METHOD OF TREATING KEOIDS OR HYPERTROPHIC SCARS USING ANTISENSE COMPOUNDS TARGETING CONNECTIVE TISSUE GROWTH FACTOR (CTGF)

Applicant: Excaliard Pharmaceuticals, Inc., New York, NY (US)

Inventors: Nicholas M. Dean, Encinitas, CA (US); Lincoln Krochmal, Los Gatos, CA (US); Gregory Hardee, Del Mar, CA (US); J. Gordon Foulkes, Encinitas, CA (US); Niall O’Donnell, St. Louis, MO (US); Leroy Young, Wildwood, MO (US); Mark Jewell, Eugene, OR (US)

Assignee: EXCALIARD PHARMACEUTICALS, INC., New York, NY (US)

Appl. No.: 14/865,652
Filed: Sep. 25, 2015

Related U.S. Application Data
Continuation of application No. 13/364,547, filed on Feb. 2, 2012, now Pat. No. 9,173,894.

Publication Classification
Int. Cl.
C12N 15/113 (2006.01)

U.S. Cl.
CPC ...... C12N 15/1136 (2013.01); C12N 2310/14 (2013.01); C12N 2320/30 (2013.01); C12N 2310/341 (2013.01); C12N 2310/3341 (2013.01); C12N 2310/222 (2013.01); C12N 2310/11 (2013.01)

ABSTRACT
This invention provides methods of preventing formation of, or treating, fibrotic lesions, including skin scars such as keloids and hypertrophic scars which comprise administering to the subject by one or more injection a compound which comprises a modified oligonucleotide, such as a modified antisense oligonucleotide, siRNA, or oligodeoxyribonucleotide, which inhibits expression of protein involved in fibrosis. Dosing of the antisense using an intradermal threading technique is also described.
FIGURE 1A

ASO Diffusion and Clearance in Rabbit Skin

Direction of ASO injection
5 mg ASO in 100 μl PBS
3.0 cm full thickness incision (closed w/2 staples)
0.5 cm diameter biopsies

FIGURE 1B

ASO Diffusion and Clearance in Rabbit Skin

Skin ASO Conc (μg ASO/gram tissue)

Day

0 1000 2000 3000

Injection Site
0.5 cm distal
1.0 cm distal
1.5 cm distal
FIGURE 3A (hypertrophic scar 24 weeks post revision surgery)

Placebo

EXC 001

Expert VAS Score = -34.9mm
Physician Overall = -2
Subject Overall = -3

FIGURE 3B (keloid scar 24 weeks post revision surgery)

EXC 001

Placebo

Expert VAS Score = -73.5mm
Physician Overall = -4
Subject Overall = -6
FIGURE 4: Hypertrophic scar 12 weeks post abdominoplasty surgery.

Expert WAS Score = -47.2mm
Physician Overall = -2
Subject Overall = -1
FIGURE 5: Example of limited lateral diffusion of EXC 001 distal to injection site.

Abdominoplasty Study (#202)
Week 12
FIGURE 6

EXC 001 mediated reduction in abdominal scar severity and CTGF protein expression.

EXC 001 Treated

Placebo Treated

Physician overall score: 2

Physician overall score: 6

CTGF Immunohistochemistry (protein staining)
FIGURE 7A

EXC 001-Mediated Reduction of CTGF
13 weeks – 2 or 3 weeks post-final dose EXC 001

- EXC 001 dosed 5 mg/cm at:
  - 2, 4, 6, 8 & 10 weeks (cohort 1)
  - 2, 5, 8 and 11 weeks (cohort 2)
  - 2, 6 and 10 weeks (cohort 3)

PLACEBO
EXC 001 5 mg/cm

CTGF mRNA (% Unwounded Skin)

Cohort 1  Cohort 2  Cohort 3  Cohorts 1-3

P=0.0087  P=0.0018  P=0.0613

Unwounded Skin
FIGURE 7B

**EXC 001-Mediated Reduction of Col3A1**

13 weeks – 2 or 3 weeks post-final dose EXC 001

- EXC 001 dosed 5 mg/cm at:
  - 2, 4, 6, 8 & 10 weeks (cohort 1)
  - 2, 5, 8 and 11 weeks (cohort 2)
  - 2, 6 and 10 weeks (cohort 3)

![Bar chart showing Co3A1 mRNA levels](image-url)

FIGURE 7C

**EXC 001-Mediated Reduction of ELASF**

13 weeks – 2 or 3 weeks post-final dose EXC 001

- EXC 001 dosed 5 mg/cm at:
  - 2, 4, 6, 8 & 10 weeks (cohort 1)
  - 2, 5, 8 and 11 weeks (cohort 2)
  - 2, 6 and 10 weeks (cohort 3)

![Bar chart showing ELASF mRNA levels](image-url)
FIGURE 7D

Pannus Model Study (#201)
2 weeks post single dose EXC 001 (cohort 1)

mRNA expression 4 weeks post-wounding.
Single dose of EXC 001 2 weeks prior to sampling.

FIGURE 7E

Pannus Model Study (#201)
13 weeks - 2 weeks post last EXC 001 dose

Total of 4 EXC 001 doses (weeks 2, 5, 8 & 11)
METHOD OF TREATING KELOIDS OR HYPERTROPHIC SCARS USING ANTISENSE COMPOUNDS TARGETING CONNECTIVE TISSUE GROWTH FACTOR (CTGF)

CROSS REFERENCE TO RELATED APPLICATIONS


SEQUENCE LISTING

[0002] This application contains a Sequence Listing submitted as an electronic text file named “P035467B_Sequence_Listing.txt”, having a size in bytes of 62,000 bytes, and created on Apr. 8, 2016. The information contained in this electronic file is hereby incorporated by reference in its entirety.

INCORPORATION BY REFERENCE

[0003] Throughout this application, various patents and publications are referenced. The disclosures of these patents and publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention relates.

FIELD OF INVENTION

[0004] This invention concerns methods of preventing formation of, or treating, fibrotic lesions, including skin scars such as keloids and hypertrophic scars.

BACKGROUND OF THE INVENTION

[0005] Antisense compounds are an effective means for reducing the expression of specific gene products and may be uniquely useful in a number of therapeutic applications, for example, for the modulation of expression of proteins involved in fibrosis such as connective tissue growth factor (CTGF). (See U.S. Pat. No. 6,965,025B2 to Guadre et al.)

[0006] Antisense compounds are oligomeric compounds that are capable of hybridizing to a target nucleic acid (e.g., a target mRNA molecule) and inhibiting expression of the target nucleic acid.

[0007] Antisense compounds, compositions and methods for modulating expression of CTGF and for treating diseases associated with expression of CTGF are disclosed in U.S. Pat. No. 6,965,025B2. However, there remains a need for additional such compounds capable of providing enhanced inhibition of CTGF expression as well as other advantageous properties.

[0008] Connective tissue growth factor (CTGF; also known as c discriminatory, fibroblast inducible secreted protein, fisp-12, NOV2, insulin-like growth factor-binding protein-related protein 2, IGBPBP-RFP2, IGBPBP-8, HBGF-0.8, HesC24, and ecogin) is a member of the CCN (CTGF/ Cyr61/NOV) family of nodular proteins, named for the first family members identified, connective tissue growth factor, cysteine-rich (CYR61), and nephroblastoma overexpressed (NOV), but the family also includes the proteins ELM-1 (expressed in low-metastatic cells), WISP-3 (Wnt-1-induced secreted protein), and COP-1 (WISP-2). CCN proteins have been found to be secreted, extracellular matrix-associated proteins that regulate cellular processes such as adhesion, migration, mitogenesis, differentiation, survival, angiogenesis, arteriosclerosis, chondrogenesis, wound healing, tumorigenesis, and vascular and fibrotic diseases like sclerodema (Liao and Lam, Exp. Cell Res., 1999; 248, 44-57). The connective tissue growth factor protein was shown to stimulate DNA synthesis and promote chemotaxis of fibroblasts (Bradham et al., J. Cell Biol., 1991, 114, 1285-1294).

[0009] Connective tissue growth factor is expressed in fibroblasts during normal differentiation processes that involve extracellular matrix (ECM) production and remodeling. Connective tissue growth factor is also frequently overexpressed in fibrotic skin disorders such as systemic sclerosis, localized skin sclerosis, keloids, scar tissue, eosinophilic fasciitis, nodular fasciitis, and Dupuytren's contracture. Connective tissue growth factor mRNA or protein levels are elevated in fibrotic lesions of major organs and tissues including the liver, kidney, lung, cardiovascular system, pancreas, bowel, eye, and gingiva. In mammary, pancreatic and fibrohistiocytic tumors characterized by significant connective tissue involvement, connective tissue growth factor is overexpressed in the stromal compartment.

The Role of CTGF in Keloid Diseases

[0010] Keloid disease (KD) is a benign dermal fibroproliferative tumor characterized by an excessive accumulation of extracellular matrix proteins, leading to an overabundance of collagen formation. Abnormal skin scarring can occur, post-injury in genetically susceptible individuals. KD can also be a familial condition, occurring more commonly in ethnic groups with darker skin. The highest incidence of keloids is found in the black population, where it has been estimated to be around 4-6% and up to 16% in random samples of black Africans. Various modes of inheritance have been proposed for KD ranging from autosomal recessive to autosomal dominant with incomplete clinical penetrance and variable expression. The majority of keloids can lead to considerable cosmetic defects, but can also grow large enough to become symptomatic, by causing deformity or limiting joint mobility.

[0011] Although low levels of CTGF are expressed in normal skin, CTGF becomes up-regulated following dermal injury, and it becomes persistently over-expressed when scarring is severe, as in keloids or systemic sclerosis. Fibroblasts cultured from both hypertrophic scars, keloids, and scleroderma lesions express increased basal CTGF (Exp. Cell Res. 2000, 259: 213-224), and cells cultured from hypertrophic scars and keloids were shown to express more CTGF basally and also elaborate more CTGF in response to stimulation with TGF-β (Plast. Reconstr. Surg. 2005, 116: 1387-90). Similarly, transcription of CTGF after serum stimulation was significantly higher in keloid versus normal fibroblasts in cell culture (Ann. Surg. 2007, 246(5):886-95).

[0012] In keloid tissue, fibroblasts expressing CTGF mRNA were found distributed throughout the lesions, especially in the peripheral areas (J. Invest. Derm. 1996, 106: 729-733). CTGF mRNA expression levels have been compared in normal skin, keloid scars, hypertrophic scars, and mature scars. CTGF mRNA was strongly detected in all cases of the keloids, although not in mature scars. There was
a significant difference between levels found in keloids and normal skin (J. Japan Soc. Plastic Reconstr. Surg. 2002, 22:560-565). Recent data also suggests that, relative to normal fibroblasts, keloid scar fibroblasts synthesize 100-150-fold more CTGF in response to exogenous TGF-β1 than do normal fibroblasts (Plast. Reconstr. Surg. 2005, 116: 1387-1390). When compared to normal skin, increased localization of CTGF was seen in the basal layer of keloid epidermis and higher expression of CTGF was observed in keloid tissue extract (J. Cell Physiol. 2006, 208(2):336-43). Previously no data has been generated to validate the role of CTGF in keloid disease by showing that inhibition of CTGF expression inhibits keloid growth.

[0013] Currently, no effective single therapeutic regimen has been established for treatment of keloids or prevention of keloids growth after surgery. Existing therapeutic approaches include excisional dressings, compression therapy, intra-lesional steroid injections, cryosurgery, surgical excision, laser treatment, radiation therapy, Kenalog (triamcinolone), interferon therapy, bleomycin, 5-fluoracil, verapamil, imiquimod cream, and combinations thereof. Both silicone and non-silicone-based excisional dressings have been a widely used clinical option for keloids for the last 30 years, but all of these methods result in very limited efficacy and it is widely understood that a new therapy for keloids is urgently needed.

[0014] Various forms of radiotherapy have been attempted as a mono-therapy for keloids, but remain quite controversial because of anecdotal reports of carcinogenesis after treatment. Laser therapy using argon, CO₂, and pulse dye have been repeatedly attempted during the last 40 years, but none of them have proven to be efficacious. All three forms of laser therapy, according to multiple studies, have recurrence rates of upwards of 90%, showing little to no benefit. Cryotherapy has been used as a mono-therapy. However, side effects associated with this approach include pain at the therapeutic site and hypo- or hyper-pigmentation. Intra-lesional triamcinolone acetonide injections, a type of corticosteroid, is frequently used as first-line therapy for the treatment of keloids, but again, actual reported clinical efficacy varies widely. In addition, the need for multiple injections, along with the side effects of injection pain, skin atrophy, telangiectasias, and altered pigmentation have caused clinicians and researchers to continue seeking other means of treatment.

[0015] Consequently, there remains a long felt need for additional methods and agents to effectively prevent the formation of keloids, hypertrophic scars, and other types of fibrotic lesions as well as to treat keloids, hypertrophic scars and fibrotic lesion so as to eliminate or reduce them and/or to prevent their reoccurrence. The clinical results described herein clearly demonstrate for the first time the ability of an antisense oligonucleotide targeting CTGF to reduce the growth and severity of keloids post surgery.

Antisense Dosing into Skin

[0016] It has also been demonstrated for the first time that antisense oligonucleotides do not diffuse laterally very far after dosing into skin (see Example 2) which could lead to irregular effects of this class of drug on a linear incision/ healing scar or keloid if dosing was conducted as a single bolus type of administration, resulting in variable concentrations of antisense along the length of the developing scar. To overcome this drawback, a method for delivering antisense oligonucleotides by an intradermal threading technique has been developed. This technique effectively delivers a constant amount of antisense drug along the full length of the scar, and results in effective and consistent scar reduction along the full length of the scar or keloid. Intra-dermal threading consists of introducing a needle into the dermis at an angle as parallel to the skin as possible, and threading the needle into and along the dermis for a distance of typically between 1 and 5 cm. At this point, the needle is withdrawn and drug injected into the dermis along the full length of the needle tract as the needle is withdrawn, resulting in an equal amount and volume of drug being deposited along the full length of the needle tract.

SUMMARY OF THE INVENTION

[0017] This invention provides a method for treating a keloid, or preventing the formation, reformation, or growth of a keloid after an injury to the skin, in a subject in need thereof, which comprises administering to the subject by one or more injections at the site of the keloid or of the injury to the skin, a composition which comprises a modified oligonucleotide consisting of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a region selected from nucleotides 553-611, 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, 2728-2797, 2267-2301, 1394-1423, 1469-1508, 1559-1605, 1659-1689, 2100-2129 and 1399-1423 of SEQ ID NO: 9, or a salt or ester thereof, in an amount effective to treat, or to prevent the formation, reformation, or growth of, the keloid, wherein the effective amount is from 0.1 to 50 mg of the modified oligonucleotide per injection per linear centimeter of the keloid or of the injury to the skin.

[0018] This invention also provides a method for treating a hypertrophic scar, or preventing the formation, reformation, or growth of a hypertrophic scar after an injury to the skin, in a subject in need thereof, which comprises administering to the subject by one or more injections at the site of the hypertrophic scar or of the injury to the skin, a composition which comprises a modified oligonucleotide consisting of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a region selected from nucleotides 553-611, 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, 2728-2797, 2267-2301, 1394-1423, 1469-1508, 1559-1605, 1659-1689, 2100-2129 and 1399-1423 of SEQ ID NO: 9, or a salt or ester thereof, in an amount effective to treat, or to prevent the formation, reformation, or growth of, the hypertrophic scar, wherein the effective amount is from 0.1 to 25 mg of the modified oligonucleotide per injection per linear centimeter of the hypertrophic scar or of the injury to the skin.

[0019] The invention further provides a method for reducing formation, reformation, or growth of a scar or keloid at a site of an injury to the skin, or of treating a pre-existing scar or keloid, in a subject in need thereof, which comprises administering to the subject by one or more threading injections at the site of the injury or of the pre-existing scar or keloid, a composition which comprises a modified oligonucleotide, or a salt or ester thereof, targeted to a nucleic acid encoding a protein involved in fibrosis in an amount effective to inhibit expression of the protein and thereby reduce scar or keloid formation, reformation, or growth at the site of the injury or to treat the pre-existing scar or keloid.

[0020] This invention still further provides a method for reducing formation, reformation, or growth of a fibrotic
lesion at a site of an injury, or of treating a pre-existing fibrotic lesion, in a subject in need thereof, which comprises administering to the subject by one or more threading injections at the site of the injury or of the pre-existing fibrotic lesion, a composition which comprises a modified oligonucleotide, or a salt or ester thereof, targeted to a nucleic acid encoding a protein involved in fibrosis in an amount effective to inhibit expression of the protein and thereby reduce formation, reformation, or growth of the fibrotic lesion at the site of the injury or to treat the pre-existing fibrotic lesion.

**BRIEF DESCRIPTION OF THE FIGURES**

[0021] FIG. 1A and FIG. 1B show that 2′MOE containing antisense oligomericoloides diffuse over relatively short distances (~0.5-1.0 cm) in rabbit skin when given by intradermal injection (described in Example 2).

[0022] FIGS. 2A and 2B show that treatment of keloids in an animal model, with a CTGF antisense oligonucleotide resulted in reduction in both CTGF (FIG. 2A) and Col3A1 (FIG. 2B) mRNA expression in intact human keloid tissue transplanted into mice (described in Example 3).

[0023] FIGS. 3A and 3B show that treatment with a CTGF antisense oligonucleotide, (EXC 001 or SEQ ID NO: 39) inhibits growth of both hypertrophic scars and keloids at 24 weeks post scar revision surgery in humans. The scores below each set of pictures represent the degree of improvement between placebo- and EXC 001-treated keloids. A negative score represents an improvement in scar resulting from EXC 001 treatment. FIG. 3A shows placebo- and EXC 001-treated hypertrophic scars 24 weeks post scar revision surgery. FIG. 3B shows placebo- and EXC 001-treated keloid scars 24 weeks post scar revision surgery (described in Example 4).

[0024] FIG. 4 shows that treatment with CTGF antisense oligonucleotide, (EXC 001 or SEQ ID NO: 39) inhibits the formation and growth of a hypertrophic scar 12 weeks post abdominoplasty surgery. The scores below the pictures represent the degree of improvement between placebo- and EXC 001-treated scar. A negative score represents an improvement in scar resulting from EXC 001 treatment (described in Example 5).

[0025] FIG. 5 shows the limited diffusion of EXC 001 when dosed adjacent to a scar (described in Example 5). The section of the abdominoplasty scar on the right side of the scar (to the right of the vertical line) was treated with EXC 001 whereas the scar to the left of the vertical line did not receive any treatment. Clearly the scar severity to the right is less than to the left. This example demonstrates that the EXC 001 therapeutic benefit is limited to the region of scar directly adjacent to the site of drug delivery by intradermal threading. Therefore the drug appears to have limited diffusion away from the site of administration and will require dosing immediately adjacent to and along the length of the potential scar site, for example by intradermal threading.

[0026] FIG. 6 shows an example of the ability of EXC 001 to reduce the growth and formation of hypertrophic scars (described in Example 6). In this example, two matching 2 cm abdominal scars are shown, one treated with 5 mg/cm EXC 001 and one with placebo. The severity of the EXC 001 treated scar is less than the placebo treated scar. Histological analysis of these two scars also revealed an EXC 001 mediated reduction in the expression of CTGF protein (by immunohistochemistry) clearly demonstrating that EXC 001 is functioning to reduce the expression of its intended target (CTGF).

**DETAILED DESCRIPTION OF THE INVENTION**

[0027] FIGS. 7A-7E show the effects of EXC 001 on mRNA expression in abdominal scars of various genotypes at various times post treatment (described in Example 6). FIG. 7A shows the effect of EXC 001 in suppressing CTGF mRNA expression. FIG. 7B shows the effect of EXC 001 in suppressing Collagen III-a1 (Col3A1) mRNA expression. FIG. 7C shows the effect of EXC 001 in suppressing elastin (ELASF) mRNA expression. FIGS. 7D and 7E show there was no significant inhibition of either SMAD3 or TGF-β1 mRNA expression by EXC 001 as compared to placebo.

