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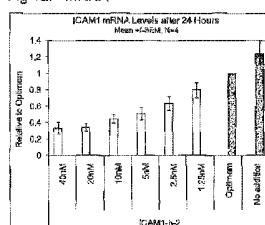
Fig 1: Knockdown of ICAM-1 in the H292 human epithelial cell line by ICAM1-h-2
Fig 1a: mRNA

Fig 1b: Protein

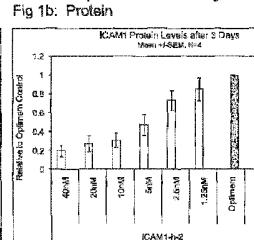
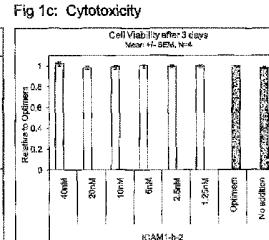


Fig 1c: Cytotoxicity



(57) Abstract: An isolated si RNA comprising a sense sequence and an antisense sequence, wherein the sense and the antisense sequence are substantially complementary to each other to form a double-stranded structure that is 17-25 base pairs in length, and wherein the antisense sequence comprises a nucleotide sequence sufficiently complementary to a target human ICAM-1 nucleotide sequence selected from the group of sequences shown in Table 1.

NOVEL SIRNA INHIBITORS OF HUMAN ICAM-1

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FIELD OF THE INVENTION

The present invention is related to a double-stranded nucleic acid suitable to inhibit the expression of ICAM-1 and uses thereof.

BACKGROUND OF INVENTION

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals and man mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing (PTGS) or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome and is mediated by a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. dsRNA is processed into short duplexes by the enzyme dicer which then enters the RISC complex where the antisense strand specifically targets the degradation of complementary mRNA. RNA interference can also be triggered by direct delivery of small duplexes into the cell (Elbashir *et al.*, 2001, *Nature*, 411, 494) and it is also possible to design hairpin structures (Siolas *et al.*, 2005 *Nature Biotechnol.* 23:227) which may be encoded by plasmids or viruses and also longer duplexes (Kim *et al.*, 2005 *Nature Biotechnol.* 23:222) that are cleaved by dicer to generate an siRNA of interest. This mechanism which is also existing in animal cells and in particular also in mammalian cells, appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

Intercellular adhesion molecule 1 (ICAM-I, CD54) is a member of a family of adhesion proteins and comprises five extracellular (N-terminal) immunoglobulin fold domains (D1-D5), a transmembrane helix and C-terminal cytoplasmic tail (approx 30 residues). It has a broad tissue distribution and is induced by inflammatory cytokines and

LPS. The primary role of ICAM- 1 is as an adhesion molecule involved in leukocyte trafficking; it binds the β_2 -integrins, LFA-1 and Mac-1. LFA-1 (Leukocyte function-associated antigen-1, $\alpha_L\beta_2$, CD11a/CD18), bind to the D1 domain. Mac-1 (macrophage differentiation antigen 1, $\alpha_M\beta_2$, CD11b/CD18) is reported to bind to the D3 domain. Therapeutics that inhibit human ICAM-1 expression have general application by inhibition of leukocyte trafficking in inflammatory disorders and viral and bacterial infections. In particular therapeutics that inhibit ICAM-1 could have utility in a wide therapeutic range including respiratory diseases and disorders such as asthma, COPD, acute respiratory distress syndrome, inflammatory disorders (Luster et. al., *Nature Immunology* 6, 1182–1190, 2005) such as rheumatoid arthritis, psoriasis, sepsis, Crohn's disease, ulcerative colitis, irritable bowel disease, proliferative cell disorders such as cancer including leukemia, atherosclerosis and cardiovascular disease (Kitagawa et. al., *Atherosclerosis*, Volume 160, Issue 2, February 2002, Pages 305-310, Ridker, PM. *European Heart Journal* (1998) **19**, 1119–1121). Inhibition of ICAM-1 is also useful for treating exacerbations of inflammatory disorders triggered by viral or bacterial infections in particular exacerbations of asthma and COPD, bronchiectasis and severe respiratory viruses, in particular human rhinovirus, known to be a major trigger for exacerbations in both asthmatics (Johnston, SL *et al* (1995) Br. Med. J. 310:1225) and COPD patients (Seemungal, T. *et al.* (2001) Am J Respir Crit Care Med. 164:1618).

