofection, transfection employing polyamine transfection reagents, cell sonication, gene bombardment using high velocity microprojectiles, and receptor-mediated transfection.

[0066] The present invention also includes methods for scavenging or clearing miR-137 and/or miR-138 inhibitors following treatment. The method may comprise overexpressing binding sites for the miR-137 and/or miR-138 inhibitors in cardiac, vascular, or skeletal muscle tissue. In one embodiment, the binding site regions for the miR-137 inhibitor preferably contain a sequence of the seed region for miR-137 and the binding site regions for the miR-138 inhibitor preferably contain a sequence of the seed region for miR-138. The seed region is the 5’ portion of a miRNA spanning bases 2-8, which is important for target recognition. In some embodiments, the binding site for the miR-137 inhibitor may contain a sequence complementary to the 3’UTR of one or more targets of miR-137, such as Klf2, Klf4, Sik1, Hmgn3, Lsd1, Cdc42, and Asph. In some embodiments, the binding site for the miR-138 inhibitor may contain a sequence complementary to the 3’UTR of one or more targets of miR-138, such as Sirt1.

[0067] The present invention also includes pharmaceutical compositions comprising an inhibitor of miR-137 and a pharmaceutically acceptable carrier or excipient. In another embodiment, the present invention provides pharmaceutical compositions comprising an inhibitor of miR-138 and a pharmaceutically acceptable carrier or excipient. In yet another embodiment, the present invention provides pharmaceutical compositions comprising an inhibitor of miR-137 and an inhibitor of miR-138 in the same formulation. In other embodiments, inhibitors of miR-137 and miR-138 may be administered concurrently but in separate compositions, with concurrently referring to inhibitors given within short period, for instance, about 30 minutes of each other. In some other embodiments, inhibitors of miR-137 and miR-138 may be administered in separate
compositions at different times. In embodiments where additional therapeutic agents are included, the additional agents may be administered concurrently but in separate formulations or sequentially. In other embodiments, additional therapeutic agents may be administered at different times prior to after administration of a miR-137 inhibitor and/or a miR-138 inhibitor. Prior administration includes, for instance, administration of the first agent within the range of about one week to up to 30 minutes prior to administration of the second agent. Prior administration may also include, for instance, administration of the first agent within the range of about 2 weeks to up to 30 minutes prior to administration of the second agent. After or later administration includes, for instance, administration of the second agent within the range of about one week to up to 30 minutes after administration of the first agent. After or later administration may also include, for instance, administration of the second agent within the range of about 2 weeks to up to 30 minutes after administration of the first agent. Where clinical applications are contemplated, pharmaceutical compositions will be prepared in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[0068] In one embodiment, the pharmaceutical composition comprises an effective dose of a miR-137 inhibitor and a pharmaceutically acceptable carrier. In another embodiment, the pharmaceutical composition comprises an effective dose of a miR-138 inhibitor and a pharmaceutically acceptable carrier. In yet another embodiment, the pharmaceutical composition comprises an effective dose of a miR-137 inhibitor, an effective dose of a miR-138 inhibitor, and a pharmaceutically acceptable carrier. For instance, the pharmaceutical composition comprises an effective dose of a modified oligonucleotide inhibitor targeting miR-137 and/or an effective dose of a modified oligonucleotide inhibitor targeting miR-138 as described herein. In some embodiments, the pharmaceutical composition comprises a modified oligonucleotide inhibitor having a sequence selected from the group consisting of SEQ ID NOs: 27-28. In some other embodiments, the pharmaceutical composition comprises a modified oligonucleotide inhibitor having a sequence selected from the group consisting of SEQ ID NOs: 29-30. In yet some other embodiments, the pharmaceutical composition comprises a modified oligonucleotide inhibitor having a sequence selected from the group consisting of SEQ ID NOs:
27-28 and a modified oligonucleotide inhibitor having a sequence selected from the group consisting of SEQ ID NOs: 29-30. An “effective dose” is an amount sufficient to effect a beneficial or desired clinical result. An effective dose of an oligonucleotide inhibitor of the invention may be from about 1 mg/kg to about 100 mg/kg, about 2.5 mg/kg to about 50 mg/kg, or about 5 mg/kg to about 25 mg/kg. The precise determination of what would be considered an effective dose may be based on factors individual to each patient, including their size, age, type of disorder (e.g. hypertension, renovascular hypertension, pulmonary arterial hypertension, myocardial infarction, heart failure, coronary or peripheral artery disease), and nature of inhibitor (e.g. antagonir, expression construct, oligonucleotide inhibitor, decoy, etc.). Therefore, dosages can be readily ascertained by those of ordinary skill in the art from this disclosure and the knowledge in the art.