[0028] This invention provides a method for treating a keloid, or preventing the formation, reformation, or growth of a keloid after an injury to the skin, in a subject in need thereof, which comprises administering to the subject by one or more injections at the site of the keloid or of the injury to the skin, a composition which comprises a modified oligonucleotide consisting of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a region selected from nucleotides 553-611, 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, 2728-2797, 2267-2301, 1394-1423, 1469-1508, 1559-1605, 1659-1689, 2100-2129 and 1399-1423 of SEQ ID NO: 9, or a salt or ester thereof, in an amount effective to treat, or to prevent the formation, reformation, or growth of, the keloid, wherein the effective amount is from 0.1 to 50 mg of the modified oligonucleotide per injection per linear centimeter of the keloid or of the injury to the skin.

[0029] This invention also provides a method for treating a hypertrophic scar, or preventing the formation, reformation, or growth of a hypertrophic scar after an injury to the skin, in a subject in need thereof, which comprises administering to the subject by one or more injections at the site of the hypertrophic scar or of the injury to the skin, a composition which comprises a modified oligonucleotide consisting of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a region selected from nucleotides 553-611, 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, 2728-2797, 2267-2301, 1394-1423, 1469-1508, 1559-1605, 1659-1689, 2100-2129 and 1399-1423 of SEQ ID NO: 9, or a salt or ester thereof, in an amount effective to treat, or to prevent the formation, reformation, or growth of, the hypertrophic scar, wherein the effective amount is from 0.1 to 25 mg of the modified oligonucleotide per injection per linear centimeter of the hypertrophic scar or of the injury to the skin.

[0030] This invention further provides a method for reducing formation, reformation, or growth of a scar or keloid at a site of an injury to the skin, or of treating a pre-existing scar or keloid, in a subject in need thereof, which comprises administering to the subject by one or more threading injections at the site of the injury or of the pre-existing scar or keloid, a composition which comprises a modified oligonucleotide, or a salt or ester thereof, targeted to a nucleic acid encoding a protein involved in fibrosis in an amount effective to inhibit expression of the protein and thereby reduce scar or keloid formation, reformation, or growth at the site of the injury or of treat the pre-existing scar or keloid. This invention still further provides a method for
reducing formation, reformation, or growth of a fibrotic lesion at a site of an injury, or of treating a pre-existing fibrotic lesion, in a subject in need thereof, which comprises administering to the subject by one or more threading injections at the site of the injury or of the pre-existing fibrotic lesion, a composition which comprises a modified oligonucleotide, or a salt or ester thereof, targeted to a nucleic acid encoding a protein involved in fibrosis in an amount effective to inhibit expression of the protein and thereby reduce formation, reformation, or growth of the fibrotic lesion at the site of the injury or to treat the pre-existing fibrotic lesion.

[0031] In one embodiment of the preceding methods, the one or more threading injections comprise multiple intradermal threading injections per scar.

[0032] In the preceding methods, the protein involved in fibrosis may be connective tissue growth factor, transforming growth factor beta-1, mothers against decapentaplegic homolog-3, early growth response-1, monocyte chemoattractant protein-1, a collagen, or an elastin. Examples of suitable collagens are Collagen 3A1, Collagen 1A2, and Collagen 1A1.

[0033] In certain embodiments of the methods of this invention, the effective amount is from 0.1 to 50 mg, e.g. 0.1 to 25 mg, of the modified oligonucleotide per injection per linear centimeter of the site of the injury to the skin or of the pre-existing scar.

[0034] In certain embodiments, the modified oligonucleotide is administered at least once every two weeks for at least four weeks, i.e. at least twice.

[0035] In other embodiments, the modified oligonucleotide is administered at least once every three weeks for at least six weeks, i.e. at least twice.

[0036] In still other embodiments, the modified oligonucleotide is administered at least once every four weeks for at least eight weeks. In yet other embodiments, the modified oligonucleotide is administered at least once every eight weeks for at least sixteen weeks.

[0037] It is currently contemplated that it may be preferable that the modified oligonucleotide is administered over a period of at least nine weeks, for example over a period of 26 weeks.

[0038] In certain embodiments, the modified oligonucleotide consists of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a region selected from the group consisting of nucleotides 535-611, 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, 2728-2797, 2267-2301, 1394-1423, 1469-1508, 1559-1605, 1659-1689, 2100-2129, and 1399-1423 of SEQ ID NO: 9.

[0039] In certain embodiments, at least a 12 nucleobase sequence portion of the modified oligonucleotide is present within the nucleobase sequence set forth in any of the sequences set forth in SEQ ID NO: 28, 30, 39, 40, 43, 44, 45, 50, Si, 52, 56, 78, 125, or 166.

[0040] In certain embodiments, the modified oligonucleotide consists of at least 14, e.g. 20, linked nucleosides.

[0041] In certain embodiments, the modified oligonucleotide is a single-stranded oligonucleotide. In others, the modified oligonucleotide is a double-stranded oligonucleotide.

[0042] In still other embodiments, the modified oligonucleotide comprises at least one oligodeoxyribonucleotide or at least one oligoribonucleotide.

[0043] In certain embodiments, the modified oligonucleotide has a sequence which is 100% identical over its length to a portion of any one of the sequences set forth in SEQ ID NO: 28, 30, 39, 40, 43, 44, 45, 50, 51, 52, 56, 78, 125, or 166.

[0044] In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage, e.g. a phosphorothioate internucleoside linkage, such that some or all of the internucleoside linkages may be phosphorothioate internucleoside linkages.

[0045] In certain embodiments, at least one nucleoside in the modified oligonucleotide comprises a modified sugar, such as a bicyclic sugar. In some such embodiments, at least one of the modified sugar comprises 2'-O-methoxyethyl.

[0046] In other embodiments, the modified oligonucleotide comprises at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

[0047] In some such embodiments, each of the at least one tetrahydropyran modified nucleoside has the structure:

[0048] In certain embodiments, at least one nucleoside comprises a modified nucleobase, e.g. a modified deoxyribonucleoside, a ribonucleoside, or a 5'-methylcytosine.

[0049] In certain embodiments, the modified oligonucleotide comprises:

[0050] (a) a gap segment consisting of linked deoxyribonucleosides;

[0051] (b) a 5' wing segment consisting of linked modified nucleosides; and

[0052] (c) a 3' wing segment consisting of linked modified nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside within each wing segment comprises a modified sugar.

[0053] In certain currently preferred embodiments, the modified oligonucleotide comprises:

[0054] (a) a gap segment consisting of thirteen linked deoxyribonucleosides;

[0055] (b) a 5' wing segment consisting of two linked modified nucleosides; and

[0056] (c) a 3' wing segment consisting of five linked modified nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each modified nucleoside within each wing segment comprises a 2'-O-methoxyethyl sugar, and wherein each internucleoside linkage is a phosphorothioate linkage.

[0057] In certain embodiments, the sequence of the nucleobase is the sequences set forth in SEQ ID NO: 39.
In other embodiments, the sequence of the nucleobase is the sequences set forth in SEQ ID NO: 40.

In still other embodiments, the sequence of the nucleobase is the sequences set forth in SEQ ID NO: 45.

In yet other embodiments, the sequence of the nucleobase is the sequences set forth in SEQ ID NO: 52.

In yet other embodiments, the sequence of the nucleobase is the sequences set forth in SEQ ID NO: 166.

In certain embodiments, the composition comprises the modified oligonucleotide or a salt thereof, and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the modified oligonucleotide directly or indirectly inhibits expression of collagen or elastin or both, so as to treat the keloid, prevent the formation, reformation, or growth of the keloid, treat the hypertrophic scar, prevent the formation, reformation, or growth of the hypertrophic scar, reduce scar formation at the site of the injury, treat the pre-existing scar, reduce formation of the fibrotic lesion at the site of the injury, or treat the pre-existing fibrotic lesion.

It is currently contemplated that the preceding methods further comprise administering to the subject a second compound.

In certain embodiments, the second compound may be an antisense compound targeting the same or a different sequence, and the modified oligonucleotide and the second compound may be administered simultaneously or sequentially.

In certain embodiments, the modified oligonucleotide is present in a conjugate with a moiety which enhances uptake of the compound into, and/or increases residence time of the compound in, the subject, wherein the residence time is preferably 7 to 60 days. The conjugate moiety is polyethylene glycol, hyaluronic acid, cholesterol, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-(5-(hexadecyl)gluceral, hexadecylglycerol, hexadecylamine, geranyloxyhexyl, palmitic acid, myristic acid, spermine, spermidine, folate acid, vitamin E, a carbohydrate cluster, a peptide (including antennapedia helix, HIV Tat fragments, integrin binding peptide), transportin, or porphyrin.

In certain embodiments, the modified oligonucleotide is administered in a delivery system which enhances uptake of the compound into, and/or increases residence time of the compound in, the subject, wherein the residence time is preferably 7 to 60 days. The delivery system comprises a cationic lipid, a liposome, a microparticle, a nanoparticle, a liquid formulation with suspended particles with or without drug in the solution for immediate release or with drug depot in particles (particularly PLGA and poly-Arg particles), a liquid formulation that gels after injections such as thermosetting/responsive liquids (e.g. pluronic liquids), liquids that contain a polymer and drug in a biocompatible solvent that precipitate when the solvent is diluted by body fluids (e.g. atrigel), or a gel, a semi-solid formulation such as hydrogel (with a matrix backing or as a spray solution), a powder to be sprinkled on during surgery, a resorbable suture, or a fast dissolving gel or polymer strip.

In certain embodiments, the modified oligonucleotide is administered to the subject following a surgical excision of the keloid, scar, or fibrotic lesion.

In certain embodiments, the injury to the skin is the result of a surgical incision, a biopsy, a skin piercing, a skin removal, a burn, or a wound.

In certain embodiments, the effective amount is about 5 mg of the modified oligonucleotide per injection per linear centimeter of the keloid, the hypertrophic scar, the injury to the skin, the site of the injury, the pre-existing scar, or the pre-existing fibrotic lesion.

In certain embodiments, the modified oligonucleotide is administered for up to 6 months. In other embodiments, the modified oligonucleotide is administered for up to 1 year.

In certain embodiments, the preceding methods further comprise administering to the subject another therapeutic agent which may be a steroid, a silicone wrap, TGF-β3 (i.e. Juvista), collagenase (i.e. Xyflex), 17β-estradiol (i.e. Zosteem), IL-10 (i.e. Prevascar), mannose 6-phosphate (i.e. Juvidx), a smooth muscle relaxant (i.e. AZX100, a 24-amino acid synthetic peptide), a stem cell therapy (i.e. GBT009), serum amyloid protein, antibodies targeting integrin αvβ6, CTGF, TGFβ, or molecules that inhibit the activity of ALK-4 and/or ALK-5 (the TGF beta receptors), any inhibitor designed to block TNF activity (for example etanercept), occlusive dressings, compression therapy, cryosurgery, surgical excision, laser treatment, radiation therapy, interferon therapy, bleomycin, 5-fluorouracil, verapamil, imiquimod cream, one capable of promoting wound healing, such as Dermagraft, Apligraf, PDGF (i.e. Regranex), electrical stimulation, “growth factors” as a category, dressings as a category, small intestinal submucous (SIS), Promogran, hyperbaric oxygen, or combinations thereof.

In certain embodiments, the modified oligonucleotide is administered by means of a formulation, ultrasound, electroporation, iontophoresis or micro-needle.

In other embodiments, the modified oligonucleotide is administered adjacent to the keloid, the hypertrophic scar, the injury to the skin, the site of the injury, the pre-existing scar, or the pre-existing fibrotic lesion.

In other embodiments, the modified oligonucleotide is administered along the entire length of the keloid, the hypertrophic scar, the injury to the skin, the site of the injury, the pre-existing scar, or the pre-existing fibrotic lesion.

In still other embodiments, the modified oligonucleotide is administered along each side of the keloid, the hypertrophic scar, the injury to the skin, the site of the injury, the pre-existing scar, or the pre-existing fibrotic lesion.

In yet other embodiments, the modified oligonucleotide is administered directly into the keloid, the hypertrophic scar, the injury to the skin, the site of the injury, the pre-existing scar, or the pre-existing fibrotic lesion.

In certain embodiments, the subject is genetically predisposed to formation of keloids or hypertrophic scars or both.

In certain embodiments, the modified oligonucleotide is administered intradermally.

In other embodiments, the modified oligonucleotide is administered intradermally by threading technique.

In still other embodiments, the modified oligonucleotide is administered sub-cutaneously.

In yet other embodiments, the modified oligonucleotide is administered topically.

This invention also provides a kit which comprises:

a. a device pre-filled with the composition comprising the modified oligonucleotide; and

b. instruction for use.
In a certain embodiment the antisense oligonucleotide is complementary to a portion of the region of CTGF targeted by active oligonucleotides which stretches from target sites 1396 through 1424. This is the sequence space targeted by oligo 418899, 412295 and 412294/EXC 001 (SEQ ID NOs: 166, 40 and 39, respectively).

This invention also provides a modified oligonucleotide comprising at least 12, preferably at least 14, linked nucleosides, the nucleoside sequence of which is a portion of one of the nucleoside sequences set forth in SEQ ID NOs: 28, 30, 39, 40, 43, 44, 45, 50, 51, 52, 56, 78, 125 and 166.

The antisense compounds described herein can comprise an oligonucleotide having 12 to 30, 12 to 20, and preferably 14 to 20 linked nucleosides.

In one embodiment of the invention, the modified oligonucleotide is a single-stranded or a double-stranded oligonucleotide.

The present invention employs oligomeric compounds, particularly antisense oligonucleotides, for use in modulating the function of nucleic acid molecules encoding proteins involved in fibrosis, ultimately modulating the amount of protein produced. This is accomplished by providing antisense compounds which specifically hybridize with one or more nucleic acids encoding a protein involved in fibrosis. As used herein, the terms “target nucleic acid” and “nucleic acid encoding connective tissue growth factor” encompass DNA encoding a protein involved in fibrosis, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA.

The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as “antisense”. The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of connective tissue growth factor. In the context of the present invention, “modulation” means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

It is preferred to target specific nucleic acids for antisense. “Targeting” an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process.

It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

In one embodiment, a target region is a structurally defined region of the nucleic acid. For example, a target region may encompass a 5' UTR, a 3' UTR, an exon, an intron, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for the nucleic acid can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In other embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the target region.

Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In other embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In one embodiment, target segments within a target region are separated by no more than about 500 nucleotides. In other embodiments, target segments within a target region are separated by no more than about 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid. In another embodiment, target segments within a target region are separated by no more than about 5 nucleotides on the target nucleic acid. In additional embodiments, target segments are contiguous.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, or an exon. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifically exclude a certain structurally defined region such as the start codon or stop codon.

The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In one embodiment, reductions in CTGF mRNA levels are indicative of inhibition of CTGF expression. Reductions in levels of a CTGF protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of CTGF expression.

Antisense Compounds

In the context of this invention, the term “oligonucleotide” refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent inter
When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 3' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7309-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi et al (J. Natl. Cancer Inst. 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL in vitro and in vivo. Furthermore, this oligonucleotide demonstrated potent antitumor activity in vivo.

Maher and Dolnick (Nuc. Acid. Res. 16:3341-3358, 1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Bhanot et al. (PCT/US2007/068401) provided short antisense compounds, including compounds comprising chemically-modified high-affinity monomers 8 to 16 monomers in length. These short antisense compounds were shown to be useful for reducing target nucleic acids and/or proteins in cells, tissues, and animals with increased potency and improved therapeutic index. Short antisense compounds were effective at lower doses than previously described antisense compounds, allowing for a reduction in toxicity and cost of treatment. In addition, the described short antisense compounds have greater potential for oral dosing.

Hybridizations

Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

In the context of this invention, “hybridization” means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding.
between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds.

Identity

[0114] The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific oligo (Isis) number. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

[0115] In one embodiment, the antisense compounds are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

Modifications

[0116] In a certain embodiment of the invention, modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases.

[0117] In one embodiment of the invention the compound comprises at least one modification selected from the group consisting of a modified internucleoside linkage, a modified sugar, and a modified nucleobase.

[0118] Although antisense oligonucleotides containing a variety of modified internucleoside linkages may be employed, the currently preferred modified internucleoside linkage is a phosphorothioate linkage between one or more of the nucleosides or wherein all of the internucleoside linkages are phosphorothioate internucleoside linkages.

[0119] In general, it is also preferred that the antisense oligonucleotide contains at least one and typically more than one modified sugar, wherein the sugar is a bicyclic sugar. Although various modified sugars may be employed it is presently preferred to employ a 2'-O-methoxyethyl sugar.

[0120] Further, at least one and typically more than one of the nucleobases contained in the antisense oligonucleotide will be a modified nucleotide such as a 5-methylcytosine.

[0121] A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

[0122] Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

[0123] Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

[0124] The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

[0125] Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphorothiesters, methylphosphonates, phosphoramidates, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

[0126] In one embodiment, antisense compounds targeted to a CTGF nucleic acid comprise one or more modified internucleoside linkages. In some embodiments, the modified internucleoside linkages are phosphorothioate linkages. In other embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

[0127] As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound.

[0128] In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.
Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphorothiester, aminkylphosphorothiester, methyl and other alkyl phosphonates including 3′-alkylene phosphonates, 5′-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramic acids including 3′-amino phosphoramidates and aminomethylphosphoramide, thiono phosphoramidates, thioalkylphosphonates, thioalkylphospho-riesters, selenophosphates and boronophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of these, and those having inverted polarity wherein one or more internucleoside linkages is a 3′ to 3′, 5′ to 5′ or 2′ to 2′ linkage. Preferred oligonucleotides having inverted polarity comprise a single 3′ to 3′ linkage at the 3′-most internucleoside linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,521,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,559,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,650, certain of which are commonly owned with this application, and each of which is herein incorporated by reference. Various salts, mixed salts and free acid forms are also included.

Preferred modified oligonucleotide backbones that do not include a phosphorus atom wherein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methyleneformacetyl and thioformacetyl backbones; riboacyclic backbones; alkene containing backbones; sulfinate backbones; methylenemino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and C12 component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,935; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,886; 5,602,240; 5,610,289; 5,618,704; 5,625,070; 5,603,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeromic compound, an oligonucleotide mimic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an amidominethylene glycol backbone. The nucleobases are retained and are bound directly or indirectly to azanitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497-1500.

Most preferred embodiments of the invention are oligonucleosides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular —CH2—NH—O—CH2—, —CH2—N(CH3)—O—CH2— [known as a methylene (methylimino) or MMIm backbone], —CH2—O—N(CH3)—CH2—, —CH2—N(CH3)—N (CH3)—CH2— and —O—N(CH3)—CH2—CH2— wherein the native phosphodiester backbone is represented as —O—P—O—CH2— of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleosides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,634,506.

Modified Sugar Moieties

Modified oligonucleotides may also contain one or more substituted sugar moieties. For example, the furanose sugar ring can be modified in a number of ways including substitution with a bicyclic nucleic acid “BNA” and substitution of the 4′-O with a heteroatom such as S or N(R) as described in U.S. Pat. No. 7,399,845 to Seth et al., hereby incorporated by reference herein in its entirety. Other examples of BNA and siloxane backbones are described in published International Patent Application No. WO2007/146511, hereby incorporated by reference herein in its entirety.

Antisense compounds of the invention can optionally contain one or more nucleosides having modified sugar moieties. Sugar modifications may impart nuclease stability, binding affinity or some other beneficial biological property to the antisense compounds. The furanose sugar ring of a nucleoside can be modified in a number of ways including, but not limited to: addition of a substituent group, particularly at the 2′ position; bridging of two non-geminal ring atoms to form a bicyclic nucleic acid (BNA); and substitution of an atom or group such as —S—, —N(R)— or —C(R1)(R2) for the ring oxygen at the 4′-position. Modified sugars include, but are not limited to: substituted sugars, especially 2′-substituted sugars having a 2′-F, 2′-OCH3 (2′-OMe) or a 2′-O(CH2)2—OCH3 (2′-O-methoxyethyl or...
2'-MOE) substituent group; and bicyclic modified sugars (BNAs), having a 4'-(CH)n-O-2' bridge, where n=1 or n=2. Methods for the preparations of modified sugars are well known to those skilled in the art.

In certain embodiments, a 2'-modified nucleoside has a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety is a D sugar in the alpha configuration. In certain such embodiments, the bicyclic sugar moiety is a D sugar in the beta configuration. In certain such embodiments, the bicyclic sugar moiety is an L sugar in the alpha configuration. In certain such embodiments, the bicyclic sugar moiety is an L sugar in the beta configuration.