There is an ongoing need in the art for means of silencing or knocking down the expression levels of ICAM-1 *in vitro* and *in vivo*, including the use of siRNA for the treatment of various diseases. The present invention addresses these unmet needs through discovery of compositions, methods of using and processes of making siRNA directed to ICAM-1.

25

SUMMARY OF THE INVENTION

Various aspects of this invention provide compounds suitable for inhibiting the expression of ICAM-1 in cells. Compositions comprising these compounds are also provided. Also described herein are methods of making and using compounds disclosed herein.

In one aspect, the invention includes an isolated siRNA comprising a sense sequence and an antisense sequence, wherein the sense and the antisense sequence are substantially complementary to each other to form a double-stranded structure or duplex that is 17 to 25

base pairs in length, and wherein the antisense sequence comprises a nucleotide sequence sufficiently complementary to a target human ICAM-1 nucleotide sequence selected from the group of sequences shown in Table 1. All DNA and RNA sequences are annotated from the 5' phosphate residue to the 3' hydroxyl commonly described in the art as 5' to 3'.

5

Table 1:

SEQ ID NO:2	GATTGATGGATGTTAAA
SEQ ID NO:3	CCCTTGATGATATGTAT
SEQ ID NO:4	CCAACCCTTGATGATAT
SEQ ID NO:5	TGCACACCTAAACACT

In one embodiment, the target ICAM-1 nucleotide sequence is selected from the group of sequences shown in Table 2.

Table 2

SEQ ID NO:6	AGACATGATTGATGGATGTTAAA
SEQ ID NO:7	CCCCAACCCCTTGATGATATGTAT
SEQ ID NO:8	CTGACCCAACCCCTTGATGATAT
SEQ ID NO:9	GGTACCTGCACACCTAAACACT

10

In another embodiment, the antisense strand sequence is 17 to 25 nucleotides in length and includes any of the following sequences, wherein the first nucleotide of the antisense sequence begins at nucleotide position 1 of each of the following sequences:

15

SEQ ID NO: 11 **UUUAACAUCCAUCAAUC**;
 SEQ ID NO: 13 **AUACAUAUCAUCAAGGG**;
 SEQ ID NO: 15 **AUAUCAUCAAGGGUUGG**; or
 SEQ ID NO: 17 **AGUGUUUUAGGUGUGCA**.

Nucleotide position 1 is shown above in bold.

In one embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:18, and the antisense strand sequence comprises the sequence of SEQ ID NO:19. In another embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:20, and the antisense strand sequence comprises the sequence of SEQ ID NO:21. In another embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:22, and the antisense strand sequence comprises the sequence of SEQ ID NO:23. In another embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:24, and the antisense strand sequence comprises the sequence of SEQ ID NO:25. In yet another embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:26, and the antisense strand sequence comprises the sequence of SEQ ID NO:27. In another embodiment,

the sense strand sequence comprises the sequence of SEQ ID NO:28, and the antisense strand sequence comprises the sequence of SEQ ID NO:29. In yet another embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:30, and the antisense strand sequence comprises the sequence of SEQ ID NO:31. In another embodiment, the sense strand sequence comprises the sequence of SEQ ID NO:32, and the antisense strand sequence comprises the sequence of SEQ ID NO:33.

In one example, the antisense strand and the sense strand can each be 17 to 25 nucleotides in length. In one embodiment, the double stranded structure or duplex region is from 17 to 25 nucleotides in length, e.g. 23 nucleotides.

The siRNAs according to the present invention can be blunt ended at both ends, or have an overhang at one end and a blunt end at the other; or have an overhang at both ends.

The siRNAs according to the present invention can be modified on the sense and/or antisense strands by one or more modifications. For example, alternating nucleotides on the sense and/or antisense strands can be modified. In one example, the alternating nucleotides on both the sense and antisense strands are modified at the 2'-hydroxyl group of the ribose ring and the modification is amino, fluoro, O-methyl, alkoxy or alkyl. Also one or more phosphodiester groups may be modified to a phosphorothioate group. In one example, each of the odd numbered nucleotides are modified in the antisense strand numbering from 5' to 3' and each of the even numbered nucleotides are modified in the anti-parallel sense strand numbering from 3' to 5'. In another example, a first stretch of the sense strand and a second stretch of the antisense strand each consist of contiguous alternating single 2'-O-methyl modified and single unmodified ribonucleotides, wherein each modified ribonucleotide in the first stretch is base paired with the unmodified ribonucleotide in the second stretch.