[0069] Colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes, may be used as delivery vehicles for the oligonucleotide inhibitors of miR-137 and/or miR-138, or constructs expressing particular miRNA inhibitors.

[0070] Liposomal formulations may be particularly useful compositions of the subject invention. In one embodiment, the oligonucleotides are contained within the liposome. Examples of liposomes are unilamellar, multilamellar, and paucilamellar liposomes, which may or may not contain phospholipids. Such compositions can be prepared by combining the oligonucleotide inhibitor with a phospholipid, such as dipalmitoylphosphatidyl choline, cholesterol and water. Commercially available fat emulsions that are suitable for delivering the oligonucleotide inhibitors of the invention to cardiac and skeletal muscle tissues include Intralipid®, Liposyn®, Liposyn® II, Liposyn® III, Nutrilipid, and other similar lipid emulsions. A preferred colloidal system for use as a delivery vehicle in vivo is a liposome (i.e., an artificial membrane vesicle). The preparation and use of such systems is well known in the art. Exemplary formulations are also disclosed in US 5,981,505; US 6,217,900; US 6,383,512; US 5,783,565; US 7,202,227; US 6,379,965; US 6,127,170; US 5,837,533; US 6,747,014; and WO03/093449, which are herein incorporated by reference in their entireties.
In certain embodiments, liposomes used for delivery are amphoteric liposomes such as SMARTICLES® (Marina Biotech, Inc.) which are described in detail in U.S. Pre-grant Publication No. 20110076322. The surface charge on the SMARTICLES® is fully reversible which make them particularly suitable for the delivery of nucleic acids. SMARTICLES® can be delivered via injection, remain stable, and aggregate free and cross cell membranes to deliver the nucleic acids.

One will generally desire to employ appropriate salts and buffers to render delivery vehicles stable and allow for uptake by target cells. Aqueous compositions of the present invention comprise an effective amount of the delivery vehicle comprising the inhibitor polynucleotides (e.g., liposomes or other complexes or expression vectors) dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The phrases “pharmaceutically acceptable” or “pharmacologically acceptable” refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, “pharmaceutically acceptable carrier” includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or polynucleotides of the compositions.

The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention may be via any common route so long as the target tissue is available via that route. This includes oral, nasal (inhalational), or buccal. Alternatively, administration may be by intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or by direct injection into cardiac tissue. Pharmaceutical compositions comprising oligonucleotide inhibitors or expression constructs comprising oligonucleotide inhibitors may also be administered by catheter systems or...

[0074] In another embodiment of the invention, compositions comprising miR-137 and/or miR-138 inhibitors as described herein may be formulated as a coating for a medical device, such as a stent, balloon, or catheter. Particularly useful in methods of treating cardiovascular disorders in a subject, the inhibitors of miR-137 and/or miR-138 can be used to coat a metal stent to produce a drug-eluting stent. A drug-eluting stent is a scaffold that holds open narrowed or diseased arteries and releases a compound to prevent cellular proliferation and/or inflammation. The inhibitors of miR-137 and/or miR-138 may be applied to a metal stent imbedded in a thin polymer for release of the agonists or inhibitors over time. Methods for device-based delivery and methods of coating devices are well known in the art, as are drug-eluting stents and other implantable devices. See, e.g., U.S. Pat. Nos. 7,294,329, 7,273,493, 7,247,313, 7,236,821, 7,232,573, 7,156,869, 7,144,422, 7,105,018, 7,087,263, 7,083,642, 7,055,237, 7,041,127, 6,716,242, and 6,589,286, and WO 2004/004602, which are herein incorporated by reference in their entireties. Thus, the present invention includes a medical device, such as a balloon, catheter, or stent, coated with a miR-137 inhibitor and/or a miR-138 inhibitor. In some embodiments, the miR-137 inhibitor and/or miR-138 inhibitor can be used in combination with other therapeutic agents (e.g. anti-restenosis compounds) to produce a formulation for incorporation into drug-eluting stents.