In certain embodiments, the bicyclic sugar moiety comprises a bridge group between the 2' and the 4-carbon atoms. In certain such embodiments, the bridge group comprises from 1 to 8 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises from 1 to 4 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises 2 or 3 linked biradical groups. In certain embodiments, the bicyclic sugar moiety comprises 2 linked biradical groups. In certain embodiments, a linked biradical group is selected from 2'-MOE (Singh et al., Chem. Commun., 1998, 4, 455-456).
LNA and LNA analogs display very high duplex thermal stabilities with complementary DNA and RNA (Tm = +3 to +10°C), stability towards 3'-exonucleolytic degradation and good solubility properties. Other preferred bridge groups include the 2'-deoxy-2'-CH₂OCH₂-4' bridge. LNAs and preparation thereof are described in published International Patent Application Nos. WO 98/39352 and WO 99/14226.

[0147] Other preferred modifications include 2'-methoxy (2'-O—CH₃), 2'-aminoproxy (2'-OCH₂CH₂NH₂), 2'-allyl (2'-CH₃—CH=CH₂), 2'-O-allyl (2'-O—CH₂—CH=CH₂) and 2'-fluoro (2'-F). The 2'-modification may be in the arabinof (up) position or ribo (down) position. A preferred 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics or surrogates (sometimes referred to as DNA analogs) such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety. In certain embodiments, nucleosides are modified by replacement of the ribosyl ring with a surrogate ring system such as a morpholino ring, a cyclohexenyl ring, a cyclohexyl ring or a tetrahydropyranyl ring such as one having one of the formulae:

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{Bx} & \quad \text{OCH₃}
\end{align*}
\]

[0148] Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for example review article: Leumann, Christian J.). Such ring systems can undergo various additional substitutions to enhance activity.

[0149] In one embodiment of the invention, the compound comprising at least one tetrahydropyran modified nucleoside wherein a tetrahydropyran ring replaces the furanose ring.

[0150] In another embodiment of the invention, wherein each of the at least one tetrahydropyran modified nucleoside has the structure:

wherein Bx is an optionally protected heterocyclic base moiety.

Modified Nucleobases

[0151] Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. Oligonucleotides modifications or substitutions are structurally distinguishable from, yet functionally interchangable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds. Modified nucleobases include synthetlc and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an antisense compound against a target nucleic acid. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanhivi, Y. S., Crooke, S. T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278).

[0152] Additional unmodified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenosine and guanine, 2-propyl and other alkyl derivatives of adenosine and guanine, 2-thiouracil, 2-thiophen and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (—C=—CH₂) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (psuedouracil), 4-thiouracil, 8-halo, 8-amino, 8-hiio, 8-thioalkyl, 8-hydrox and other 8-substituted adenosines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-aza-sugarine and 8-azadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[0153] Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azaprimidines and N2, N6 and 0-6 substituted purines, including 2-aminopyridinamine, 5-propynyluracil and 5-propynylcytosine.

[0154] In one embodiment, antisense compounds targeted to a CTGF nucleic acid comprise one or more modified nucleobases. In an additional embodiment, gap-widened antisense oligonucleotides targeted to a CTGF nucleic acid comprise one or more modified nucleobases. In some
embodiments, the modified nucleobase is 5-methylcytosine. In further embodiments, each cytosine is a 5-methylcytosine.

[0155] As used herein, “unmodified” or “natural” nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-meC), 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-thiouridine and 2-thiocytosine, 5-haloaracil and cytosine, 5-propynyl (—C≡C—CH₃) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracyl (pseudouracil), 4-thiouracil, 8-halo, 8-aminoguanine, 8-thiouracil, 8-hydroxopyrimidines, 2-thiopyrimidines, 2,4-diamino pyrimidines, 2-amino-6-alkyl adenine, 8-aza guanine and 8-azidoguanine, and 7-deazaguanine and 7-deazadenine and 3-deazaguanine and 3-deazadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine(1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), 6-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminomethyl)-1H-pyrimido [5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]pyrido[2,3-d]pyrimidin-2-one), pyridoxalol cytidine (1H-pyrido[3',4':5,6]pyrido[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopuridine and 2-prise. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia of Polymer Science And Engineering, pages 858-859, Kroschwitz, J.L., ed. John Wiley & Sons, 1990, those disclosed by English et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S.T. and Lebleu, B., eds., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 9-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2′-O-methoxymethyl sugar modifications.

[0156] Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,727; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,001; 5,614,617; 5,645,985; 5,830,653; 5,763,588; 6,005,916; and 5,681,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, which has an owner in common with the owners of the instant application and also herein incorporated by reference.

Antisense Compound Motifs

[0157] In certain embodiment of the invention, the compound comprises a modified oligonucleotide comprised of (a) a gap segment consisting of linked deoxynucleosides, preferably consists of a thirteen linked modified deoxynucleosides; (b) a 5’ segment consisting of linked modified nucleosides, preferably consists of two linked modified nucleosides; and (c) a 3’ segment consisting of linked modified deoxynucleosides, preferably consists of five linked deoxynucleosides wherein the gap segment is positioned between the 5’ gap segment and the 3’ gap segment, and wherein each modified nucleoside within each gap segment comprises a modified sugar, preferably comprises a 2′-O-methoxymethyl sugar; and wherein each intranucleoside linkage is a phosphorothioate linkage.

[0158] These patterns of modified nucleotides in an antisense compound are called motif. These motifs, confer to the antisense compounds properties such as the enhancement of the inhibitory activity, increase binding affinity for a target nucleic acid, or resistance to degradation by in vivo nucleases.

[0159] In certain embodiments, antisense compounds targeted to a CTGF nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as the enhancement of the inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by in vivo nucleases.

[0160] Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of a RNA:DNA duplex.

[0161] Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer for a target nucleic acid comprising a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap region generally serves as the substrate for endonuclease cleavage, while the gap segments comprise modified nucleosides. In a preferred embodiment, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxy-ribonucleosides, 2′-modified nucleosides (such 2′-modified nucleobases may include 2′-MOE, and 2′-O—CH₂, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4′-(CH₂)n-O-2′ bridge, where n=1 or n=2). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as “X—Y—Z”, where “X” represents the length of the 5′ wing region, “Y” represents the length of the gap region, and “Z” represents the length of the 3′ wing region. Any of the
antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleotides. Thus, gapmers of the present invention include, but are not limited to, for example 2-13-5, 5-10-5, 4-8-4, 4-12-3, 4-12-4, 3-14-3, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1 or 2-8-2.

In some embodiments, the antisense compound as a “wingmer” motif, having a wing-gap or gap-wing configuration, i.e. an X—Y or Y—Z configuration as described above for the gapmer configuration. Thus, wingmer configurations of the present invention include, but are not limited to, for example 5-10-8, 4-12-8, 4-12-4, 3-14-6, 16-2, 18-1, 10-3, 2-10-1, 2-10-1 or 8-2.

In one embodiment, antisense compounds targeted to a nucleic acid possess a 2-13-5 gapmer motif.

In some embodiments, an antisense compound targeted to a CTGF nucleic acid has a gap-widened motif. In other embodiments, an antisense oligonucleotide targeted to a CTGF nucleic acid has a gap-widened motif.

In one embodiment, a gap-widened antisense oligonucleotide targeted to a CTGF nucleic acid has a gap segment of fourteen 2-deoxyribonucleotides positioned between wing segments of three chemically modified nucleosides. In one embodiment, the chemical modification comprises a 2’-sugar modification. In another embodiment, the chemical modification comprises a 2’-MOE sugar modification.

Antisense compounds having a gapmer motif are considered “chimeric” antisense compounds or “chimeras,” which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide, in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide.

An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In a preferred embodiment, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2’-modified nucleosides (such as 2’-modified nucleosides may include 2’-MOE), and bicyclic sugar modified nucleosides.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. The compounds of the invention can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polynucleotides, polynucleotides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugates groups include cholesterol, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve oligomer uptake, enhance oligomer resistance to degradation, and/or strengthen sequence-specific hybridization with RNA. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve oligomer uptake, distribution, metabolism or excretion. Representative conjugate groups are disclosed in International Patent Application PCT/US92/09196, filed Oct. 23, 1992 the entire disclosure of which is incorporated herein by reference. Conjugate moieties include but are not limited to lipid moieties such as a cholesterol moiety (Lesfinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053-1060), a thioether, e.g., hexyl-5-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-320; Manoharan et al., Bioorg. Med. Chem. Lett., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saisin-Beahmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabarnov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., dihexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycerol-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shear et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamanate acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmitoyl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexy-
lamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937. Oligonucleotides of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-propranofen, carprofen, dapsylsarcosine, 2,3,5-triodobenzoic acid, flufenamic acid, folic acid, a benzothiadiazide, chlo-rothiazide, a diazepine, indomethacin, a barbiturate, a cephalosporin, a sulfon drug, an antidiabetic; an antibacterial or an antibiotic. Oligonucleotide-drug conjugates and their preparation are described in U.S. patent application Ser. No. 09/334,130 (filed Jun. 15, 1999) which is incorporated herein by reference in its entirety.

[0171] Representative United States patents that teach the method of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,857,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,355; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,649; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, certain of which are custom owned with the instant application, and each of which is herein incorporated by reference.

[0172] In another embodiment of the invention, the compound comprises the modified oligonucleotide consists of 20 linked nucleosides.

[0173] In a preferred embodiment of the invention, the compound comprises the nucleobase sequence is the sequence set forth in SEQ ID Nos: 39, 40, 45, 52 and 166.

[0174] In one embodiment of the invention the composition comprises a modified oligonucleotide comprising linked nucleosides, the nucleobase sequence of which is a sequence set forth in one of SEQ ID Nos: 28, 30, 39, 40, 43, 44, 45, 50, 51, 52, 56, 78, 125 and 166 or a salt thereof, and a pharmaceutically acceptable carrier or diluent. Examples of pharmaceutically acceptable salts are well known to those skilled in the art.

[0175] In one embodiment of the invention, the antisense compound is complementary within a range of nucleotides on the cTGF sequence. In certain embodiments the antisense compound is complementary within the range of nucleotides 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, or 2267-2301 of SEQ ID NO: 9. In a certain embodiment the antisense compound is complementary within the range of nucleotides 2728-2797 of SEQ ID NO: 10. Compounds targeted to these ranges demonstrate at least 50% inhibition (i.e. SEQ ID Nos: 15, 29, 31, 42, 46-49, 53, 72, 81, 82, 152-154, 164, and 165). Certain target sites listed in Table 1 also demonstrate at least 50% inhibition (i.e. SEQ ID Nos: 12, 20, 33, 34, 76, 107, 129, 132, 134, 136, and 146). In certain embodiments the antisense compound is complementary within the range of nucleotides 553-611, 1304-1423, 1469-1508, 1559-1605, 1650-1689 or 2100-2129 of SEQ ID NO: 9 and 2623-2647 of SEQ ID NO: 10. Compounds targeted to therein demonstrate at least 60% inhibition (i.e. SEQ ID Nos: 27, 28, 38, 39, 40, 43, 44, 45, 49, 50, 51, 52, 54, 55, 56, 77, 78, 79, 138 and 139). Certain additional target sites listed in Table 1 also demonstrate at least 60% inhibition (i.e. SEQ ID Nos: 24, 30, 61, 63, 67, 69, 73, 86, 125, 128, and 161). In certain embodiments the antisense compound is complementary within the range of nucleotides 1399-1423. Compounds targeted therein demonstrate at least 70% inhibition (i.e. SEQ ID Nos: 39 and 40). Certain target sites listed in Table 1 also demonstrate at least 70% inhibition (i.e. SEQ ID Nos: 28, 30, 44, 45, 51, 56, 78, 128, and 138). One target site listed in Table 1 also demonstrates at least 80% inhibition (i.e. SEQ ID NO: 44). In certain embodiments, the percent inhibition is achieved when the antisense compound is delivered to HuVec cells at a concentration of 50 nm. Refer to Example 8, provided herein below, for more details.

[0176] In an embodiment of the composition, the modified oligonucleotide is a single-stranded or double stranded oligonucleotide. In another embodiment of the invention, comprising a modified oligonucleotide, wherein the modified oligonucleotide consists of 20 linked nucleosides.

[0177] In another embodiment of the invention, provides a method for inhibiting expression of connective tissue growth factor in a cell or a tissue which comprises contacting the cell or tissue with the compound of interest under conditions such that expression of connective tissue growth factor is inhibited.

[0178] Antisense oligonucleotides may be combined with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[0179] Antisense compound targeted to a nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a nucleic acid and a pharmaceutically acceptable diluent. In one embodiment, the pharmaceutically acceptable diluent is PBS. In another embodiment, the pharmaceutically acceptable diluent is pharmaceutical grade saline or pharmaceutical grade PBS. In other embodiments, the antisense compound is an antisense oligonucleotide.

[0180] Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

[0181] A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound. In particular, prodrug versions of the oligonucleotides of the
The term “pharmacologically acceptable salts” refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention; i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects.

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N′-dibenzylethylendiamine, chloroprocain, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., “Pharmaceutical Salts,” J. of Pharma Sci., 1977, 66, 1-19). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a “pharmaceutical addition salt” includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propanic acid, glycic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, lactic acid, oxalic acid, gluconic acid, gluaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-amino-saliclyc acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, adipic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid. Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible.

For oligonucleotides, preferred examples of pharmaceutically acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamino acids such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methylsulfonic acid, p-toluensulfonic acid, naphthalene-disulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chloride, bromine, and iodine.

In certain embodiments of the invention, a pharmaceutically acceptable carrier or diluent is an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable as a solvent, suspending agent or any other pharmaceutically inert vehicle for delivering one or more nucleic acids to a human or non-human animal. Pharmaceutical carriers are well known to those skilled in the art.

Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, “carrier compound” or “carrier” can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate oligonucleotide in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid (Mi-yao et al., Antisense Res. Dev., 1995, 5, 115-121; Takakura et al., Antisense & Nucl. Acid Drug Dev., 1996, 6, 177-183).

Excipients

In contrast to a carrier compound, a “pharmaceutical carrier” or “excipient” is a pharmaceutically acceptable solvent, suspending agent or any other pharmaceutically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium
hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzolate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

[0188] Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which does not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

[0189] Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

[0190] In one embodiment of the invention, the composition comprises a modified oligonucleotide comprises a single-stranded or a double-stranded oligonucleotide, and wherein the modified oligonucleotide consists of 20 linked nucleosides.

[0191] In another embodiment of the invention involves a method for inhibiting expression of connective tissue growth factor in a cell or a tissue which comprises contacting the cell or tissue with any one of the above-mentioned compounds under conditions such that expression of connective tissue growth factor is inhibited.

[0192] In certain embodiment of the invention involves a method of treating an animal having a disease or condition associated with expression of connective tissue growth factor which comprises administering to the animal an amount of the compound described hereinabove effective to inhibit expression of connective tissue growth factor so as to thereby treat the animal.

[0193] In the practice of the method of this invention, an animal includes a human as well as a non-human animal, preferably human.

[0194] The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be by intradermal administration, including intradermal threading of the injection needle, subcutaneous, topical (including opthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intranasal; subcutaneous; intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

[0195] Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful. Preferred intradermal and topical formulations include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidylDOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltrimethylammoniumpropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). Oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters include but are not limited arachidonic acid, oleic acid, eicosanonic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylacyclosheptan-2-one, an acetylcarnitine, an acetycholine, or a C11-10 alkyl ester (e.g. isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. patent application Ser. No. 09/315,298 filed on May 20, 1999 which is incorporated herein by reference in its entirety.

[0196] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0197] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both; and, if necessary, shaping the product.

[0198] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, syringes, pre-filled syringes, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethyl-cellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0199] In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The preparation of such compositions and formulations is generally known to those skilled in the
pharmaceutical and formulation arts and may be applied to the formulation of the compositions of the present invention.

Liposomes

[0200] There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term “liposome” means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

[0201] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages in vivo.

[0202] In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

[0203] Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245).

[0204] Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

[0205] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes. As the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

[0206] Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for intradermal and topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[0207] Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis.

[0208] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

[0209] Liposomes which are pH-sensitive or negatively charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., Journal of Controlled Release, 1992, 19, 269-274).

[0210] One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[0211] Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) were ineffective (Weiner et al., Journal of Drug Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis et al., Antiviral Research, 1992, 18, 259-265).

[0212] Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. S.T.P. Pharma. Sci., 1994, 4, 6, 466).

[0213] Liposomes also include “sterically stabilized” liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{44}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol.
(PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., FEBS Letters, 1987, 223, 42; Wu et al., Cancer Research, 1993, 53, 3765).

[0214] Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (Ann. N.Y. Acad. Sci., 1987, 507, 64) reported the ability of monoauroganglioside galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylethanolamine are disclosed in WO 97/13499 (Lim et al.).

[0215] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, 2C_{12} 15 G, that contains a PEG moiety. Illum et al. (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycolic results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polylkylene glycols (e.g., PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Khilanov et al. (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of dioleoylphosphatidylethanolamine (DOPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al.). U.S. Pat. No. 5,540,935 (Miyazaki et al.) and 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

[0216] A limited number of liposomes comprising nucleic acids are known in the art. WO 96/00622 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an antisense RNA. U.S. Pat. No. 5,665,710 to Kalman et al. describes certain methods of encapsulating oligodeoxy-nucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising antisense oligonucleotides targeted to the raf gene.

[0217] Transferrosomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transferrosomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transferrosomes are adaptable to the environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transferrosomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transferrosomes have been used to deliver serum albumin to the skin. The transferrase-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

[0218] Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the “head”) provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

[0219] If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propanoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

[0220] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl tartarates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[0221] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

[0222] If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.
The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides, to the skin of animals. Most drugs are present in solution in both ionized and non-ionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition, aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants: In connection with the present invention, surfactants (or “surface-active agents”) are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligonucleotides through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylhexane-20-cetyl ether (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92); and perfluorooalkanes, such as FC-43. Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

Fatty Acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lactic acid, capric acid (n-decanic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicartrate, tricartrate, monoolein (1-monoooleyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monononanoate, 1-dodecylacyclocerylheptan-2-one, acyclarnitines, acycl cholines, C10-18 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and diglycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).


Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotides through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanillic), N-acetyl derivatives of collagen, lau reth-9 and N-amino acyl derivatives of beta-diketones (enamines) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al., J. Control Rel., 1990, 14, 43-51).

Non-Chelating Non-Surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligonucleotides through the alimentary mucosa (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylacycloalkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621-626).

Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al., U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of oligonucleotides.

Other agents may be utilized to enhance the penetration of the administered nucleic acids into and through the skin, including gels such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthene.
Other Components

[0233] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels.

[0234] Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0235] Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0236] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

[0237] The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

Certain Indications

[0238] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotide drugs, including ribozymes, have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

[0239] In one embodiment of the invention the method comprises treating a disease or condition, wherein the disease or disorder is a fibrotic disease. In one embodiment of the method of the invention, the fibrotic disease is hypertrophic scarring, keloids, skin scarring, liver fibrosis, pulmonary fibrosis, renal fibrosis, cardiac fibrosis, or restenosis.

[0240] In another embodiment of the invention, the method further comprises treating the above-mentioned disease or condition, wherein the disease or disorder is joint fibrosis (including frozen shoulder syndrome, tendon and peripheral nerve damage), surgical adhesions, spinal cord damage, coronary bypass, abdominal and peritoneal adhesions (including endometriosis, uterine leiomyomata and fibroids), radial keratotomy and photorefractive kerectomy, retinal reattachment surgery, device mediated fibrosis (in for example diabetes), tendon adhesions, Dupuytren contracture, or scleroderma.

[0241] In another embodiment of the invention also provides a method for reducing hypertrophic scarring or keloids resulting from dermal wound healing in a subject in need thereof which comprises administering to the subject an amount of compound of an antisense oligonucleotide effective to inhibit expression of connective tissue growth factor (CTGF) in the subject so as to thereby reduce scarring.

[0242] The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. In general, dosage from 0.1 to 25 mg per injection per linear cm of scar is used, and may be given daily, weekly, every 3 weeks, monthly or bi-monthly.

[0243] In another embodiment of this invention, the method further comprises reducing hypertrophic scarring or keloids resulting from dermal wound healing, wherein wound healing is healing at a wound selected from the group consisting of skin breakage, surgical incisions and burns.