The present invention includes a vector having an siRNA as described above. The present invention further includes a cell having an siRNA as described above. Also within the present invention is a composition, such as a pharmaceutical composition including an siRNA as described herein

The present invention further includes a method of generating an siRNA as described above, comprising contacting a chain of ribose nucleotides with Dicer such that the products of Dicer activity are siRNAs as described herein.

The present invention further includes an siRNA delivery system such as an RNA binding protein, cell penetrating pepides, cationic polymers, cationic lipods, carbon nanotudes, chitosan, conjugation with antibodies, conjugation with polycations, lipoplex, or a

liposome comprising an siRNA according to any preceding claim. In one embodiment, the delivery system can include one or a combination of the following: RNA binding proteins, cell penetrating peptide (e.g. antennapedia homeodomain peptide), targeting agents (e.g., peptides, antibodies or aptamers, natural ligands or small molecules) endosomal release agents (e.g., certain viral coat peptides) cationic polymers and other polycations e.g., poly(L-lysine), polyethylenimine, polymethacrylate or carbohydrate-based polymers e.g. chitosan and β -cyclodextrin, lipids, cholesterol including analogues and derivatives, substances containing polyethylene glycol or other substances known to assist oligonucleotide delivery such as carbon nanotubes, Gold or silica nanoparticles and quantum dots and where the 5 siRNA is covalently bonded or non-covalently complexed with the delivery system and exists in solution or in the form of oligomeric complexes or nanoparticles, dendrimers, liposomes, 10 lipoplexes, biodegradable polymeric vectors or polymeric hydrogels. In one example, the liposome includes

15 a) about 50 mol% β -arginyl-2,3-diaminopropionic acid-N-palmityl-N-oleyl-amide trihydrochloride, preferably (β -(L-arginyl)-2,3-L-diaminopropionic acid-N-palmityl-N-oleyl-amide tri-hydrochloride);

b) about 48 to 49 mol% 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE); and

20 c) about 1 to 2 mol% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polyethyleneglycole, preferably N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.

The present invention further includes a use or a method of a siRNA for the treatment of a disease or disorder. The disease or disorder is any disease or disorder that might respond to the modulation of ICAM-1 expression such as respiratory disorders including COPD, rhinovirus infection and asthma, acute lung injury and acute respiratory distress syndrome, inflammatory disease or disorders including sepsis and organ damage such as follows myocardial infarction, autoimmune disease or disorders, cancer and proliferative disease or disorders and cardiovascular disorders including atherosclerosis.

30

BRIEF DESCRIPTION OF DRAWINGS

Further features, embodiments and advantages may be taken from the following figures:

Fig 1a-c depict bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in the H292 epithelial cell line after treatment with siRNA ICAM1-h-2 (siRNA duplex of SEQ ID NO: 18 and SEQ ID NO: 19) over various time periods.

5 **Fig 2a-c** depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in the H292 epithelial cell line after treatment with siRNA ICAM1-hc-3 (siRNA duplex of SEQ ID NO: 20 and SEQ ID NO: 21) over various time periods.

10 **Fig 3a-c** depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in the H292 epithelial cell line after treatment with siRNA ICAM1-hc-6 (siRNA duplex of SEQ ID NO: 22 and SEQ ID NO: 23) over various time periods.

15 **Fig 4a-c** depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in the H292 epithelial cell line after treatment with siRNA ICAM1-h-26 (siRNA duplex of SEQ ID NO: 24 and SEQ ID NO: 25) over various time periods.

20 **Fig 5a-c** depict bar graphs showing mRNA knockdown and protein knockdown of ICAM-1 and cell viability in the H292 cell line after treatment with a control siRNA specific for luciferase over various time periods.

25 **Fig 6a-c** depict bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in primary human epithelial cells after treatment with siRNA ICAM1-h-2 (siRNA duplex of SEQ ID NO: 18 and SEQ ID NO: 19) over various time periods.

30 **Fig 7a-c** depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in primary human epithelial cells after treatment with siRNA ICAM1-hc-3 (siRNA duplex of SEQ ID NO: 20 and SEQ ID NO: 21) over various time periods.