[0075] The active compounds may also be administered parenterally or intraperitoneally. By way of illustration, solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as
hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally contain a preservative to prevent the growth of microorganisms.

[0076] The pharmaceutical forms suitable for injectable use or catheter delivery include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0077] Sterile injectable solutions may be prepared by incorporating the active compounds in an appropriate amount into a solvent along with any other ingredients (for example as enumerated above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients, e.g., as enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof.
[0078] The compositions of the present invention generally may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, acid addition salts (formed with the free amino groups of the protein) derived from inorganic acids (e.g., hydrochloric or phosphoric acids), or from organic acids (e.g., acetic, oxalic, tartaric, mandelic, and the like). Salts formed with the free carboxyl groups of the protein can also be derived from inorganic bases (e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides) or from organic bases (e.g., isopropylamine, trimethylamine, histidine, procaine and the like).

[0079] Upon formulation, solutions are preferably administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations may easily be administered in a variety of dosage forms such as injectable solutions, drug release capsules, eye drops, intravitreal injections, drug-eluting stents or other coated vascular devices, and the like. For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic for example with sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous, intravitreal and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0080] In certain embodiments of the invention, the pharmaceutical compositions of the invention are packaged with or stored within a device for administration. Devices for injectable formulations include, but are not limited to, injection ports, autoinjectors, injection pumps, and injection pens. Devices for aerosolized or powder formulations include, but are not limited to, inhalers, insufflators, aspirators, and the like. Thus, the present invention includes
administration devices comprising a pharmaceutical composition of the invention for treating or preventing one or more of the disorders described herein.

[0081] This invention is further illustrated by the following additional examples that should not be construed as limiting. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made to the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0082] All patent and non-patent documents referenced throughout this disclosure are incorporated by reference herein in their entirety for all purposes.

EXAMPLES:

Example 1: Animal models of arterial stiffness

[0083] Various rodent models are used to study hypertension and arterial stiffness. For example, aged mice or aged spontaneously hypertensive rats (SHR) serve as a useful model to study arterial stiffness (Komatsu et al., Hypertension, 25(2):207-213, 1995). These rodents mimic the pathophysiology of arterial stiffness quite well because even in the absence of other risk factors such as high blood pressure, aging alone can result in arterial stiffening. Additional models include a DOCA-rat model and a SHR/L-NAME model, which serve as models for vascular stiffening in the presence of high blood pressure. In the DOCA (deoxycorticosterone acetate) model, rats are subjected to nephrectomy followed by a dose of deoxycorticosterone. In the SHR/L-NAME model, adult spontaneously hypertensive rats (SHR) are treated with L-NG-Nitroarginine Methyl Ester (L-NAME). L-NAME is a nonspecific nitric oxide (NO) synthase inhibitor. Treatment of adult SHRs with L-NAME results in pathophysiology that is similar to aged SHRs, including endothelial dysfunction, severe hypertension, and end-stage renal disease.

Example 2: Profiling of miRNAs in rodent models of arterial stiffness

[0084] The role of miRNAs in arterial stiffness was investigated by profiling miRNAs from the aorta of rats that had undergone unilateral nephrectomy followed by implantation of a deoxycorticosterone acetate (DOCA) pellet. These rats exhibit hypertension and vascular
stiffness as assessed by aortic pulse-wave velocity (PWV). The real-time PCR analysis of RNA revealed that the expression of miR-137 and miR-138 is significant upregulated in the aorta after 4 weeks of DOCA treatment (FIG. 1).

Example 3: Inhibition of miR-137 and miR-138 reduces arterial stiffness and blood pressure