[0244] In certain embodiments, the invention provides methods of treating an individual comprising administering one or more pharmaceutical compositions of the present invention. In certain embodiments, the individual has one of the above mentioned disorders. In certain embodiments, the individual is at risk for one of the above mentioned disorders. In certain embodiments, the individual has been identified as in need of therapy. In certain embodiments the invention provides methods for prophylactically reducing CTGF expression in an individual.

[0245] Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a CTGF nucleic acid.

[0246] In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a CTGF nucleic acid is accompanied by monitoring of CTGF levels in the skin and/or serum of an individual, to determine an individual’s response to administration of the antisense compound. An individual’s response to administration of the antisense compound is used by a physician to determine the amount and duration of therapeutic intervention.
In one embodiment, administration of an antisense compound targeted to a CTGF nucleic acid results in reduction of CTGF expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In one embodiment, administration of an antisense compound targeted to a CTGF nucleic acid results in a change in a measure of CTGF as measured by a standard test, for example, but not limited to, CTGF. In some embodiments, administration of a CTGF antisense compound decreases the measure by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments pharmaceutical composition comprising an antisense compound targeted to CTGF is used for the preparation of a medicament for treating a patient suffering or susceptible to any one of the above-mentioned disorders.

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease or condition as the one or more pharmaceutical compositions of the present invention. In certain embodiments, such one or more other pharmaceutical agents are designed to treat a different disease or condition as the one or more pharmaceutical compositions of the present invention. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired effect of one or more pharmaceutical compositions of the present invention. In certain embodiments, one or more pharmaceutical compositions of the present invention are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions of the present invention and one or more other pharmaceutical agents are prepared separately.

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include a second therapeutic agent. In certain such embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition of the present invention include, but are not limited to second therapeutic agent. In certain such embodiments, the second therapeutic agent is administered prior to administration of a pharmaceutical composition of the present invention. In certain such embodiments, the second therapeutic agent is administered following administration of a pharmaceutical composition of the present invention. In certain such embodiments the second therapeutic agent is administered at the same time as a pharmaceutical composition of the present invention. In certain such embodiments the dose of a co-administered second therapeutic agent is the same as the dose that would be administered if the second therapeutic agent was administered alone. In certain such embodiments the dose of a co-administered second therapeutic agent is lower than the dose that would be administered if the second therapeutic agent was administered alone. In certain such embodiments the dose of a co-administered second therapeutic agent is greater than the dose that would be administered if the second therapeutic agent was administered alone.

In certain embodiments, the co-administration of a second compound enhances the therapeutic effect of a first compound, such that co-administration of the compounds results in a therapeutic effect that is greater than the effect of administering the first compound alone, a synergistic effect. In other embodiments, the co-administration results in therapeutic effects that are supra-additive of the effects of the compounds when administered alone. In other embodiments, the first compound is an antisense compound. In some embodiments, the second compound is an antisense compound.

This invention is illustrated in the Examples Section which follows. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to limit in any way the invention as set forth in the claims which follow thereafter.

EXAMPLES

Example 1

Selection of Lead Human Connective Tissue Growth Factors (CTGF) Antisense Oligonucleotides Candidate

Introduction

A series of oligonucleotides were designed to target different regions of the human connective tissue growth factor RNA, using published sequences (GenBank accession number NM_001901.2, incorporated herein as SEQ ID NO: 9, and GenBank accession number NT_025741.14, incorporated herein as SEQ ID NO: 10).

This study analyzes available sequence space and modified antisense oligonucleotides targeting both exonic and intronic space of CTGF. Approximately 150 novel sequences per target were synthesized and evaluated for activity against CTGF in cell-culture. The oligonucleotides are shown in Table 1. All compounds in Table 1 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of either ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotides "wings" or 13 2'-deoxy-nucleotides, which is flanked on both sides (5' and 3' directions) by two- and five-nucleotides "wings," respectively. The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5'-methylcytidines. The compounds were analyzed for their effect on human connective tissue growth factor mRNA levels by quantitative real-time PCR as described later. Data are averages from two experiments. If present, "N.D." indicates "no data."
<table>
<thead>
<tr>
<th>ISIS #</th>
<th>REGION</th>
<th>SEQ ID NO.</th>
<th>TARGET SITE</th>
<th>SEQUENCE</th>
<th>% INHIB</th>
<th>SEQ ID NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>124173</td>
<td>CDS</td>
<td>9</td>
<td>380</td>
<td>CACGCTGCTTGGCCGAGACC</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>124189</td>
<td>CDS</td>
<td>9</td>
<td>1003</td>
<td>GCCAGAAGAGCTCAAACTTTGA</td>
<td>57</td>
<td>12</td>
</tr>
<tr>
<td>124212</td>
<td>3’-UTR</td>
<td>9</td>
<td>1783</td>
<td>CCACACAGTCTCCACTCTAA</td>
<td>47</td>
<td>13</td>
</tr>
<tr>
<td>124235</td>
<td>3’-UTR</td>
<td>9</td>
<td>2267</td>
<td>GTTCACACTTCTCACAAATA</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>124238</td>
<td>3’-UTR</td>
<td>9</td>
<td>2282</td>
<td>AAACATGTAACCTTTGGTCA</td>
<td>53</td>
<td>15</td>
</tr>
<tr>
<td>412271</td>
<td>5’-UTR</td>
<td>9</td>
<td>4</td>
<td>GCGAAAGAGTTGTTGTGAGA</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>412272</td>
<td>5’-UTR</td>
<td>9</td>
<td>38</td>
<td>AGGGTGGAGTGGGACTGGCT</td>
<td>46</td>
<td>17</td>
</tr>
<tr>
<td>412273</td>
<td>CDS</td>
<td>9</td>
<td>228</td>
<td>ACAGAAAGCCACGCGAGCGAG</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>412274</td>
<td>CDS</td>
<td>9</td>
<td>265</td>
<td>GCGAGCGGCGGCGCGGCTGC</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>412275</td>
<td>CDS</td>
<td>9</td>
<td>475</td>
<td>GTGGCCAGACGCCGACCTTGC</td>
<td>52</td>
<td>20</td>
</tr>
<tr>
<td>412276</td>
<td>CDS</td>
<td>9</td>
<td>483</td>
<td>TCTTTGGCGCTCGACACCC</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>412277</td>
<td>CDS</td>
<td>9</td>
<td>489</td>
<td>GCACCGATCTTTGGCCGTCGA</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>412278</td>
<td>CDS</td>
<td>9</td>
<td>496</td>
<td>GCAGGGAGCAGACGACATTCG</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>412279</td>
<td>CDS</td>
<td>9</td>
<td>501</td>
<td>AGGATCCAGGGGACCCACCCAT</td>
<td>63</td>
<td>24</td>
</tr>
<tr>
<td>412280</td>
<td>CDS</td>
<td>9</td>
<td>507</td>
<td>CCACCCCAAGAGTCCAGGAGC</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>412281</td>
<td>CDS</td>
<td>9</td>
<td>512</td>
<td>CCCTACACCAGAGATCCAG</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>412282</td>
<td>CDS</td>
<td>9</td>
<td>553</td>
<td>GTATCGGACGTCTCTGGA</td>
<td>68</td>
<td>27</td>
</tr>
<tr>
<td>412283</td>
<td>CDS</td>
<td>9</td>
<td>592</td>
<td>GGCCATGCGCCACCCACGCCC</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>412284</td>
<td>CDS</td>
<td>9</td>
<td>718</td>
<td>AGGCCCACCCACGCGTTGTCG</td>
<td>59</td>
<td>29</td>
</tr>
<tr>
<td>412285</td>
<td>CDS</td>
<td>9</td>
<td>723</td>
<td>AGGCCAGGCGCCCAACACGAG</td>
<td>79</td>
<td>30</td>
</tr>
<tr>
<td>412286</td>
<td>CDS</td>
<td>9</td>
<td>732</td>
<td>TAACCCGAGGGCCAGGCC</td>
<td>55</td>
<td>31</td>
</tr>
<tr>
<td>412287</td>
<td>CDS</td>
<td>9</td>
<td>829</td>
<td>CCCCAGGGTTCTGGAGACAGG</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>412288</td>
<td>CDS</td>
<td>9</td>
<td>939</td>
<td>AGATGCCACACCACAGGTC</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>412289</td>
<td>3’-UTR</td>
<td>9</td>
<td>1273</td>
<td>CCAGTCTAATAGTTAATTGAT</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td>412290</td>
<td>3’-UTR</td>
<td>9</td>
<td>1281</td>
<td>TTCAAGTCCTCAGTCTAATGA</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>412291</td>
<td>3’-UTR</td>
<td>9</td>
<td>1361</td>
<td>TTTTTTTTTTTTTTTTTTTTTTTTTTT</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>412292</td>
<td>3’-UTR</td>
<td>9</td>
<td>1389</td>
<td>CACACAGTCTTGGGAACTTG</td>
<td>50</td>
<td>37</td>
</tr>
<tr>
<td>412293</td>
<td>3’-UTR</td>
<td>9</td>
<td>1394</td>
<td>ACATGCGCAATGTTTTGAGA</td>
<td>67</td>
<td>38</td>
</tr>
<tr>
<td>412294</td>
<td>3’-UTR</td>
<td>9</td>
<td>1399</td>
<td>GTCTACACATGCGACACAAAT</td>
<td>73</td>
<td>39</td>
</tr>
<tr>
<td>412295</td>
<td>3’-UTR</td>
<td>9</td>
<td>1404</td>
<td>TATTTTTTTTTTTTTTGACGCA</td>
<td>74</td>
<td>40</td>
</tr>
<tr>
<td>412296</td>
<td>3’-UTR</td>
<td>9</td>
<td>1412</td>
<td>TGATAGACATTTGGTGGAC</td>
<td>35</td>
<td>41</td>
</tr>
<tr>
<td>412297</td>
<td>3’-UTR</td>
<td>9</td>
<td>1457</td>
<td>GTTCACACTGCTTCATTTA</td>
<td>55</td>
<td>42</td>
</tr>
<tr>
<td>412298</td>
<td>3’-UTR</td>
<td>9</td>
<td>1469</td>
<td>TGATCAATGTTATAGTTCCACT</td>
<td>69</td>
<td>43</td>
</tr>
<tr>
<td>412299</td>
<td>3’-UTR</td>
<td>9</td>
<td>1482</td>
<td>CATCCTGCTCTGGTTGACTA</td>
<td>86</td>
<td>44</td>
</tr>
<tr>
<td>412300</td>
<td>3’-UTR</td>
<td>9</td>
<td>1489</td>
<td>TAATATACATTGCTGGTGTCG</td>
<td>76</td>
<td>45</td>
</tr>
<tr>
<td>ISIS # REGION</td>
<td>SOURCE NO</td>
<td>TARGET SITE NO</td>
<td>TARGET SITE SEQUENCE</td>
<td>% INHIB</td>
<td>SEQ ID NO</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>----------------</td>
<td>----------------------</td>
<td>---------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>412301 3'-UTR</td>
<td>9</td>
<td>1495</td>
<td>AACCCTTAATATACATTCTG</td>
<td>54</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>412302 3'-UTR</td>
<td>9</td>
<td>1562</td>
<td>TAAAGGCACACTTATATA</td>
<td>54</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>412303 3'-UTR</td>
<td>9</td>
<td>1520</td>
<td>GTACCTTCCTACGCTCTTA</td>
<td>53</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>412304 3'-UTR</td>
<td>9</td>
<td>1554</td>
<td>AAGATGCTATCTGATGATTAC</td>
<td>52</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>412305 3'-UTR</td>
<td>9</td>
<td>1559</td>
<td>CATAAGATGCTATCTGAT</td>
<td>69</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>412306 3'-UTR</td>
<td>9</td>
<td>1577</td>
<td>AATAACAGGGCATATTACTCG</td>
<td>74</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>412307 3'-UTR</td>
<td>9</td>
<td>1586</td>
<td>TACACTTCAATATACAGCCA</td>
<td>66</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>412308 3'-UTR</td>
<td>9</td>
<td>1591</td>
<td>TCAATACACCTCAGATTG</td>
<td>50</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>412309 3'-UTR</td>
<td>9</td>
<td>1659</td>
<td>GGAAGATGCGATCTTACACTG</td>
<td>66</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>412310 3'-UTR</td>
<td>9</td>
<td>1665</td>
<td>ATGCCCTGAGGACAGCATC</td>
<td>60</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>412311 3'-UTR</td>
<td>9</td>
<td>1670</td>
<td>TCTTGATGGCTGAGAATGCC</td>
<td>71</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>412312 3'-UTR</td>
<td>9</td>
<td>1729</td>
<td>GAATCAAGATGCTAGAGCTG</td>
<td>37</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>412313 3'-UTR</td>
<td>9</td>
<td>1946</td>
<td>CATGGAAATATGAAAGCTT</td>
<td>0</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>412314 3'-UTR</td>
<td>9</td>
<td>1952</td>
<td>GGCTACACTTGGAAATATCCA</td>
<td>25</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>412315 3'-UTR</td>
<td>9</td>
<td>1958</td>
<td>AATTGAGCTGACATTGAAA</td>
<td>1</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>412316 3'-UTR</td>
<td>9</td>
<td>1965</td>
<td>GTTCAGAAATGAGGCTAACC</td>
<td>65</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>412317 3'-UTR</td>
<td>9</td>
<td>1971</td>
<td>TATGGTTCAGAAGATGAG</td>
<td>13</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>412318 3'-UTR</td>
<td>9</td>
<td>1976</td>
<td>CTACCTATGTGCTTGACAAA</td>
<td>61</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>412319 3'-UTR</td>
<td>9</td>
<td>1982</td>
<td>TACATTCTACTTATGATTT</td>
<td>38</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>412320 3'-UTR</td>
<td>9</td>
<td>1991</td>
<td>GACAAGCTTCTTTAACATTCC</td>
<td>24</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>412321 3'-UTR</td>
<td>9</td>
<td>1996</td>
<td>GATCGAGCAAGCTTTACATT</td>
<td>37</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>412322 3'-UTR</td>
<td>9</td>
<td>2007</td>
<td>ATGCTTGCAGCTAGACAGCA</td>
<td>64</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>412323 3'-UTR</td>
<td>9</td>
<td>2012</td>
<td>ATTTCTGCTTGGAAAGAGATC</td>
<td>44</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>412324 3'-UTR</td>
<td>9</td>
<td>2018</td>
<td>GTATCCATTCATCTTCTGAA</td>
<td>60</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>412325 3'-UTR</td>
<td>9</td>
<td>2026</td>
<td>CCATATAAATGCTACCTTCA</td>
<td>48</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>412326 3'-UTR</td>
<td>9</td>
<td>2032</td>
<td>GAAATTCACATATGATACC</td>
<td>28</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>412327 3'-UTR</td>
<td>9</td>
<td>2040</td>
<td>TCTGACGAGAAATTTATGAT</td>
<td>59</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>412328 3'-UTR</td>
<td>9</td>
<td>2050</td>
<td>TGCTATGCTATCGAACAGA</td>
<td>61</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>412329 3'-UTR</td>
<td>9</td>
<td>2060</td>
<td>TTGACGAGACCTGATATCTA</td>
<td>47</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>412330 3'-UTR</td>
<td>9</td>
<td>2070</td>
<td>AACAATGCTTTGACAGGAC</td>
<td>48</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>412331 3'-UTR</td>
<td>9</td>
<td>2088</td>
<td>TGATGCCCTCCCTTGACATAA</td>
<td>53</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>412332 3'-UTR</td>
<td>9</td>
<td>2100</td>
<td>GACCAAGGACACTGATGCTT</td>
<td>68</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>412333 3'-UTR</td>
<td>9</td>
<td>2105</td>
<td>CAGCTGCCCAAGACACTGA</td>
<td>75</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>412334 3'-UTR</td>
<td>9</td>
<td>2110</td>
<td>GAAATGACGCTGGCAAGGAC</td>
<td>60</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>412335 3'-UTR</td>
<td>9</td>
<td>2115</td>
<td>ACCTGAAATCAAGCTGCAA</td>
<td>46</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>ISIS # REGION</td>
<td>SEQ ID</td>
<td>TARGET SITE</td>
<td>SEQUENCE</td>
<td>% INHIB</td>
<td>SEQ ID NO.</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>-------------</td>
<td>----------</td>
<td>---------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>412336</td>
<td>9</td>
<td>2120</td>
<td>TTCTACCTAGAAATACGCC</td>
<td>51</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>412337</td>
<td>9</td>
<td>2128</td>
<td>TACCACATTCCTACCTAGA</td>
<td>59</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>412338</td>
<td>9</td>
<td>2134</td>
<td>TGAGCTACCACTATTCTCTTA</td>
<td>0</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>412339</td>
<td>9</td>
<td>2140</td>
<td>TAAAAGTGGAGCTACACAT</td>
<td>48</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>412340</td>
<td>9</td>
<td>2213</td>
<td>CAAACCTCCAGTGAAAAA</td>
<td>49</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>412341</td>
<td>9</td>
<td>2219</td>
<td>TAGAAACAAATGCTTCCAGG</td>
<td>66</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>412342</td>
<td>9</td>
<td>2230</td>
<td>TCATATCAAGATGAAACCA</td>
<td>12</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>412343</td>
<td>9</td>
<td>2242</td>
<td>TCCGAAAACAGTGCTATCAG</td>
<td>24</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>412369</td>
<td>Intron 1</td>
<td>10</td>
<td>1308</td>
<td>ACCGGCTCGAGGGGCGAG</td>
<td>0</td>
<td>89</td>
</tr>
<tr>
<td>412369</td>
<td>Intron 1</td>
<td>10</td>
<td>1313</td>
<td>CTTTACCAGCGGATGCGAG</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>412370</td>
<td>Intron 1</td>
<td>10</td>
<td>1410</td>
<td>GACAGGCCTCGACGGCGCC</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>412371</td>
<td>Intron 2</td>
<td>10</td>
<td>1730</td>
<td>AGGCCGAGGGGTTTCCTTT</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>412372</td>
<td>Intron 2</td>
<td>10</td>
<td>1735</td>
<td>AACTCAGTCGAGCCGTTTC</td>
<td>19</td>
<td>93</td>
</tr>
<tr>
<td>412373</td>
<td>Intron 2</td>
<td>10</td>
<td>1740</td>
<td>AAGAAACTCTCAGTCGGAGCC</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td>412374</td>
<td>Intron 2</td>
<td>10</td>
<td>1745</td>
<td>TGAGGAAAAGAATGCAGTC</td>
<td>45</td>
<td>95</td>
</tr>
<tr>
<td>412375</td>
<td>Intron 2</td>
<td>10</td>
<td>1750</td>
<td>GCACTTGAGGAAAGAATCC</td>
<td>14</td>
<td>96</td>
</tr>
<tr>
<td>412376</td>
<td>Intron 2</td>
<td>10</td>
<td>1755</td>
<td>TGCGACAGCTGAGAAAGA</td>
<td>46</td>
<td>97</td>
</tr>
<tr>
<td>412377</td>
<td>Intron 2</td>
<td>10</td>
<td>1887</td>
<td>AGAGACGACCATCTTTGGCT</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>412378</td>
<td>Intron 3</td>
<td>10</td>
<td>2125</td>
<td>TACCCCGAGGGCGGACGCC</td>
<td>33</td>
<td>99</td>
</tr>
<tr>
<td>412379</td>
<td>Intron 3</td>
<td>10</td>
<td>2137</td>
<td>GAAAGAGTCAGACTCACGCG</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>412380</td>
<td>Intron 3</td>
<td>10</td>
<td>2142</td>
<td>TTAGAGGAAGACCTGACCTCA</td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td>412381</td>
<td>Intron 3</td>
<td>10</td>
<td>2150</td>
<td>ACCCTGACTTAGAGGGAGAC</td>
<td>47</td>
<td>102</td>
</tr>
<tr>
<td>412382</td>
<td>Intron 3</td>
<td>10</td>
<td>2155</td>
<td>TACGACCCCTGACTTAGAG</td>
<td>31</td>
<td>103</td>
</tr>
<tr>
<td>412383</td>
<td>Intron 3</td>
<td>10</td>
<td>2160</td>
<td>GAGAATCCAGACCCATGCTT</td>
<td>2</td>
<td>104</td>
</tr>
<tr>
<td>412384</td>
<td>Intron 3</td>
<td>10</td>
<td>2165</td>
<td>TGGGAGGAATACACGACCTT</td>
<td>31</td>
<td>105</td>
</tr>
<tr>
<td>412385</td>
<td>Intron 3</td>
<td>10</td>
<td>2170</td>
<td>CTCCCCTGGAGAGATCAGG</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td>412386</td>
<td>Intron 3</td>
<td>10</td>
<td>2191</td>
<td>GGTCCGACAGCTAGGATC</td>
<td>53</td>
<td>107</td>
</tr>
<tr>
<td>412387</td>
<td>Intron 3</td>
<td>10</td>
<td>2196</td>
<td>GTTTGCGGCGACGAGTGA</td>
<td>30</td>
<td>108</td>
</tr>
<tr>
<td>412390</td>
<td>Intron 3</td>
<td>10</td>
<td>2216</td>
<td>CCTCGATAAGGTATTTCCC</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td>412391</td>
<td>Intron 3</td>
<td>10</td>
<td>2235</td>
<td>ACAACACATGGAATAGAGCC</td>
<td>11</td>
<td>110</td>
</tr>
<tr>
<td>412392</td>
<td>Intron 3</td>
<td>10</td>
<td>2256</td>
<td>TAAGCTGCAAGCGAAGACA</td>
<td>2</td>
<td>113</td>
</tr>
<tr>
<td>412393</td>
<td>Intron 3</td>
<td>10</td>
<td>2261</td>
<td>GTCCGTTAAGCTGCGAGAC</td>
<td>23</td>
<td>114</td>
</tr>
<tr>
<td>412394</td>
<td>Intron 3</td>
<td>10</td>
<td>2266</td>
<td>TCCAGOTGCTGAAAGGTC</td>
<td>15</td>
<td>115</td>
</tr>
<tr>
<td>ISIS #</td>
<td>REGION</td>
<td>SEQ ID</td>
<td>TARGET SITE</td>
<td>SEQUENCE</td>
<td>% INHIB</td>
<td>SEQ ID NO.</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>--------</td>
<td>-------------</td>
<td>---------------------------</td>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>412395</td>
<td>Intron 4</td>
<td>10</td>
<td>2472</td>
<td>ACATGACCTTTAATGTTCTC</td>
<td>0</td>
<td>116</td>
</tr>
<tr>
<td>412396</td>
<td>Intron 4</td>
<td>10</td>
<td>2477</td>
<td>GGCGAGACATGACCTTAAAG</td>
<td>0</td>
<td>117</td>
</tr>
<tr>
<td>412397</td>
<td>Intron 4</td>
<td>10</td>
<td>2482</td>
<td>TAGGACGACAGACGATACCT</td>
<td>9</td>
<td>118</td>
</tr>
<tr>
<td>412398</td>
<td>Intron 4</td>
<td>10</td>
<td>2487</td>
<td>GTTAAATAGGACGACGATG</td>
<td>19</td>
<td>119</td>
</tr>
<tr>
<td>412399</td>
<td>Intron 4</td>
<td>10</td>
<td>2496</td>
<td>TGAAAAATAGTAAATGGAG</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>412400</td>
<td>Intron 4</td>
<td>10</td>
<td>2511</td>
<td>CCACTGTTTTTCCGGTAA</td>
<td>10</td>
<td>121</td>
</tr>
<tr>
<td>412401</td>
<td>Intron 4</td>
<td>10</td>
<td>2525</td>
<td>AAGTTAGCTCTATCCACTG</td>
<td>28</td>
<td>122</td>
</tr>
<tr>
<td>412402</td>
<td>Intron 4</td>
<td>10</td>
<td>2530</td>
<td>GCTTCCGTTGACGCTTATC</td>
<td>20</td>
<td>123</td>
</tr>
<tr>
<td>412403</td>
<td>Intron 4</td>
<td>10</td>
<td>2535</td>
<td>CAAGACCCGTTAGTGCCCCG</td>
<td>0</td>
<td>124</td>
</tr>
<tr>
<td>412404</td>
<td>Intron 4</td>
<td>10</td>
<td>2540</td>
<td>CGTGGACAGGGCGTACTTTCC</td>
<td>64</td>
<td>125</td>
</tr>
<tr>
<td>412405</td>
<td>Intron 4</td>
<td>10</td>
<td>2558</td>
<td>CGGCGCTATATACACAAACG</td>
<td>6</td>
<td>126</td>
</tr>
<tr>
<td>412406</td>
<td>Intron 4</td>
<td>10</td>
<td>2563</td>
<td>GAAACCGCGTTTATACATAC</td>
<td>33</td>
<td>127</td>
</tr>
<tr>
<td>412407</td>
<td>Intron 4</td>
<td>10</td>
<td>2568</td>
<td>TTTGGATGAAACCGCTTATA</td>
<td>73</td>
<td>128</td>
</tr>
<tr>
<td>412408</td>
<td>Intron 4</td>
<td>10</td>
<td>2573</td>
<td>TAGTTTTGAGGATACAGCGCC</td>
<td>51</td>
<td>129</td>
</tr>
<tr>
<td>412409</td>
<td>Intron 4</td>
<td>10</td>
<td>2578</td>
<td>TTGATGATGTTTGGAGATAA</td>
<td>24</td>
<td>130</td>
</tr>
<tr>
<td>412410</td>
<td>Intron 4</td>
<td>10</td>
<td>2584</td>
<td>CAAATGTTTATGTTTGGG</td>
<td>36</td>
<td>131</td>
</tr>
<tr>
<td>412411</td>
<td>Intron 4</td>
<td>10</td>
<td>2589</td>
<td>CAGCTCACGTTAGTAGTACTAG</td>
<td>53</td>
<td>132</td>
</tr>
<tr>
<td>412412</td>
<td>Intron 4</td>
<td>10</td>
<td>2594</td>
<td>CAAAACGCTCAATGTTTAG</td>
<td>34</td>
<td>133</td>
</tr>
<tr>
<td>412413</td>
<td>Intron 4</td>
<td>10</td>
<td>2599</td>
<td>TCCAGCAGGAAAACAGCTCAGT</td>
<td>59</td>
<td>134</td>
</tr>
<tr>
<td>412414</td>
<td>Intron 4</td>
<td>10</td>
<td>2604</td>
<td>CTCCATCCGAGGAAAAACAGT</td>
<td>42</td>
<td>135</td>
</tr>
<tr>
<td>412415</td>
<td>Intron 4</td>
<td>10</td>
<td>2609</td>
<td>AAGCCTCTCATCCAGGAGA</td>
<td>57</td>
<td>136</td>
</tr>
<tr>
<td>412416</td>
<td>Intron 4</td>
<td>10</td>
<td>2614</td>
<td>TACACAAGCTTCTCCATCCAG</td>
<td>44</td>
<td>137</td>
</tr>
<tr>
<td>412417</td>
<td>Intron 4</td>
<td>10</td>
<td>2623</td>
<td>GGGTGCTTATACACAGCTCT</td>
<td>72</td>
<td>138</td>
</tr>
<tr>
<td>412419</td>
<td>Intron 4</td>
<td>10</td>
<td>2629</td>
<td>GGGTGCTTATACACAGCTCT</td>
<td>63</td>
<td>139</td>
</tr>
<tr>
<td>412420</td>
<td>Intron 4</td>
<td>10</td>
<td>2633</td>
<td>GAAAACGCTCAGTGTGGCTATT</td>
<td>29</td>
<td>140</td>
</tr>
<tr>
<td>412421</td>
<td>Intron 4</td>
<td>10</td>
<td>2638</td>
<td>TAGTGAGAAACAGCTGTGGTG</td>
<td>5</td>
<td>141</td>
</tr>
<tr>
<td>412422</td>
<td>Intron 4</td>
<td>10</td>
<td>2643</td>
<td>TTAACGTACCCCTGAGGAGA</td>
<td>15</td>
<td>142</td>
</tr>
<tr>
<td>412423</td>
<td>Intron 4</td>
<td>10</td>
<td>2672</td>
<td>TGCCGAGGCGTCTGAGGAT</td>
<td>4</td>
<td>143</td>
</tr>
<tr>
<td>412424</td>
<td>Intron 4</td>
<td>10</td>
<td>2677</td>
<td>TGAAATCGTCTGAGAATTAC</td>
<td>0</td>
<td>144</td>
</tr>
<tr>
<td>412425</td>
<td>Intron 4</td>
<td>10</td>
<td>2691</td>
<td>GCCGAGGCGTCTGAGGAGG</td>
<td>36</td>
<td>145</td>
</tr>
<tr>
<td>412426</td>
<td>Intron 4</td>
<td>10</td>
<td>2698</td>
<td>AAAATAGGCGGAGAGCTCT</td>
<td>59</td>
<td>146</td>
</tr>
<tr>
<td>412427</td>
<td>Intron 4</td>
<td>10</td>
<td>2703</td>
<td>TGCCGAGGCGTCTGAGGAGG</td>
<td>28</td>
<td>147</td>
</tr>
<tr>
<td>412428</td>
<td>Intron 4</td>
<td>10</td>
<td>2708</td>
<td>TGCTGCTCCAAAAATAAGCC</td>
<td>15</td>
<td>148</td>
</tr>
<tr>
<td>412429</td>
<td>Intron 4</td>
<td>10</td>
<td>2713</td>
<td>TCATTGCTGCTGCTCAGAGGC</td>
<td>28</td>
<td>149</td>
</tr>
<tr>
<td>412430</td>
<td>Intron 4</td>
<td>10</td>
<td>2718</td>
<td>GAGCTCTCATTGTGGCTATGCC</td>
<td>20</td>
<td>150</td>
</tr>
</tbody>
</table>
TABLE 1 -continued