35 **Fig 8a-c** depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in primary human epithelial cells after treatment with siRNA ICAM1-hc-6 (siRNA duplex of SEQ ID NO: 22 and SEQ ID NO: 23) over various time periods.

40 **Fig 9a-c** depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in primary human epithelial cells after treatment with

siRNA ICAM1-h-26 (siRNA duplex of SEQ ID NO: 24 and SEQ ID NO: 25) over various time periods.

Fig 10a-c depicts bar graphs showing mRNA knockdown and protein knockdown of human ICAM-1 and cell viability in primary human epithelial cells after treatment with a control siRNA specific for luciferase over various time periods.

Fig 11a-g depicts bar graphs showing the effect of varying length of ICAM1-hc-3 on ICAM1 mRNA knockdown in H292 cell line 24 hours after transfection.

Fig 12a-g depicts bar graphs showing the effect of varying length of ICAM1-hc-3 on cell viability (by WST-1) in H292 cell line 72 hours after transfection.

Fig 13a-g depicts bar graphs showing the effect of varying length of ICAM1-hc-3 on ICAM1 mRNA knockdown in H292 cell line 24 hours after transfection.

BRIEF DESCRIPTION OF THE SEQUENCES

All sequences shown are 5' to 3'.

SEQ ID NO: 1 is the mRNA sequence encoding human ICAM-1 represented as cDNA. The human ICAM-1 cDNA sequence is described in the entry identifier EMBL:M24283 and by the primary accession number M24283 in the European Molecular Biology (EMBL) database of DNA sequences.

gcattttggggccatggtaacctgcacacctaataactaggccacgcatactgtatctgtatgcataactgactaaagccaaggaggagc
aagaactcaagacatgattgtatggatgttaagtcttagcctgtatgagaggggaagtgggtggggagacatagccccaccatgaggaca
tacaactggaaatactgaaacttgcgcatttgggtatgcgaggccccacagactacagaagaagtggccctccatagacatgt
tagcatcaaaaacacaaaggcccacacttcctgacggatgccagctggcactgtctactgaccccaaccctgtatgtatgtatt
attcatttgttatttaccagctatttattgttagtgtctttatgttaggtaaatgaacataggctctgcectacggagctccagtc
acattcaaggtcaccaggtaacagtgtacagggttacactgcaggagagtgcctggaaaaagatcaaattgggctggacttcat
tggccaaacctgccttccccagaaggagtgttttatcgccacaaaagcactatatggactggtaatggtcacaggcagagatta
ccagtgaggccatttcctccctcccccacaaactgacacccctttagccaccccccacccacatacatttcgcctgttcacaatg
acactcagcggtcatgtctggacatgagtgcacaggatatgcacccagctatgcctgtcttgcatttcactggagc
ttgcactatgtcagctccagttctgcagtgatcagggtctgcaagcagtgggaaggggccaaggatgttggaggactccctcc
agctttggaaaggcctatccgcgtgtgtgtgtgtatgttagacaagctctgcctgtcaccaggctggagtgcaatgggtca
atcatggttcactgcagcttgaccccttgggtcaagtgtatccctccacccctcagcctctgatgtgggaccataggctcacaacac
cacacccatggcaatttgtttttttttttccagagacgggtctgcacacattgcacccagactcccttgtttagtaataaagcttctcaa
ctgcc (SEQ ID NO:1)

15

SEQ ID NOS: 2-9 are exemplary target human ICAM-1 DNA nucleotide sequences

SEQ ID NO:2	GATTGATGGATGTTAAA
SEQ ID NO:3	CCCTTGATGATATGTAT
SEQ ID NO:4	CCAACCCTTGATGATAT
SEQ ID NO:5	TGCACACCTAAACACT
SEQ ID NO:6	AGACATGATTGATGGATGTTAAA
SEQ ID NO:7	CCCCAACCTTGATGATATGTAT
SEQ ID NO:8	CTGACCCCAACCTTGATGATAT
SEQ ID NO:9	GGTACCTGACACCTAAACACT

SEQ ID NOs 11, 13, 15, and 17 are exemplary antisense strands of particular siRNAs.