To determine if the increased expression of miR-137 and miR-138 plays a causal role in the progression of arterial stiffness, spontaneously hypertensive rats at 16 weeks of age were subjected to four weekly 25 mg/kg subcutaneous doses of an antimiR-137 or antimiR-138. At 18 weeks, L-NG-Nitroarginine Methyl Ester (L-NAME) was administered in the drinking water (50 mg/L), given ad libitum for 2 weeks to create a therapeutic window in arterial stiffness as assessed by pulse-wave velocity (PWV) measurements. At 20 weeks of age, terminal pulse-wave velocity and blood pressure were measured. Perindopril, an ACE inhibitor, was included in the study as a positive control. All groups received L-NAME. The study outline is shown in FIG. 2. Treatment of SHR/L-NAME rats with a miR-137 oligonucleotide inhibitor showed a statistically-significant reduction in pulse-wave velocity but no effect on mean system pressure, suggesting a direct effect on the vessel wall (FIG. 3). Treatment of SHR/L-NAME rats with a miR-138 oligonucleotide inhibitor showed a statistically-significant reduction in pulse-wave velocity and mean system pressure. A control oligo did not show a similar benefit with this dose and regime, thereby showing the microRNA specificity of this therapeutic effect. Together, these results demonstrate that the inhibition of miR-137 or miR-138 has therapeutic benefits in the treatment of arterial stiffness and hypertension.^^

The inhibitory activity of the miR-137 oligonucleotide inhibitor was confirmed by measuring the miR-137 target de-repression in kidneys. The target de-repression was measured in kidneys, instead of aorta, because the measurement of pulse-wave velocity results in robust RNA degradation in the aorta. The real-time PCR analysis of two miR-137 targets, Klf2 and Klf4, from the kidneys of rats treated with saline, perindopril, or the miR-137 oligonucleotide inhibitor was performed. The results show that the miR-137 oligonucleotide inhibitor treatment, but not the perindopril treatment, resulted in significantly increased expression of Klf2 and Klf4 in the kidneys (FIG. 4). As the Klf5 are considered to be the gate-keepers of vascular biology (Atkins and Jain, Circ Res, 100(12): 1686-1695, 2007), the increase in the expression of Klf5

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with the miR-137 oligonucleotide inhibitor, but not perindopril, suggests that the miR-137 oligonucleotide inhibitor treatment may show an additive or synergistic effect with perindopril as these agents appear to be acting through separate pathways.

Example 4: Inhibition of miR-137 increases the expression of miR-137 targets in aorta and kidneys

[0087] To study the effect of inhibition of miR-137 on its targets, adults SHRs were treated subcutaneously with 25 mg/kg antimiR-137. The miR-137 target expression was analyzed 24 hours later in the aorta, kidney, and right ventricle using real-time PCR. Treatment with the miR-137 oligonucleotide inhibitor showed significant target de-repression in the aorta and kidney, but not right ventricle at 24 hours (FIG. 5).
CLAIMS:

1. A method of treating or preventing arterial stiffness in a subject in need thereof comprising administering to the subject an inhibitor of miR-137 and/or an inhibitor of miR-138.

2. The method of claim 1, wherein the subject in need thereof is diagnosed with or at risk for a condition selected from the group consisting of essential hypertension, secondary hypertension, renovascular hypertension, resistant hypertension, peripheral arterial disease, coronary artery disease, atherosclerosis, arteriosclerosis, aneurysm, angina, hypertensive heart disease, heart failure, ischemia, cor pulmonale, pulmonary hypertension, pulmonary arterial hypertension, diabetic nephropathy, diabetic retinopathy, optic neuropathy, cerebrovascular disease, stroke, hypertensive encephalopathy, myocardial infarction, vascular calcification, hypertensive retinopathy, hypertensive nephropathy, hypertensive nephrosclerosis, restenosis and thrombosis.

3. The method of claim 1, wherein the inhibitor is an oligonucleotide inhibitor.

4. The method of claim 3, wherein the oligonucleotide inhibitor of miR-137 comprises a sequence that is at least partially complementary to a mature sequence of miR-137 and/or the oligonucleotide inhibitor of miR-138 comprises a sequence that is at least partially complementary to a mature sequence of miR-138.

5. The method of claim 4, wherein the oligonucleotide inhibitor comprises at least one sugar and/or backbone modification.

6. The method of claim 5, wherein the sugar modification is a locked nucleic acid.

7. The method of claim 5, wherein the sugar modification is a 2’ O-alkyl modification.

8. The method of claim 5, wherein the sugar modification is a 2’-halo modification.
9. The method of claim 8, wherein the 2'-halo modification is a 2'-fluoro modification.

10. The method of claim 5, wherein the backbone modification is a phosphorothioate linkage.

11. The method of claim 5, wherein the oligonucleotide inhibitor further comprises a base modification.

12. The method of claim 11, wherein the base modification comprises 5-methyl cytidine.

13. The method of any one of claims 3-13, wherein the oligonucleotide inhibitor is about 6 to about 22 nucleotides in length.