<table>
<thead>
<tr>
<th>ISIS #</th>
<th>REGION</th>
<th>SEQ ID NO.</th>
<th>TARGET SITE</th>
<th>SEQUENCE</th>
<th>% INHIB</th>
<th>SEQ ID NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>412430</td>
<td>Intron 4</td>
<td>10</td>
<td>2723</td>
<td>AGTTTGAGTCTTATTTGCTA</td>
<td>30</td>
<td>151</td>
</tr>
<tr>
<td>412431</td>
<td>Intron 4</td>
<td>10</td>
<td>2728</td>
<td>GAGGAGGTTGATGCCTATT</td>
<td>55</td>
<td>152</td>
</tr>
<tr>
<td>412432</td>
<td>Intron 4</td>
<td>10</td>
<td>2763</td>
<td>CTTCGCTGTTGCTGGCTCTTG</td>
<td>55</td>
<td>153</td>
</tr>
<tr>
<td>412433</td>
<td>Intron 4</td>
<td>10</td>
<td>2778</td>
<td>CCTCTGTTGGGATGCTTCTT</td>
<td>56</td>
<td>154</td>
</tr>
<tr>
<td>412434</td>
<td>Intron 4</td>
<td>10</td>
<td>2788</td>
<td>TTTCTCAACCCTCTCTGGTT</td>
<td>15</td>
<td>155</td>
</tr>
<tr>
<td>412435</td>
<td>Intron 4</td>
<td>10</td>
<td>2796</td>
<td>GCTTGCGCTTCTTCCACCC</td>
<td>43</td>
<td>156</td>
</tr>
<tr>
<td>412436</td>
<td>Intron 4</td>
<td>10</td>
<td>2849</td>
<td>AGGAGAGCAAGGGAAAGAGC</td>
<td>20</td>
<td>157</td>
</tr>
<tr>
<td>412437</td>
<td>Intron 4</td>
<td>10</td>
<td>2884</td>
<td>TCTTAAAGGAGAAGAAGGAA</td>
<td>0</td>
<td>158</td>
</tr>
<tr>
<td>412439</td>
<td>Intron 4</td>
<td>10</td>
<td>2859</td>
<td>TGCCCTTCTGAGAGAGCAA</td>
<td>31</td>
<td>159</td>
</tr>
<tr>
<td>412439</td>
<td>Intron 2</td>
<td>10</td>
<td>1791</td>
<td>GGGATGCAAGAGTGGATGCG</td>
<td>0</td>
<td>160</td>
</tr>
<tr>
<td>412440</td>
<td>CDS</td>
<td>9</td>
<td>380</td>
<td>CCAGCTTGTGCTGGGAGGAGC</td>
<td>64</td>
<td>161</td>
</tr>
<tr>
<td>412441</td>
<td>CDS</td>
<td>9</td>
<td>1003</td>
<td>GCCAGAAAGCCCAAATTTGA</td>
<td>37</td>
<td>162</td>
</tr>
<tr>
<td>412442</td>
<td>3'-UTR</td>
<td>9</td>
<td>1783</td>
<td>CCAGAAGGCTGCACTCTTA</td>
<td>32</td>
<td>163</td>
</tr>
<tr>
<td>412443</td>
<td>3'-UTR</td>
<td>9</td>
<td>2267</td>
<td>GGTCCGACCTCTGAGAAAT</td>
<td>59</td>
<td>164</td>
</tr>
<tr>
<td>412444</td>
<td>3'-UTR</td>
<td>9</td>
<td>2282</td>
<td>AAACATGTAACCTTTTGCTA</td>
<td>55</td>
<td>165</td>
</tr>
<tr>
<td>418899</td>
<td>3'-UTR</td>
<td>9</td>
<td>1391</td>
<td>TGCATCTGGCCACTGTGGTG</td>
<td>ND*</td>
<td>166</td>
</tr>
</tbody>
</table>

*ND* - i.e. not determined in the experiment but was highly active in another assay.

**[0255]** As shown in Table 1, SEQ ID NOS 11-15, 17-20, 24, 26-34, 36-57, 59, 61, 63-82, 84-86, 88, 95, 97, 99, 102, 103, 105, 107, 108, 122, 125, 127-140, 145, 146, 149, 151-154, 156, 159, 161-165 demonstrated at least 24% inhibition of human connective tissue growth factor expression in this assay and are therefore preferred. The target sites to which these preferred sequences are complementary are herein referred to as “active sites” and are therefore preferred sites for targeting by compounds of the present invention.

**[0256]** The antisense compound is complementary within a range of nucleotides on the CTGF sequence, i.e. within the range of nucleotides 718-751, 1388-1423, 1457-1689, 2040-2069, 2120-2147, or 2267-2301 of SEQ ID NO: 9. In a certain embodiment the antisense compound is complementary within the range of nucleotides 2728-2797 of SEQ ID NO: 10. Compounds targeted to these ranges demonstrate at least 50% inhibition (i.e. SEQ ID NOs: 15, 29, 31, 42, 46-49, 53, 72, 81, 82, 152-154, 164, and 165). Certain target sites listed in Table 1 also demonstrate at least 50% inhibition (i.e. SEQ ID NOs: 12, 20, 33, 34, 36, 107, 129, 132, 134, 135, and 146).

**[0257]** In certain embodiments the antisense compound is complementary within the range of nucleotides 553-611, 1304-1423, 1469-1508, 1559-1605, 1659-1689 or 2100-2129. Compounds targeted therein demonstrate at least 60% inhibition (i.e. SEQ ID NOs: 27, 38, 43, 50, 52, 54, 55, 77, 79, and 86). Certain target sites listed in Table 1 also demonstrate at least 60% inhibition (i.e. SEQ ID NOs: 24, 61, 63, 67, 69, 73, 125, 139 and 161).

**[0258]** The antisense compound is also complementary within the range of nucleotides 1399-1423. Compounds targeted therein demonstrate at least 70% inhibition (i.e. SEQ ID NOs: 39 and 40). Certain target sites listed in Table 1 also demonstrate at least 70% inhibition (i.e. SEQ ID NOs: 28, 30, 45, 51, 56, 78, 128, and 138). One target site listed in Table 1 also demonstrates at least 80% inhibition (i.e. SEQ ID NO: 44). In certain embodiments, the percent inhibition is achieved when the antisense compound is delivered to HuVec cells at a concentration of 50 nm.

**[0259]** Multiple leads with apparent activity greater than the historical ASO lead sequence, SEQ ID No. 15 (ISIS124238), were identified in both exonic and intronic sequences.

Materials and Methods

**[0260]** Oligonucleotides were evaluated and activity confirmed at a concentration 50 nM in human umbilical vein endothelial cells (HuVEC) using Lipofectin mediated transfection. HuVEC cells from Cascade Biologies (Portland, Oreg.) maintained in Medium 200 supplemented with Low Serum Growth Supplement (from Cascade Biologies) were plated into 96-well plates at 5,000 cells per well and incubated overnight at 37°C in the presence of 5% CO2. The following day the medium was aspirated and replaced with prewarmed Opti-MEM I (Invitrogen) containing Oligo-
Lipofectamine 2000 (Invitrogen) mixture (3 mg of Lipofectamine 2000 per 1 ml of Opti-MEM I medium). After 4 hours, the transfection mixture was exchanged for fresh Medium 200 supplemented with Low Serum Growth Supplement and incubated at 37° C. in the presence of 5% CO₂. After 16-24 hours, at approximately 80% confluence, the cells were washed with phosphate buffer saline (PBS) and lysed for RNA purification with the Qiagen RNeasy Kit. CTGF message was measured by quantitative real time polymerase chain reaction (RT-PCR) (PrimerProbe sets shown below) and the results were normalized to total RNA.

Statistical Analysis

[0261] Each sample was analyzed in duplicate, and vertical bars represent the spread between the two measurements.

Results and Discussion

[0262] Of the approximately 150 novel sequences in Cell-culture, the CTGF oligonucleotides (SEQ ID NOs: 28, 30, 39, 40, 45, 52, 56, 78, 125, and 166) show excellent inhibition of human CTGF mRNA expression.

**Example 2**

A Single-Dose Intra-Dermal Pharmacokinetic Study of CTGF Antisense Oligonucleotide in Rabbits

**Study Objective**

[0263] The purpose of this pharmacokinetic study in rabbit is to evaluate the diffusion and concentration of a CTGF antisense oligonucleotide (SEQ ID NO:39, ISIS 412294) in rabbit skin at different times subsequent to a single intra-dermal injection.

**Study Design**

[0264] On day 0 of the study all animals were dosed intra-dermally (ID) with a single 100 μL injection of CTGF antisense oligonucleotide SEQ ID NO:39 at a concentration of 50 mg/mL (5 mg total dose). The animals were dosed with the antisense oligonucleotide in a site to the left of the spinal mid-line, roughly parallel to the rabbit’s shoulders and adjacent to a suture 3 cm incisional wound (FIG. 1A). The needle was inserted so that the test material was injected down towards the base of the animal’s body. On days 1, 3, 7 or 14, the rabbits were euthanized and two full-thickness 1.0 cm punch biopsies were obtained, one centered over the original injection site and the other below the injection site spaced 0.5 cm apart. The samples were snap frozen and stored at −80° C. prior to analysis of the antisense oligonucleotide drug levels using a hybridization capture method. Results represent the mean antisense oligonucleotide levels from both biopsies at the indicated time.

**Results and Conclusions**

[0265] Significant levels of the antisense oligonucleotide are present up to 14 days after intradermal dosing (see FIG. 1B). The antisense oligonucleotide also remains close to the original site of injection (~1 cm), with very limited lateral diffusion distal to the site of administration (FIG. 1D). For example, levels of ASO are very low at distances 1.5 cm distal to the injection site at all times post injection. Therefore the pharmacological effects of this class of molecule will be limited to areas of skin immediately adjacent to the injection site. To overcome this limitation, an intradermal injection threading technique has been developed (and used in clinical studies) which delivers equal amounts of antisense along the full length of the developing scar. These results demonstrate two novel findings. First, that there is a prolonged residence time of a 2MOE antisense oligonucleotide with this chemical configuration in skin; and second, antisense oligonucleotides have very limited lateral diffusion in skin. The latter limitation of this class of molecule can be overcome by dosing the antisense oligonucleotide by a threading technique as described previously.

**Example 3**

Animal Study with CTGF Antisense Oligonucleotide Targeting Human Keloids in an In Vivo Model System

**Study Objective**

[0266] The purpose of this study was to evaluate the efficacy of antisense oligonucleotides targeting human CTGF in an in vivo model system using a human keloid/mouse xenograft model. The oligonucleotide was antisense oligonucleotide No. 412300 (SEQ ID NO: 45).

**Methods**

Human Keloid/Mouse Xenograft Model.

[0267] A human keloid/mouse xenograft model using transplanted human keloid tissue into nude mice was used.

[0268] Fresh specimens of keloid tissue were obtained anonymously, from discarded tissue of patients undergoing elective excision of keloids for cosmetic reasons. The keloid samples were processed into 10×5×5 mm samples and weighed on an analytical balance. The keloid samples were then implanted into the mice as described below.

[0269] Mice were anaesthetized using 3% isoflurane. Buprenorphine was given before surgery and post-operatively, as needed (Buprenorphine at 0.3 mg/ml; 2 mg/kg; SC).

[0270] After achieving general anesthesia and prepping the animal, two 10 mm incisions were made in the skin, over the left and right scapula, and a pouch created using fine-tipped scissors, between subcutaneous fat and the fascia. One keloid sample was inserted into each pouch. The incisions were closed with veterinary grade cyanoacrylate glue (VetBond or Nexaband) and the area swabbed with 70% ethanol. The area was then dressed with sterile semi-permeable membranes (OpSite™, Smith and Nephew).

**Evaluation of ASOs In Vivo Against Keloids**

[0271] An ASO targeting human (and not mouse) CTGF was used (antisense oligonucleotide No. 412300; SEQ ID NO: 45). To test the efficacy of the ASO in the in vivo model system described above, keloid implants were injected with either ASO (dissolved in PBS) or PBS control. There were two groups of animals with 8 animals per group (with two keloids/animal). Animals were allowed to acclimate for approximately 2 weeks post keloids implantation prior to ASO dosing.
A total dose of 500 µg of oligo per keloid sample was administered in a total volume of 100 µl (from a stock solution of 5.0 mg/ml). The ASO or PBS control was administered immediately adjacent to each implant for a total of 4 weeks, twice a week. One week after the last injection, one implant was removed and subjected to qRT-PCR for gene expression analysis, and the contralateral keloid removed and analyzed histology for the collagen content using integrated density of fluorescence (IDF).

Total RNA was isolated and either CTGF or collagen, type III, alpha 1 (Col3A1) mRNA determined by quantitative real-time PCR using primer pairs that have been shown to be specific for human mRNA sequences. The mRNA levels were correlated with the GAPDH levels in the same samples, and any changes in the ASO-treated samples over the control treated by PBS alone were calculated.

**Results**

Results following 4 weeks of treatment with 500 µg of antisense oligonucleotide No. 412300 (SEQ ID NO: 45) in a total volume of 100 µl per keloid sample indicates reduction in CTGF and Col3A1 mRNA expression in individual keloid samples. Treatment with the CTGF ASO reduced CTGF mRNA expression in the keloid tissue to 67% control (p=0.082) (FIG. 2A). Treatment with the CTGF ASO reduced Col3A1 mRNA expression in the keloid tissue to 45% control (p=0.153) (FIG. 2B).

**Conclusion**

Keloids are characterized by abnormal proliferation of fibroblasts and overproduction of different forms of collagen. Histo-pathologically, they are characterized by the presence of whorls and nodules of thick, hyalinized collagen bundles or keloidal collagen with mucinous ground substance and relatively few fibroblasts in the dermis of keloid scars. The large thick collagen bundles and numerous thin fibrils are closely packed together. Type III collagen is the second most abundant collagen found in the skin, and is very abundant in keloids. Col3A1 is significantly elevated in keloids compared to normal skin.

It has been demonstrated here for the first time, the ability of 2MOE: chemically modified ASOs to reduce CTGF in intact, human keloid tissue. Treatment of the human keloid tissues with an ASO CTGF reduced the target CTGF mRNA expression by 33%. This has also resulted in a reduction in Col3A1 expression of 55%. This reduction in Col3A1 expression would lead to a significant therapeutic benefit in patients suffering from keloid growth, and demonstrate the utility of a 2MOE ASO as a novel drug for the treatment of keloids.

**Example 4**

Breast Scar Revision Study in Humans

**Study Objective**

This is a randomized, double-blind, within-subject controlled clinical study evaluating efficacy and safety of a CTGF antisense oligonucleotides (EXC 001) in subjects undergoing elective revision of scars resulting from prior breast surgery. This study requires that the subjects to have pre-existing scars of sufficient severity to warrant scar revision surgery. Thus, this study pre-screen for subjects who could be expected to have a high rate of hypertrophic scar or keloids formation in the revised placebo-treated scars.

In patients who have had prior surgery of the breast and now have bilateral matching scars at the same anatomic locations, EXC 001 (SEQ ID NO:39) or placebo was administered to the portion of the revised breast scars via intradermal threading injections. The primary objective of the study was to assess the efficacy of EXC 001 in reducing subsequent skin scarring. The secondary objective of the study was to assess the safety of EXC 001.

**Methods**

A 6 cm section of either side of the revised breast wound/scar was treated with 4 doses of EXC 001 or placebo, at 2, 5, 8, and 11 weeks after the surgical incision was closed. Randomization determined which side was treated with EXC 001 or placebo in each subject.

Dosage of EXC 001 used was 5 mg per linear centimeter. Concentration of EXC 001 used was 25 mg/ml and 100 µl of EXC 001 was injected per linear centimeter of the revised breast wound/scar. Injections were made on both sides of each incision, with half of the amount per linear centimeter administered on each side. The injections on a given side were 3 cm apart. In order to deliver placebo or EXC 001 adjacent to and along the length of the incision, intradermal needles (3 cm long) were used for injections. The needle is inserted immediately adjacent to the surgical incision on each side by a threading technique, and EXC 001 or placebo is injected as needle is withdrawn, so that equal amounts of antisense drug are administered along the length of the scar.

The study duration was approximately 31 weeks. The subjects receive the scar revisions on Day 1, followed by 4 doses of EXC 001 and placebo, at 2, 5, 8, and 11 weeks after the surgical incisions were closed. Scar observation and assessment were performed at week 24.

Efficacy was determined by rating each matched pair of incisions from individual patients (within subject analysis). Efficacy was evaluated at week 24 following scar revision surgery, using three methods of rating severity of incisional scars:

**Subject assessment of their scars.** On a scale of 1-10 the patient rated their “overall” opinion on the appearance of the scar.

**Physician (investigator) assessment of the scars.** On a scale of 1-10 the physician rated overall opinion of scar appearance.

**Expert Panel assessment of pairs of blinded scar photographs, using a 100 mm Visual Analog Scale (VAS), where 0=best possible scar and 100=worst possible scar.** This method gives information on the absolute severity of the scars as well as the differences between the two scars in the pair.

**Results**

21 subjects completed this study. Examination of the week 24 post-surgery photographs indicate that nearly all of the subjects show recurrence of hypertrophic scars on at least one side following scar revision surgery. In some cases, the scars extend beyond the boundaries of the original incisions and are therefore keloidal scars.
This study achieved statistically significant results in favor of EXC 001 in all three endpoints (a negative score represents the difference between placebo and EXC 001 treated, in favor of EXC 001—see Table 2 below).

The subject assessment of their scars:

The “overall” rating was statistically significant in favor of EXC 001 (p<0.033).

The physician assessment of the scars:

The “overall” rating was highly statistically significant in favor of EXC 001 (p<0.001).

Expert panel VAS rating of photographs was also highly statistically significant in favor of EXC 001 (p<0.001).

<table>
<thead>
<tr>
<th>Scar Assessment Scale</th>
<th>Difference between placebo and EXC 001</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject Overall</td>
<td>-2.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Physician Overall</td>
<td>-2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Expert VAS</td>
<td>-26.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

FIG. 3A shows that treatment with the CTGF antisense oligonucleotide (EXC 001 or SEQ ID NO: 39) inhibits formation and growth of a hypertrophic scar at 24 weeks post surgery. FIG. 3B shows results from a different subject, also at 24 weeks. In this second example, the patient has developed a keloid at 24 weeks post revision surgery in the placebo treated scar. In contrast, the formation and growth of a keloid scar at the adjacent scar revision site was almost completely prevented by treatment with EXC 001.

Therefore, in these two examples, the growth of both hypertrophic scars and keloid scars are inhibited by treatment with EXC 001. The scores below each set of pictures represent the degree of improvement between placebo- and EXC 001-treated scars. For example, an expert VAS score of -19.3 indicates that the keloid scar is worse than the corresponding EXC 001-treated scar by 19.3 on a 100 point scale. The physician and subject scores of ~1 means that the placebo-treated scar is worse than the corresponding EXC 001-treated scar by 1 point on a 10 point scale. The result demonstrates that EXC 001 reduces the severity of both hypertrophic scars and keloid scars.

Example 5

Primary Prevention Abdominoplasty Study in Humana

Study Objective

This is a randomized, double-blind, within-subject controlled clinical study evaluating efficacy and safety of a CTGF antisense oligonucleotide (EXC 001) in subjects undergoing elective abdominoplasty surgery.

Method

Study duration was 24 weeks. Subjects received the abdominoplasty on day 1, followed by treatment with either EXC 001 or placebo over a 9-week period.

Dosage of EXC 001 used was 5 mg per linear centimeter. Concentration of EXC 001 used was 25 mg/ml and 100 ul of EXC 001 was injected per linear centimeter of the abdominoplasty wound/scar. Injections were made on both sides of each incision, with half of the amount per linear centimeter administered on each side. Either drug or placebo was dosed along two 6 cm portions of the scar at each lateral end of the scar, and so the dosed sections are separated by at least 10 cm of untreated scar. The injections on a given side were 3 cm apart. In order to deliver placebo or EXC 001 adjacent to and along the length of the incision, intradermal needles (3 cm long) were used for injections. The needle is inserted immediately adjacent to the surgical incision on each side using a threading technique, and EXC 001 or placebo is injected as needle is withdrawn.

The subjects receive the scar revisions on Day 1, followed by 4 doses of EXC 001 and placebo, at 2, 5, 8, and 11 weeks after the surgical incisions were closed. Scar observation and assessment were performed at week 12.

Efficacy is determined by rating each matched portion of the dosed incision (placebo treated compared to EXC 001 treated) using the three methods of rating scar severity of incisional scars as described in the previous example.

Results

EXC 001 is efficacious by these criteria at week 12. An example of the efficacy is shown in FIG. 4. In this example, the reduction in scar severity resulting from EXC 001 dosing compared to placebo dosing is clearly seen. The placebo treated section of the incision has developed into a hypertrophic scar whereas the EXC 001 treated section of the scar is more fine-line.

Another example of the ability of EXC 001 to reduce the formation of hypertrophic scars is shown in FIG. 5. In this example, the section of the abdominoplasty on the right side of the scar (to the right of the vertical line) was treated with EXC 001 whereas the scar to the left of the vertical line did not receive any treatment. Clearly the scar severity to the right is less severe than to the left.

This example also demonstrates that the EXC 001 therapeutic benefit is limited to the region of scar directly adjacent to the site of drug intradermal threading. Therefore the drug appears to have limited diffusion away from the site of administration and will require dosing immediately adjacent to the scar, for example by intradermal threading.

Example 6

Biomarker Study Demonstrating Effect of EXC 001 on mRNA Expression of Various Genes in Humans

Method

13 weeks prior to an abdominoplasty procedure, an area between the umbilicus and the suprapubic hairline was used as a site to create a total of twenty 2 cm incisions. The 2 cm long incisions were in four columns of four incisions (A, B, C, D) and were used for mRNA analysis at week 13 post-incisions. Two additional incisions lateral to these columns on each side of the abdomen (a, b, and c, d) were used for 4 or 8 weeks post-incisions mRNA analysis. Each column was separated by at least 4 cm and each incision separated by at least 3 cm.

Treatments of incisional wounds with either EXC 001 or placebo were randomly assigned to two treatment groups. All four incisional wounds in each of the columns A, B, C, and D received the same EXC 001 or placebo dose. All the incisional wounds/scars on one side of the abdomen
received injections of EXC 001 and the other side received injections of placebo. All injections were blinded to the individual receiving the test agents. All incisions/scars in a given subject were treated on the same dosage schedule.

Thirty subjects were randomly assigned to one of three cohorts of 10 subjects each; each cohort was treated on a different dosage schedule (but the high and low doses were the same for all cohorts):

Cohort #1: Intradermal injections at weeks 2, 4, 6, 8, and 10
Cohort #2: Intradermal injections at weeks 2, 5, 8, and 11
Cohort #3: Intradermal injections at weeks 2, 6, and 10

A 27 gauge needle, approximately 38 mm long, was inserted intradermally 2 cm (the entire length of each incision), parallel to and approximately 3 mm from the incisional wound/scar (intradermal threading technique). Either EXC 001 or placebo was then injected while gradually withdrawing the needle so that the correct amount per linear centimeter was evenly deposited into the dermis along the incision line.

Biopsy of Incisions

One 6 mm punch biopsy on each side adjacent to the lateral incisions were taken at day 1 for mRNA analysis and used as control for unwounded skin samples. At week 4, the 9 patients in cohort 1, and at week 8, the 20 patients in cohorts 2 and 3 were biopsied from their lateral incisions (a, b, c, d). Two of these incisions received EXC 001, and two received placebo through randomization. Just prior to the abdominoplasty surgery (at week 13), each of the scars in columns A, B, C, and D were scored for scar severity (by Physician and Expert Panel Scales, see Example 5 above), and also sampled for both histological and RNA analysis.

Results

There was a statistically significant improvement observed for EXC 001 treated vs placebo treated scars for the Physician Overall Opinion when all cohorts were combined at the high dose level (8.7%, p-value=0.018). There was also a statistically significant mean difference observed for the Overall evaluation of all cohorts combined and all doses combined (5.9%, p-value=0.016). These results again demonstrate the ability of EXC 001 to reduce scar severity.

An example of the ability of EXC 001 to reduce the growth and formation of hypertrophic scars is shown in FIG. 6. In this example, two matching scars are shown, one treated with 5 mg/cm EXC 001 and one with placebo. The severity of the EXC 001 treated scar is less than the placebo treated scar.

Histological analysis of these two scars also revealed an EXC 001 mediated reduction in the expression of CTGF protein (by immunohistochemistry) clearly demonstrating that EXC 001 is functioning to reduce the expression of its intended target (CTGF). Analysis of expression of 9 different mRNA transcripts was also performed at 4, 8, and 13 weeks post wounding (Table 3).

<table>
<thead>
<tr>
<th>mRNA transcripts analyzed by XP-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Measured</td>
</tr>
<tr>
<td>Collagen1-a1 (COL1A1)</td>
</tr>
<tr>
<td>Collagen1-a2 (COL1A2)</td>
</tr>
<tr>
<td>Collagen III-a1 (COL3A1)</td>
</tr>
<tr>
<td>Connective Tissue Growth Factor (CTGF)</td>
</tr>
<tr>
<td>Transforming Growth factor-beta1 (TGF-B1)</td>
</tr>
<tr>
<td>Mothers Against Decapentaplegic Homolog 3 (SMAD3)</td>
</tr>
<tr>
<td>Elastin</td>
</tr>
<tr>
<td>Matrix metalloproteinase 1 (MMP-1)</td>
</tr>
<tr>
<td>α-smooth muscle actin (α-SMA/ACTA2)</td>
</tr>
</tbody>
</table>

Following post-surgical wound, increased mRNA expression of CTGF and collagen genes were anticipated. CTGF mRNA expression in the scar was increased from baseline unwounded skin at all three times points, and in all three cohorts (approximately 2-5 fold). Increased expression of a variety of genes known to be associated with scarring, including the collagen genes, such as Col1A2 (5-8 fold) and Col3A1 (3-7 fold), was also observed, as would be expected in developing scar tissues.

When compared to the corresponding placebo treated scar at all three time points measured, suppression of CTGF mRNA induction was observed following EXC 001 injections. As shown in FIG. 7A, at week 13, the overall (across all 3 cohorts) EXC 001 mediated reduction in CTGF mRNA induction was 53%. The degree of CTGF mRNA suppression also varied with time and cohort dosing. For example, in one cohort a single dose of 5 mg/cm reduced the induction in expression of CTGF by 74% (p=0.011) to an induction of only 140% expression compared to unwounded skin (measurement taken two weeks after the last dose of EXC 001) (FIG. 7D). In addition, the overall induced mRNA expression levels of Col1A2, Col3A1 (FIG. 7B), and elastin fibers (ELASF) (FIG. 7C) were also significantly decreased (by 40% (p=0.0013), 69% (p=0.0001), and 63% (p=0.0004), respectively) by EXC 001 treatment, when measured at week 13 (across all three cohorts).

Complete inhibition of collagen gene expression would likely not be a desirable outcome of drug treatment, as some collagen gene expression is required to facilitate normal wound repair and healing processes. As shown in FIGS. 70 and 7E, there was no significant inhibition of either SMAD3 or TGFβ1 mRNA expression by EXC 001 treatment compared to placebo at weeks 4 or 13.

These data demonstrate a mechanism of action for EXC 001 in human. These data also demonstrate a mechanism by which EXC 001 is able to reduce the severity of skin scarring.

**TABLE 3**

<table>
<thead>
<tr>
<th>Gene Measured</th>
<th>Identifier (Accession)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen1-a1 (COL1A1)</td>
<td>NM_000088</td>
</tr>
<tr>
<td>Collagen1-a2 (COL1A2)</td>
<td>NM_000089</td>
</tr>
<tr>
<td>Collagen III-a1 (COL3A1)</td>
<td>NM_000090</td>
</tr>
<tr>
<td>Connective Tissue Growth Factor (CTGF)</td>
<td>NM_001901</td>
</tr>
<tr>
<td>Transforming Growth factor-beta1 (TGF-B1)</td>
<td>NM_000660</td>
</tr>
<tr>
<td>Mothers Against Decapentaplegic Homolog 3 (SMAD3)</td>
<td>NM_005902</td>
</tr>
<tr>
<td>Elastin</td>
<td>M_36860</td>
</tr>
<tr>
<td>Matrix metalloproteinase 1 (MMP-1)</td>
<td>NM_002421</td>
</tr>
<tr>
<td>α-smooth muscle actin (α-SMA/ACTA2)</td>
<td>NM_001613</td>
</tr>
</tbody>
</table>
-continued
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Positive control oligonucleotide directed to human H-ras

<400> SEQUENCE: 1

tcgtcatcg ctctccaggg

<210> SEQ ID NO 2
<211> LENGTH: 2075
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (130) ..(180)