14. The method of any one of claims 3-13, wherein the oligonucleotide inhibitor of miR-137 has a sequence that is completely complementary to a nucleotide sequence of miR-137 and/or the oligonucleotide inhibitor of miR-138 has a sequence that is completely complementary to a nucleotide sequence of miR-138.

15. The method of claim 3, wherein the oligonucleotide inhibitor of miR-137 comprises a sequence selected from the group consisting of SEQ ID NOs: 27-28 and/or the oligonucleotide inhibitor of miR-138 comprises a sequence selected from the group consisting of SEQ ID NOs: 29-30.

16. The method of claim 1, wherein the inhibitor is administered to the subject intravenously.

17. The method of claim 1, wherein the inhibitor is administered to the subject by an inhalational route of administration.

18. The method of claim 1, wherein the subject is human.

19. The method of claim 1, further comprising administering an additional therapeutic agent to the subject.
20. The method of claim 19, wherein the additional therapeutic agent is perindopril.

21. The method of any one of the preceding claims, wherein the function or activity of miR-137 and/or miR-138 is reduced in the cells of the subject following administration.

22. The method of claim 21, wherein the cells of the subject in which the function or activity of miR-137 and/or miR-138 is reduced are selected from the group consisting of cells of aorta, kidney, heart, lung, endothelial cells, and vascular smooth muscle cells.
FIG. 1
FIG. 2
FIG. 4
FIG. 5
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/57760

A. CLASSIFICATION OF SUBJECT MATTER

IPC (8) - A61K 31/7088, A61K 31/712, C12N 15/113 (2016.01)
CPC - A61K 31/7088, C12N 15/113, C12N 2310/113

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61K 31/7088, A61K 31/712, C12N 15/113 (2016.01)
CPC: A61K 31/7088, C12N 15/113, C12N 2310/113

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC: A61K 31/713, C12N 2310/3233, C12N 2310/35

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWest, PatBase, Google Scholar, google Patents: arterial stiffness, miR-137, miR-138, nitric oxide, 2'-halo, O'-alkyl, lock, nucleic acid, oligonucleotide, modif", inhibit", essential hypertension, secondary hypertension, renovascular hypertension, resistant hypertension, peripheral arterial disease, coronary artery disease, atherosclerosis, ar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>SUGAWARA et al., Effect of Systemic Nitric Oxide Synthase Inhibition on Arterial Stiffness in Humans, Hyportono Roo, December 2006, Vol. 30, No. 527, pag3 4 11-415; abstract, page 4 11, col 2, para 1:414, col 1, para 3;</td>
<td>1-13 and 15-20</td>
</tr>
<tr>
<td>Y</td>
<td>US 2014/007929 A1 (KALIDINI) 20 March 2014 (20.03.2014); para [0015]</td>
<td>2, 13/2</td>
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<tr>
<td>Y</td>
<td>US 2013/0281514 A1 (SAMPATH) 24 October 2013 (24.10.2013); para [0009], [0044], [0048], [0051], [0053]</td>
<td>3-13, 15-17</td>
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<tr>
<td>Y</td>
<td>US 2014/0094501 A1 (PURI et al.) 3 April 2014 (03.04.2014); abstract; para [0120], [0122]</td>
<td>8-9, 13/8-9</td>
</tr>
<tr>
<td>Y</td>
<td>US 2013/0174286 A1 (RANA) 4 July 2013 (04.07.2013); abstract, [0037]</td>
<td>11-12, 13/1-12</td>
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<tr>
<td>Y</td>
<td>US 2005/0059005 A1 (Tuschl et al.) 17 March 2005 (17.03.2005); para [0022], Table 3, SEQ ID NO: 165</td>
<td>15</td>
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</tbody>
</table>

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "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)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "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
  "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
  "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
  "a" document member of the same patent family

Date of the actual completion of the international search
14 January 2016

Date of mailing of the international search report
0 FEB 2016

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-272-8500

Authorized officer: Lee W. Young

PCT receipt: 571-272-4300
PCT OSP: 571-272-3774

Form PCT/ISA/210 (second sheet) (January 2015)
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.: 14, 21, 22
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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.