<400> SEQUENCE: 2

cocggcgcag cacgcccgag cgcacggcgg ggctctcgg gttcgcaacc agt acc gcc agc agt atg ggc ccc gtc cgc gtc ggc ttc gtt
Met Thr Ala Ala Ser Met Gly Pro Val Arg Val Ala Phe Val
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

gtc ctc ctc ggc ctc ctc aag cgg cgg ggc gtc ggc cag aac tgc aag
Val Leu Leu Ala Leu Cys Ser Arg Pro Ala Val Gly Gin Amn Cys Ser
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

ggg ccc tgc ccc ccc gac ggg gcc cgg ggc cgc ccc ccc cct ggc
Gly Pro Cys Arg Cys Pro Asp Glu Pro Ala Pro Arg Cys Pro Ala Gly
35 36 37 38 39 40 41 42 43 44 45

gtg aac ctc gtt ctc gac ggc tgc ggc tgc tgc gtc gcc aag
Val Ser Leu Val Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Lys
50 51 52 53 54 55 56 57 58 59 60

cag tgc ggg gag cag tgc aag cgc gac ccc tgc gcc cag aagg
Gln Leu Gly Glu Leu Cys Thr Glu Arg Asp Pro Cys Pro His Lys
65 66 67 68 69 70 71 72 73 74 75

ggc ctc ttc tgt gcc ttc ccc ggc aac cgc aag gtt ggc
Gly Leu Phe Cys Asp Phe Gly Ser Pro Ala Amn Arg Lys Ile Gly Val
80 81 82 83 84 85 86 87 88 89 90

tgc acc gcc aac gat ggt gct ccc tgc aca ttc ggg tgc gtt ggt ttt
tgc ttt aac aca
Cys Thr Ala Lys Arg Pro Ala Pro Phe Gly Gly Thr Val Tyr
95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110

cgc aca gga gag tcc ttc cag aca aca aca cag
Arg Ser Gly Glu Ser Phe Gin Ser Ser Cys Lys Tyr Gin Cys Thr Cys
115 116 117 118 119 120 121 122 123 124 125 126

cag acc gcc cag cgc ccc tgg cgc cgg aag tgg gc
Leu Asp Gly Glu Val Gly Cys Met Pro Leu Cys Ser Met Asp Val Arg
130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145

cgg cac cgc ctc gcc ccc gcc cgg aag cag tgc aag cgg ccc tgg
Leu Pro Ser Pro Asp Phe Pro Arg Arg Arg Val Leu Pro Gly
145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160

aaa tgc tgc gag gag tgt tgt gac gag ccc aag cag ccc aac acc gtt
Lys Cys Cys Glu Glu Thr Val Cys Asp Glu Pro Lys Asp Gin Thr Val
160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175

gtt ggc cgc ctc gcc gcc gtt tac cag ctg cga gac acg ttt ggc cca
Val Gly Pro Ala Leu Ala Ala Tyr Arg Leu Glu Asp Thr Phe Gly Pro
176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191

gac cca act atg att aag gcc aac tgc ctg gcc cag acc aca gac tgt
Asp Pro Thr Met Ile Arg Ala Cys Leu Val Gin Thr Thr Glu Trp
195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
agt ggc tgt tcc aag acc tgt ggg agc atc tcc acc cgg gtt acc
Ser Ala Cys Ser Lys Thr Cys Gly Met Gly Ile Ser Thr Arg Val Thr
210 215 220
aat gac aac ggc tgt tcc aag cag aag cag aag cgc tgt tgc atg
Asn Asp Asn Ala Ser Cys Arg Leu Glu Lys Gin Ser Arg Leu Cys Met
225 230 235
gtc cgg cct tgc gaa gct gac ctc gaa gag cac acc att aag aag ggc aag
Val Arg Pro Cys Glu Ala Asp Leu Glu Glu Arg Asn Ile Lys Lys Gly Lys
240 245 250
agt tgc atc cgt act acc aaa atc tcc aag cct aag ttt gag ctt
Lys Cys Ile Arg Thr Pro Lys Ile Ser Lys Pro Ile Lys Phe Glu Leu
255 260 265
939
tct ggc tgc acc aag cag cag gct gag aat ttc tgt gga gta
Ser Gly Cys Thr Ser Met Lys Thr Tyr Arg Ala Lys Phe Cys Gly Val
275 280 285
987
tgt acc gac ggc cga tgc tgc acc ccc ccc aca aca acc acc ctc cgg
Cys Thr Asp Gly Arg Cys Thr Pro His Arg Thr Thr Leu Pro
290 295 300
1035
gtg gag tgg tgg tcc gct gac ggc gag gtc atg aag aag aac atg atg
Val Glu Phe Lys Pro Asp Gly Glu Val Met Lys Aam Met Met
305 310 315
1083
tcc atc aag acc tgt gcc tgc cat tac acc cgg gga gac aat gac
Phe Ile Lys Thr Cys Ala Cys His Tyr Aam Cys Pro Gly Aam Aam
320 325 330
1131
ttc ttt gaa tgc tcc tcc cag aag atg tgc gcc gac atg gca tga a
Ile Phe Glu Ser Leu Tyr Tyr Lys Met Tyr Gly Asp Met Ala
335 340 345
1180
gccgagagt gagagacatt aaccctattg actggaacct gaaaggtaa atactcctatt
1240
ttcctgtaaa aatgattcct gtagacacgt ttatattaat ctggttttttt aacctggggga
1300
aaagatccc accaatcctc accatgtgrt ccagtcaataa caaatagttc atctttccca
1360
gcactgtgt tgtaggatgt taacttgtaa cgagtaatgg cccttcggcc aagcagcccg
1420
aatgtatatt aagtggttggg ttgaggacgt gttggagcgg atcgggccgg gcggatctattc
1480
cagatgcct cttatacg agtatagctc gctattttga gtagaattg aagaggaatat
1540
ttttagctgt tcaagtgctc gcctgtcgct ccagtcgcg ctaggattgg cattttcggg
1600
ccatccgct actgtagcc tctgccctc attagcgcc aagcagatccgcc gctcttgagct
1660
tctgattgca atagctgctc tccagctggt gaaactgcgc gattgtatgg gcagcatcgt
1720
ggagcagaa tttgctgtct acacgcagcg ttatatattg ataatcttgt
1780
ttgactgtcg tgtggagact tatatatat aagtctggtc atatctgat aattatatattg
1840
tgcttttggc tttttatttt ttttattgttat ctaatggta gcctccatttt
1900
tgagagcct gattgtatgg gtaaagctc tgtgattctt ctaagcatg aatggatcct
1960
tatagagaaa tttcttggtat ctagatgcgt aacaggttat cttgaagagg
2020
gagcagatcg tttctttggca ggtccttattc tattagagaa atcgctttgc tcag
2075

<210> SEQ ID NO 3
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORIGIN: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward PCR primer directed to human
collagenous connective tissue growth factor (CTGF)
<400> SEQUENCE: 3
acaggggct ctctcttgac tt 22

<210> SEQ ID NO 4
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse PCR primer directed to human connective
tissue growth factor (CTGF)

<400> SEQUENCE: 4
ggtacacgt accacogaag at 22

<210> SEQ ID NO 5
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR probe directed to human connective tissue
growth factor (CTGF)

<400> SEQUENCE: 5
tgtgcaccc caagatggtg gct 23

<210> SEQ ID NO 6
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward PCR primer directed to human GAPDH

<400> SEQUENCE: 6
gaaaggtaag gtacaggtc 19

<210> SEQ ID NO 7
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse PCR primer directed to human GAPDH

<400> SEQUENCE: 7
gaagatggtg atgggatttc 20

<210> SEQ ID NO 8
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR probe directed to human GAPDH

<400> SEQUENCE: 8
cagctttccc gtcttcagcc 20

<210> SEQ ID NO 9
<211> LENGTH: 2358
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (207) .. (1256)

<400> SEQUENCE: 9
aaactcaaac aacaactttt cccgegtgag aggagacagc cagtgccgact coaccctcca
60
gctgagggc agcggcggcc gccgacagcc ccggagcagc agcggggcgg gcggcggtcc
120
coacccgga ccacccgagc gctgccggc cccgtaggc cccgagctcc gcacccgacg
180
cctccgctcc gcggcgactg ccaacc atg acc gcc ggc agt atg ggc ccc gtc
233
Met Thr Ala Ala Ser Met Gly Pro Val 1 5
cgc gtc gcc ttc gtt gtc ctc gtc gcc ttc gtc acg cgg ccg gcc gtc
Arg Val Ala Phe Val Val Leu Leu Leu Cys Ser Arg Pro Ala Val
10 15 20 25
281
ggc cag aac tgc acg ggg ccc gtc cgg ctc cgg gac gag ccg ccg
Gly Gln Asn Cys Ser Gly Pro Cys Arg Cys Pro Asp Glu Pro Ala Pro
30 35 40
329
cgc tgc ccc cgg cgg tgt agc ctc gtt ctc gag ggc ggc tgc tgc
Arg Cys Pro Ala Gly Val Ser Leu Ala Leu Asp Gly Cys Cys Cys
45 50 55
377
cgc gtc tgc gcc aag cag ctc ggg gag ctc tgc acc acc gag cgc gac ccc
Arg Val Cys Ala Lys Gln Leu Gly Leu Leu Cys Thr Glu Arg Asp Pro
60 65 70
425
tgc gcc ccg cag aag ggg ccc gtc ttc tgt gac ttc gcc ttc ccc gcc aac
Cys Asp Pro His Lys Gly Phe Cys Asp Pro Gly Ser Pro Ala Asn
75 80 85
473
cgc aag atc ggc tgt tgc acc gcc aag gat ggt gct ccc tgc atc ttc
Arg Lys Ile Gly Val Cys Thr Ala Lys Asp Gly Ala Pro Cys Ile Phe
90 95 100 105
521
ggt aag gtt tac cgc aag gga gag ttc ttc cag aag acc cgt aag
Gly Gly Thr Val Tyr Arg Ser Gly Ser Phe Gln Ser Ser Cys Lys
110 115 120
569
tac cag tgc aag tgc ctc gcc cgg gcc gtt gcc tgc atg ccc ctc tgc
Tyr Cys Thr Cys Leu Asp Gly Ala Val Gly Cys Met Pro Leu Cys
125 130 135
617
agc atg gac gtt cct cgg gcc ctg ccc gcc tgc aag ccc cct cgg aag
Ser Met Asp Val Arg Leu Pro Ser Asp Cys Pro Arg Arg
140 145 150
665
gtc aag ctc ccc ggg aag tgc tgc gag ggc gtt gtt gtt cgc atg cag gag
Val Lys Leu Pro Gly Lys Cys Glu Glu Trp Val Cys Asp Glu Pro
155 160 165
713
aag gag cca acc gtt gtt ggg cct gcc tcc gcc gct tac cca ctg gaa
Lys Asp Gln Thr Val Val Gly Pro Ala Leu Leu Ala Tyr Arg Leu Glu
170 175 180 185
761
gac acg tgt gcc cca gcc cca act atg att aca ggc aac tgc atg gtc
Asp Thr Phe Gly Pro Asp Pro Thr Met Ile Arg Ala Asn Cys Leu Val
190 195 200
809
cag acc cca gag tgg acc gcc ccc tgt ctc aag acc tgg ggg atg ggc atc
Gln Thr Thr Glu Trp Ser Ala Leu Leu Lys Cys Thr Cys Met Gly Ile
205 210 215
857
tcc acc cgg gtt acc aat gac gcc tcc tgc agg cta gag aag cag
Ser Thr Arg Val Thr Asn Asp Ala Ser Cys Arg Leu Glu Glu Gln
220 225 230
905
agc ccc ctg atg gtc cgg gca gct cgg cta ggg cgg gtc cgc
Ser Arg Leu Cys Met Val Arg Pro Cys Glu Ala Asp Leu Glu Glu
235 240 245
953
att aag aag ggc aag aag tgc atc gct act ccc aca aat tcc aag cct
Ile Lys Lys Gly Lys Lys Cys Ile Arg Thr Pro Lys Ile Ser Lys Pro
250 255 260 265
1001
atc aag tgt gag ctg ttc ggc acc aag atg aca taa cgt gct
1049
<table>
<thead>
<tr>
<th>aa</th>
<th>tt</th>
<th>tt</th>
<th>cc</th>
<th>cc</th>
<th>tt</th>
<th>cc</th>
<th>cc</th>
<th>cc</th>
<th>cc</th>
<th>cc</th>
<th>cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>Phe</td>
<td>Glu</td>
<td>Ser</td>
<td>Gly</td>
<td>Cys</td>
<td>Thr</td>
<td>Ser</td>
<td>Met</td>
<td>Lys</td>
<td>Thr</td>
<td>Tyr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>275</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>285</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>290</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>295</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>305</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>310</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>315</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>325</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>330</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>335</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>340</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>345</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>350</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>355</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>360</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>365</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>370</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>375</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>380</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>385</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>390</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>395</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>405</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>410</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>415</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>420</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>425</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>430</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>435</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>440</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>445</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>450</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>455</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>460</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>465</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>470</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>475</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>480</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>485</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>490</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>495</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>505</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>510</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>515</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>520</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>525</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>530</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>535</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>540</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>545</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>550</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>555</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>560</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>565</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>570</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>575</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>580</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>585</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>590</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>595</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<210> SEQ ID NO 10  
<211> LENGTH: 6001  
<212> TYPE: DNA  
<213> ORGANISM: Homo sapiens  
<400> SEQUENCE: 10  

tatatttcc atctgtcact ttagtttata tccagataca acaggggtaca ctgtcttcttg 60  
aatgggaact gcctctctat tttaacagaa caaaccasat ccaatccacaa tttaagatt 120  
ataggttttta atataagaa atatgcaccct ttctcaaaaga ccctagtgaa gctgtgtttta 180  
aatgtcttaa ggtgaacccc cctttgatcct cggtgcttccc accctggacac ccagacgccc 240  
tacctaccac acacagacac atttgctcttg atagacaaact ggtacctcttt ttctggaaac 300
atgattgccc actctcccct tgtctttgccc tatataaacct tctacatatt ataaagagaa 360
aactaagca gaagttttgga aatctgccccc agagactgct atcttgagtc acaogctctc 420
ttgctctct tgtgctccaa acaagcttac tctcaagtgac aatagcataa atctcaaaaata 480
tgacctgtgg gattttaag tgtagccatct tgtggattttt aatgaagttt aacgaactca 540
cctctctct ctctctcattg gagtttcaag aagccatagc ctttaaacaag ccaatacttg 600
cctagggcg ccggtcggcgc accttttcga atttttttag atttcttctgt gtgtgctctc 660
tccagctatct actctcctaaa aagagatgtat gtcagttgac aagacaagggc aaaccttacc 720
gaaaaaagaa taagaactatc ttgccccagtgt gtgtataaat gatataaat aagagtttgtg 780
cgaagaggtatat agggaaaaaa aatatttgg tctggtctggga aatactgctgc ttttttttttttt 840
cctttttttt tttttttttttt agctgtgatgt tgtcagcttt ttcagagcaga ggaatgtgta 900
gttgctcaaggggt cggcagttctg atcggctttt ggtgtgtgag gcggaggtt gccggaggaat 960
gcggaggtactcttttcgcttggttagaactctcctc cactcagctc atggtggcagc gcgcggccc 1020
cggaggtctat aaagacggcg ggcggcggcgc ccacaactc caacaacaatt ctcgcgcgcctg 1080
gagagacgccg ccagagttgg gagctcagctcg acgcgtgacc ggggagggcc gcggagcc 1140
gcgcagagcc gcagccagcgg cagctcctcg gtcctcacct ctgcaacgaa 1200
ggccccgcgc ctccgcctgc gcgcgcacgc ggccgctcgcc tgcgcgcacca 1260
tggagccgagc cagagattgg cccgctccgc tcggttctggt gttctcctggt gcgtctcgtgc 1320
gccggtgatgc gcgcggcgcc gcgcgcgcgg gcgggggagt gcaggggggc 1380
gcgggggcgg gcggtggcgcc gcgcggcgg ggcggctagc gcgggtgcctgcc 1440
gcgggtgctg ccaagactgc aggcggggtg gcgggtgcgc gcggcgcggc 1500
gcccgccggcagg cggctgacct gcgcgggctg gctgcggctgc tgtcgcgcac 1560
gagtggggga gtggtgaccc gacgcggcccc accgcggcgg accggcggcg cctattttgctc 1620
aactagctgct ccggcgccag cccggaagag gaggccgtgac cgctgaaccc 1680
aactaggtgt gcgggtgcct gccggcgccgc cggggcgccgc gcggcggccgc gaaaaagacggc 1740
gtctcgcgtcg agtggcctct cctccaggtcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc 1800
aactagcatc ctcgagctgt ggatggcgaga gtcgcgctgc gtgcgtcggcct cggggtgctctc 1860
aaccctctct ctctctctct ctctctctgc aatggtgggt ctctctctgt ctctggggtct 1920
aogtgtaacc gcggccggac gtcctctccag agaagctgca aagttcagcagt cagctgctgtc 1980
gacggggggt tggggctgtac gcgcgctgct gcagctgaaa cttgtcgtgcc ggcggggtac 2040
tgcccccccc cggagggggt caagggcgcg gggaaatgtc gcggaggttg ggtgtgtgctc 2100
gacggcgaag accaaaacggt ggtggggcct gcctggggcg ggtggggcgag ttcctcctctg 2160
agtcaggtgtc gtgtgtctct cccagggagg gcgcgtcctac gttcgccgaccc gcacgggggga 2220
aataccttat ccaagggttct tcatggtgtg tgtgtgctgt tcgcgcgcgct gcgttggctc 2280
tgagcgcacgc gcgggcgcacg gacgccaaaat ctagtaggc acacttgtgtc gtcgcgcgca 2340
cagacggtgg gcgggctgcac aagctggtgt ggatgggctgc ctcgaccccgg gttacaacctg 2400
acagcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc 2460
cgcgcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc 2520
aaacagcgtgg aagagacatt aaggtcagct tttgtgctct attaacttt ttttcacggag 2580
aaacagcgtgg aagagacatt aaggtcagct tttgtgctct attaacttt ttttcacggag 2580
cttccaaacc tacctaaacc ttagctgttt ttgctggaat gagagcttgt gttaatacga 2640
cocacagttt tcaactcaaa aatcttccca aagggtaagg attoaagao attoaagag 2700
aggcctgcgc tatattttttt gatagcaaat gagaatcca aatcctcctcc tcaaaatata 2760
aacagacagc agacacacgc aagacttaca acagaggttt gaagaagaagccc atctctcttg 2820
tagatcggct gatttttttt tttctctctc cttttctctt gtttctcttt gaagggcaca 2880
aagtgccattc tgcactccaa aatcttaaacc cctatcagtt ttgagctttc ttggtgacc 2940
agatcgaaga cagacacagc taaactcgtt ggatgaagta ctcgagggcc atgcgtgacc 3000
cccagaaagca cccagatcct gcgggtgagg ttcaagtgcg ctagagcga ggtcatgaag 3060
agaaacatga tggtagctca gacgtagtgcc tgcggattaca aagtctcccg agacacagc 3120
atctctgtga aagctgtctca caaggaagatg tacggagaca tgtgcatgaag ccaagaggtg 3180
agagagcatta actcatgagca cttggaacttg aactgattca cattccttttt ttcgtaaaaa 3240
atgattccgc cagccagctt tatattttttt tggattttta ctctgagggg aagatctcaca 3300
cocatcaca aacatttcgcc cattgtaacaa aatactctta tcaagcgcac aagtaacttta 3360
ggaagatgtt aaggaatgagc agtgggaacta catgtagata caagagacagca atgtatatta 3420
aggtgtggct ttagagcagc tggagggtca cccagagaaa gtggtagtgat taccgagatac 3480
atcttataag agtaaatagc tctgcatattg aagttgaatt gagaagaaaattttagcttg 3540
gcttggaagctggctctgatg cccagatgagc aagctgattg tggatcctcgc agcctactaa 3600
agacagacgc aagctgtctca ctagctggag aagctgatgctg ctctctcctt atgctagttc 3660
gagaagacgc cttgaaagc gggagcgttg tggagcagct gttgcagcct gtggcaaggt 3720
aattggcctg taacagcaca gatttttttt aattttattt tgaataatttg tggcctgtgt 3780
ttggtgtgtga tctatattata ttagagtctg tttctctgct aatattaagt ttggtgtgct 3840
tttattatttt tattttttttt tggctgtatt gcctgctattt ctgaacacga 3900
tagtagataa ctagaagcgttt cctgatgattt caaagcaatga aagtgaatat tatattggaa 3960
tctgtctcg tctaggtgac agtgcgtcaaa aacagatgctggcagagggcagctcaagc 4020
tgtcctgagc aggtctggtt tagtaggaa aagtggtagct ctaacctttt aatgcaaaca 4080
tgctgcttttc taaaagacta gtagcctctct atagttctagct aagttttttttt cttggaagga 4140
ttgtttttttta gattttttttt gcaagttttt tttgtgtgagc gttgcagcc 4200
aagtttaagc ttggtctgcc tctgatctgg aataaagggt atatatttttt tataaagggc 4260
tgtgttcttt tttatctcttt tcaatacactt ctggctctttt tctggcaataa ataggaagt 4320
ttttctattc atagtaataat tccccactaa tttctttaattt attctctacta 4380
tatatatattc gatttttctttta gattttttttt atgtaactcact gaacacacagc 4440
tgcacacgctg cagcagccttc acacccatac gcctcttgttttt tttgtttctttc 4500
aoaagattaac atagattaact tttttatatatt ctgctttagc taatctcagt ctttattaca 4560
cottgccca tacatgctgt ctttctgtgc ttttgcttcc tggacggtcct atagttctctg 4620
ttcacacgct cctcaatgac aacatgtcta atgtgcctgc agctggaagaa tttttctttt 4680
atttttata cttatcgttct aggctgctctg ctggacacgct cttttttctttt gttttttatt 4740
aatattatac aattgaaatcct tcatattaat ggatgtattt ctaaagttttttt gtggagatt 4800
atccggtttt tgtagcggat gataaaacat gcaacgacacggagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagagaga
<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 11
ccagctgtct ggccgagacg 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 12
gccgaaagc tcaaacttga 20

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 13
gccgaaagc tcaaacttga 20
ccacaagtctccagtctttaa 20

<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 14

ggtcacacttcacacaata 20

<210> SEQ ID NO 15
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 15

aaacatgtacttttgtcaca 20

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 16

ggsagagttgttsgctgsga 20

<210> SEQ ID NO 17
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 17

aggtggagtctgagtcggtct 20

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 18

acgagggcaacggagacgg 20

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)
<400> SEQUENCE: 19

gccgcaggcc gcgcggtgc

<210> SEQ ID NO 20
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 20

ggtgcacag ccgatctttgc

<210> SEQ ID NO 21
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 21
	tcttgcagc gtacagtgc

<210> SEQ ID NO 22
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 22

gcaaccttt tgcgctgtca

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 23

gcagggac gcatcctttg

<210> SEQ ID NO 24
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 24

aagatgcagg gcagcaccatc

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
**OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)**

**SEQUENCE: 25**

```
ccaacgaaga tgcagggagc 20
```

**SEQ ID NO 26**

**LENGTH: 20**

**TYPE: DNA**

**ORGANISM: Artificial Sequence**

**FEATURES:**

**OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)**

**SEQUENCE: 26**

```
cgtaaccacc gaagatgcag 20
```

**SEQ ID NO 27**

**LENGTH: 20**

**TYPE: DNA**

**ORGANISM: Artificial Sequence**

**FEATURES:**

**OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)**

**SEQUENCE: 27**

```
gtacttgcag ctgctctgga 20
```

**SEQ ID NO 28**

**LENGTH: 20**

**TYPE: DNA**

**ORGANISM: Artificial Sequence**

**FEATURES:**

**OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)**

**SEQUENCE: 28**

```
ggcatgcag cccacccgcc 20
```

**SEQ ID NO 29**

**LENGTH: 20**

**TYPE: DNA**

**ORGANISM: Artificial Sequence**

**FEATURES:**

**OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)**

**SEQUENCE: 29**

```
agcccaccacc aggttttgtg 20
```

**SEQ ID NO 30**

**LENGTH: 20**

**TYPE: DNA**

**ORGANISM: Artificial Sequence**

**FEATURES:**

**OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)**

**SEQUENCE: 30**

```
agggcaggcc caaccaaggt 20
```

**SEQ ID NO 31**

**LENGTH: 20**

**TYPE: DNA**
<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 31

taagccgcga ggcagagtcc

<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 32

cccacaggtc ttggaacagc

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 33

agatgcccct cccacaggtc

<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 34

ccagtctaat gatgtaatgt

<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 35

ttcaagttcc agtctaatgt

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 36

ttttccccca gtagaaaaa

<210> SEQ ID NO 37
-continued

<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 37

cacatagttt tgaatgyggt 20

<210> SEQ ID NO 38
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 38

acagggcaca atgttttgga 20

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 39

gttggacat gcacagttt 20

<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 40

tatgtgtgtg acagggcaca 20

<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 41

tgatagasta ttgttttgac 20

<210> SEQ ID NO 42
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 42

gttccactgt caagttctta 20
...-continued

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 43

tgtactaatg tagttccact

<210> SEQ ID NO 44
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 44

cattctggtg ctgtgtacta

<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 45

taatatacat tctgytgctg

<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 46

aacottaat ataattctga

<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 47

taagccaca ccttaaatata

<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 48
gtacctccc actgtctccta

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 49
aagatgtcat cgtgatgatac

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 50
cgtataagat gctatctgat

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 51
aatagcagc atattaatcag

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 52
tacaccttcc atacaggca

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 53
tcaattatac ttaaatagc

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
<400> SEQUENCE: 54

```
ggagaatgca cactctagct
```

<210> SEQ ID NO 55
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 55

```
atggctgag aatgcacatc
```

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 56

```
ttggatgac tggagaatgc
```

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 57

```
gaatcagaa gtctagcttg
```

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 58

```
cattgaata toaagcatt
```

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 59

```
ggtaacatt gaaatcattc
```

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)
-continued

growth factor (CTGF)

<400> SEQUENCE: 60

aattgaggct aacattgaaa 20

<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 61

gttcagaaat tgagctaac 20

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 62

tatggtgttc agaattgag 20

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 63

c tacctatgg tgcagaa

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 64

tacattctac ctatggtgtt

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 65

gacaagottt acatttaccc

<210> SEQ ID NO 66
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
FEATURE: OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 66

gatcgacac gcgttacatt

SEQ ID NO: 67
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence

FEATURE: OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 67

atgctttgas cgatcgacac

SEQ ID NO: 68
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence

FEATURE: OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 68

atctcatgt tgaacgatc

SEQ ID NO: 69
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence

FEATURE: OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 69

gatccattt atgctttgas

SEQ ID NO: 70
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence

FEATURE: OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 70

catataagt atcatcctca

SEQ ID NO: 71
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence

FEATURE: OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 71

gatcttcc ataatgatcc
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 72

tctgacgaga atttcccatat

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 73

tgtcattcata tgtgacgaga

<210> SEQ ID NO 74
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 74
	ttgacggac tgtcattcata

<210> SEQ ID NO 75
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 75
	acaatcgtgt tttgacggac

<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 76
	tgagccccct cctttgacaa

<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 77

tgccaaggagc actgatgcct
AntiSense directed to human connective tissue growth factor (CTGF)

SEQ ID NO: 78
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
SEQUENCE: 78
cagcctgcac aggacactga

SEQ ID NO: 79
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
SEQUENCE: 79
gaaatcagcc tgcacaagac

SEQ ID NO: 80
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
SEQUENCE: 80
acctgaasat cagcctgcac

SEQ ID NO: 81
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
SEQUENCE: 81
tcctactcta gaaatcagcc

SEQ ID NO: 82
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
SEQUENCE: 82
taccatatt ctcacactga

SEQ ID NO: 83
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
SEQUENCE: 83
tgaggctacc acatttcot
<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 84

taaagtgag gcctaccacat

<210> SEQ ID NO 85
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 85

cgaatgttc caggtgaaga

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 86

tagaaacaa tgggtccagg

<210> SEQ ID NO 87
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 87

tcatatcga gtagaaacaa

<210> SEQ ID NO 88
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 88

tcgggaac aagcatatac

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 89

tcgggaac aagcatatac
accgggtgc agagggcgag

<210> SEQ ID NO 90
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 90

cgcttaccg gctgcagagc

<210> SEQ ID NO 91
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 91
gacagggcg tcagggcgagc

<210> SEQ ID NO 92
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 92
agttcgaggg gttttctttt

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 93
aactcgcttc gacggttttc

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 94
aaagaaactc agtccgagcg

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
-continued

<400> SEQUENCE: 95
tggagaaaga aactcgtc

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 96
gcacgtggag aaaaaaactc

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 97
tggcgcgcc tggagaaaga

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 98
agggacagc atctttggtc

<210> SEQ ID NO 99
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 99
tcaccgcgca ggccagcccc

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 100
ggagactcg actcaccgcc

<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
-continued

"<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 101

ttagaggaag actgacacta
  20

<210> SEQ ID NO 102
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 102

acctgacct agagagac
  20

<210> SEQ ID NO 103
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 103

tcagacacct gccttagagg
  20

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 104

gagaactcag acctgacct
  20

<210> SEQ ID NO 105
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 105

tgggagagaa tcagacacct
  20

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 106

cctccctgga gagaatcagc
  20

<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: DNA
ORGANISM: Artificial Sequence

FEATURE:
OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 107

ggtcgccccag gcacagactc 20

SEQ ID NO 108
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 108
cgtcgcgcgc gcacagactc 20

SEQ ID NO 109
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 109
cctggataeg gattgcccc 20

SEQ ID NO 110
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 110
acaacacaag cgttaaaagcc 20

SEQ ID NO 111
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 111
acgcacacaag ccaccagta 20

SEQ ID NO 112
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 112
tgtagcgcac gacacacacac 20

SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 113

taagctgcga gagcagacga 20

<211> SEQ ID NO 114
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 114

gtcgtaagc tgcagagcga 20

<211> SEQ ID NO 115
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 115

ttcactgagc taaaggtctc 20

<211> SEQ ID NO 116
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 116

acatgtacct taatgttctc 20

<211> SEQ ID NO 117
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 117

gcagaacatg taccttaatg 20

<211> SEQ ID NO 118
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 118

taggagcga acatgtacct 20
<210> SEQ ID NO 119
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 119

gttaatagga gcagaacatg

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 120
tgaaaaatag ttaatagga

<210> SEQ ID NO 121
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 121
ccactgtttt tccgtggaas

<210> SEQ ID NO 122
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 122
aagttgggtc ctatecaactg

<210> SEQ ID NO 123
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 123
gccctagtt ggtctctctc

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 124
-continued

cagagccctaagttgggtc

<210> SEQ ID NO 125
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 125
cagtgcgaagacgcccttaaggtgggtc

<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 126
cagtgcagacgcccttaaggtgggtc

<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 127
cagtgcagacgcccttaaggtgggtc

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 128
cagtgcagacgcccttaaggtgggtc

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 129
cagtgcagacgcccttaaggtgggtc

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)
-continued

400> SEQUENCE: 130

```
ttagatagttttgagatas
```

210> SEQ ID NO: 130
211> LENGTH: 20
212> TYPE: DNA
213> ORGANISM: Artificial Sequence
220> FEATURE:
223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

400> SEQUENCE: 131

```
caatgggttagatatggttgg
```

210> SEQ ID NO: 131
211> LENGTH: 20
212> TYPE: DNA
213> ORGANISM: Artificial Sequence
220> FEATURE:
223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

400> SEQUENCE: 132

```
cagctcaatgttagatagt
```

210> SEQ ID NO: 132
211> LENGTH: 20
212> TYPE: DNA
213> ORGANISM: Artificial Sequence
220> FEATURE:
223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

400> SEQUENCE: 133

```
caacacagctcaatggtgtag
```

210> SEQ ID NO: 133
211> LENGTH: 20
212> TYPE: DNA
213> ORGANISM: Artificial Sequence
220> FEATURE:
223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

400> SEQUENCE: 134

```
tccgcaaaaagctcaatgct
```

210> SEQ ID NO: 134
211> LENGTH: 20
212> TYPE: DNA
213> ORGANISM: Artificial Sequence
220> FEATURE:
223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)

400> SEQUENCE: 135

```
ctcatttcgcaacacagct
```

210> SEQ ID NO: 135
211> LENGTH: 20
212> TYPE: DNA
213> ORGANISM: Artificial Sequence
220> FEATURE:
223> OTHER INFORMATION: Anti-sense directed to human connective tissue growth factor (CTGF)
-continued

growth factor (CTGF)

<400> SEQUENCE: 136
aagcttcataaagctagaaaa 20

<210> SEQ ID NO 137
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 137
tacacaagcttcataaagctag 20

<210> SEQ ID NO 138
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 138
ggtgtgttgtaacacaagtcc 20

<210> SEQ ID NO 139
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 139
cattcattattcattacaca 20

<210> SEQ ID NO 140
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 140
gttctggtgatggttcatt 20

<210> SEQ ID NO 141
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense directed to human connective tissue growth factor (CTGF)

<400> SEQUENCE: 141
tgcggtggttcattcattaca 20

<210> SEQ ID NO 142
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
FEATURE: AntiSense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 142

ttaactaacc ctggtgaaga

SEQ ID NO 143
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 143
tgtcttgast ttaactaacc

SEQ ID NO 144
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 144
tggsatgtct tgaattaact

SEQ ID NO 145
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 145
gccaggcct ccttggtgaast

SEQ ID NO 146
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 146

aaaatatagcc agagcctctc

SEQ ID NO 147
LENGTH: 20
TYPE: DNA
ORGANISM: Artificial Sequence
OTHER INFORMATION: AntiSense directed to human connective tissue growth factor (CTGF)

SEQUENCE: 147
tgtccaaaaa tagccagagc

SEQ ID NO 148
LENGTH: 20
<table>
<thead>
<tr>
<th>SEQ ID NO</th>
<th>LENGTH</th>
<th>ORGANISM</th>
<th>TYPE</th>
<th>FEATURE</th>
<th>OTHER INFORMATION</th>
<th>SEQUENCE:</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>20</td>
<td>Artificial Sequence</td>
<td>DNA</td>
<td></td>
<td>AntiSense directed to human connective tissue growth factor (CTGF)</td>
<td>tgctatgtcc aaaaatagcc</td>
</tr>
<tr>
<td>150</td>
<td>20</td>
<td>Artificial Sequence</td>
<td>DNA</td>
<td></td>
<td>AntiSense directed to human connective tissue growth factor (CTGF)</td>
<td>tcatttgcta tgtccaaaaa</td>
</tr>
<tr>
<td>151</td>
<td>20</td>
<td>Artificial Sequence</td>
<td>DNA</td>
<td></td>
<td>AntiSense directed to human connective tissue growth factor (CTGF)</td>
<td>agtttgagtc tcatttgcta</td>
</tr>
<tr>
<td>152</td>
<td>20</td>
<td>Artificial Sequence</td>
<td>DNA</td>
<td></td>
<td>AntiSense directed to human connective tissue growth factor (CTGF)</td>
<td>gaggaagttt gagttcctatt</td>
</tr>
<tr>
<td>153</td>
<td>20</td>
<td>Artificial Sequence</td>
<td>DNA</td>
<td></td>
<td>AntiSense directed to human connective tissue growth factor (CTGF)</td>
<td>cttctgttgt ctgacttttgt</td>
</tr>
</tbody>
</table>
<210> SEQ ID NO 154
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor [CTGF]

<400> SEQUENCE: 154

cctctggttt ttagtctttct

<210> SEQ ID NO 155
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor [CTGF]

<400> SEQUENCE: 155

tttcttcaacc cctctggttt

<210> SEQ ID NO 156
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor [CTGF]

<400> SEQUENCE: 156

ggagtggttt cttcaaccc

<210> SEQ ID NO 157
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor [CTGF]

<400> SEQUENCE: 157
	aggagagcg gggagagag

<210> SEQ ID NO 158
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor [CTGF]

<400> SEQUENCE: 158
	ttcacgaag cagcagaga

<210> SEQ ID NO 159
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AntiSense directed to human connective tissue growth factor [CTGF]

<400> SEQUENCE: 159

lgcccttctca aggagagc
AntiSense directed to human connective tissue growth factor (CTGF)

```plaintext
ggatgcgcgt tggatctgg
```

AntiSense directed to human connective tissue growth factor (CTGF)

```plaintext
ccagctgctt ggcgcgagcgc
```

AntiSense directed to human connective tissue growth factor (CTGF)

```plaintext
gccagaagc tcaacctga
```

AntiSense directed to human connective tissue growth factor (CTGF)

```plaintext
ccacaagctg tcaagttcag
```

AntiSense directed to human connective tissue growth factor (CTGF)

```plaintext
ggcaccaactc tcaacaata
```

AntiSense directed to human connective tissue growth factor (CTGF)

```plaintext
```
1. A method for treating a keloid, or preventing the formation, reformation, or growth of a keloid after an injury to the skin, in a subject in need thereof, which comprises administering to the subject by one or more injections at the site of the keloid or of the injury to the skin, a composition which comprises a modified oligonucleotide consisting of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a sequence selected from the group consisting of SEQ ID NOs: 38, 39, 40 and 166, or a salt or ester thereof, in an amount effective to treat, or to prevent the formation, reformation, or growth of, the keloid, wherein the effective amount is from 0.1 to 50 mg of the modified oligonucleotide per injection per linear centimeter of the keloid or of the injury to the skin.

2. A method for treating a hypertrophic scar, or preventing the formation, reformation, or growth of a hypertrophic scar after an injury to the skin, in a subject in need thereof, which comprises administering to the subject by one or more injections at the site of the hypertrophic scar or of the injury to the skin, a composition which comprises a modified oligonucleotide consisting of 12-30 linked nucleosides, at least a 12 nucleobase sequence portion of which is present within a sequence selected from the group consisting of SEQ ID NOs: 38, 39, 40 and 166, or a salt or ester thereof, in an amount effective to treat, or to prevent the formation, reformation, or growth of, the hypertrophic scar, wherein the effective amount is from 0.1 to 25 mg of the modified oligonucleotide per injection per linear centimeter of the hypertrophic scar or of the injury to the skin.

3. A method for reducing formation, reformation, or growth of a scar or keloid at a site of an injury to the skin, or of treating a pre-existing scar or keloid, in a subject in need thereof, which comprises administering to the subject by one or more threading injections at the site of the injury or of the pre-existing scar or keloid, the composition of claim 1, or a salt or ester thereof, targeted to a nucleic acid encoding a connective tissue growth factor (CTGF) protein involved in fibrosis in an amount effective to inhibit expression of the protein and thereby reduce scar or keloid formation, reformation, or growth at the site of the injury or to treat the pre-existing scar or keloid.

4. The method of claim 3, wherein the one or more threading injections comprise multiple intradermal threading injections per scar.

5. A method for reducing formation, reformation, or growth of a fibrotic lesion at a site of an injury, or of treating a pre-existing fibrotic lesion, in a subject in need thereof, which comprises administering to the subject by one or more threading injections at the site of the injury or of the pre-existing fibrotic lesion, the composition of claim 1, or a salt or ester thereof, targeted to a nucleic acid encoding a connective tissue growth factor (CTGF) protein involved in fibrosis in an amount effective to inhibit expression of the protein and thereby reduce formation, reformation, or growth of the fibrotic lesion at the site of the injury or to treat the pre-existing fibrotic lesion.

6-13. (canceled)

14. The method of claim 3, wherein the effective amount is from 0.1 to 25 mg of the modified oligonucleotide per injection per linear centimeter of the site of the injury to the skin or of the pre-existing scar.

15. The method of claim 3, wherein the modified oligonucleotide is administered at least once every two weeks for at least four weeks, at least once every three weeks for at least six weeks, at least once every four weeks for at least eight weeks, at least once every eight weeks for at least sixteen weeks, over a period of at least nine weeks, or over a period of 26 weeks.

16-20. (canceled)

21. The method of claim 6, wherein the modified oligonucleotide consists of 12-30 linked nucleosides, at least a 10 nucleobase sequence portion of which is present within a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 38, 39, 40 and 166.
22-24. (canceled)

25. The method of claim 21, wherein the modified oligonucleotide consists of 12-30 linked nucleosides, at least a 10 nucleobase sequence portion of which is present within a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 38, 39, and 40.

26. (canceled)

27. The method of claim 25, wherein the modified oligonucleotide exhibits at least 67% inhibition of human CTGF mRNA expression.

28. (canceled)

29. The method of claim 22, wherein the modified oligonucleotide has a sequence which is 100% identical over its length to a portion of any one of the sequences set forth in SEQ ID NOs: 38, 39, 40, or 166.

30. The method of claim 29, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

31-32. (canceled)

33. The method of claim 29, wherein at least one nucleoside comprises a modified sugar and wherein at least one nucleoside comprises a modified nucleobase.

34-42. (canceled)

43. The method of claim 33, wherein the modified oligonucleotide comprises: (a) a gap segment consisting of thirteen linked deoxynucleosides; (b) a 5' wing segment consisting of two linked modified nucleosides; and (c) a 3' wing segment consisting of five linked modified nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each modified nucleoside within each wing segment comprises a 2'-O-methoxymethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

44. (canceled)

45. The method of claim 22, wherein the sequence of the nucleobase is the sequence set forth in SEQ ID NO: 40.

46-54. (canceled)

55. The method of claim 3, wherein the modified oligonucleotide is present in a conjugate with a moiety which enhances uptake of the compound into, and/or increases residence time of the compound in, the subject, wherein the residence time is 7 to 60 days or in a delivery system which enhances uptake of the compound into, and/or increases residence time of the compound in, the subject, wherein the residence time is 7 to 60 days.

56-60. (canceled)

61. The method of claim 3, wherein the effective amount is about 5 mg of the modified oligonucleotide per injection per linear centimeter of the keloid, the injury to the skin, the site of the injury, or the pre-existing scar.

62-66. (canceled)

67. The method of claim 3, wherein the modified oligonucleotide is administered adjacent to the keloid, the injury to the skin, the site of the injury, or the pre-existing scar; along the entire length of the keloid, the injury to the skin, the site of the injury, or the pre-existing scar; along each side of the keloid, the injury to the skin, the site of the injury, or the pre-existing scar; or directly into the keloid, the injury to the skin, the site of the injury, or the pre-existing scar.

68. (canceled)

69. The method of claim 67, wherein the modified oligonucleotide is administered adjacent each side of the keloid, the hypertrophic scar, the injury to the skin, the site of the injury, the pre-existing scar, or the pre-existing fibrotic lesion.

70-71. (canceled)

72. The method of claim 3, wherein the modified oligonucleotide is administered intradermally by threading technique.

73-76. (canceled)