SEQ ID NO: 11	UUUAACAUCCAUCAUC
SEQ ID NO: 13	AUACAUAUCAUCAAGGG
SEQ ID NO: 15	AUAUCAUCAAGGGUUGG
SEQ ID NO: 17	AGUGUUUUAGGUGUGCA

20

SEQ ID NOs 10, 12, 14, and 16 are exemplary sense strands of particular siRNAs

SEQ ID NO: 10	GAUUGAUGGAUGUUAAA
SEQ ID NO: 12	CCCUUGAUGAU AUGUAU
SEQ ID NO: 14	CCAACCCUUGAUGAU AU
SEQ ID NO: 16	UGCACACCUAAAACACU

SEQ ID NO: 18-33 are exemplary siRNAs having particular sense and antisense strands which form a double stranded structure.

SEQ ID NO: 18	AGACAUUGAUUGAUGGAUGUAAA
SEQ ID NO: 19	UUUAACAUCCAUCAAUCAGUCU
SEQ ID NO: 20	CCCCAACCCUJUGAUGAUUAUGUAU
SEQ ID NO: 21	AUACAUUAUCAUCAAGGGUJUGGGG
SEQ ID NO: 22	CUGACCCCAACCCUJUGAUGUAU

SEQ ID NO: 23	AUAUCAUCAAGGGUUGGGGUUCAG
SEQ ID NO: 24	GGUACCUGCACACCUAAAACACU
SEQ ID NO: 25	AGUGUUUUAGGUGUGCAGGUACC
SEQ ID NO: 26	AUGAUUGAUGGAUGGUAAA
SEQ ID NO: 27	UUUAACAUCCAUCAAUCAU
SEQ ID NO: 28	AACCCUUGAUGAU AUGUAU
SEQ ID NO: 29	AUACAUAUCAUCAAGGGUU
SEQ ID NO: 30	CCCCAACCCUUGAUGAU AU
SEQ ID NO: 31	AUAUCAUCAAGGGUUGGGG
SEQ ID NO: 32	CCUGCACACCUAAAACACU
SEQ ID NO: 33	AGUGUUUUAGGUGUGCAGG

DETAILED DESCRIPTION

The present invention relates to compositions comprising short interfering RNA (siRNA) or a physiologically acceptable salt thereof, directed to an expressed RNA transcript of ICAM-1 (target nucleic acid). The siRNA of the invention are nucleic acid molecules comprising a double stranded or duplex region. The present invention further relates to methods of using the siRNA compositions to reduce the expression level of ICAM-1. As used herein, the terms “silence” or “knock-down” when referring to gene expression means a reduction in gene expression. The reduction in gene expression can include where a cell expressing the ICAM-1 when in contact with the siRNA agent will express at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% less of ICAM-1 compared to a similar cell which is not in contact with the gene product.

As used herein, “disorders associated with ICAM-1 expression” refer to any biological or pathological state where the disorder is mediated at least in part by the expression of ICAM-1 and whose outcome can be affected by reducing the level of ICAM-1. Diseases or disorders include respiratory disorders including asthma, COPD, acute respiratory distress syndrome, rheumatoid arthritis, psoriasis, sepsis, Crohn’s disease, ulcerative colitis, irritable bowel disease, cancer, atherosclerosis and cardiovascular disease. Exacerbations of inflammatory disorders triggered by viral or bacterial infections in particular exacerbations of asthma and COPD, bronchiectasis and severe cancer leukemia. Inhibition of infection by respiratory viruses, in particular human rhinovirus, known to be a major trigger for exacerbations in both asthmatics (Johnston, SL *et al* (1995) Br, Med. J. 310:1225) and COPD patients (Seemungal, T. *et al.* (2001) Am J Respir Crit Care Med. 164:1618).

In one aspect, the target nucleic acid is an RNA expressed from a mammalian ICAM-1 gene. In one the target nucleic acid is an RNA expressed from human ICAM-1. In another

embodiment the target nucleic acid is a human ICAM-1 mRNA. In another embodiment the target nucleic acid is a human ICAM-1 hnRNA. In another embodiment the target nucleic acid is ICAM-1 DNA corresponding to the sequence of SEQ ID NO: 1.

The siRNA of the present invention are suitable to inhibit the expression of ICAM-1.

5 In one non-limiting embodiment expression is inhibited by a mechanism which is referred to as RNA interference (RNAi). The siRNA according to the present invention is thus suitable to trigger the RNA interference response resulting in the reduction of the ICAM-1 mRNA in a mammalian cell. The siRNA according to the present invention are further suitable to decrease the expression of ICAM-1 protein by decreasing gene expression at the level of
10 mRNA.

In one embodiment, the invention includes a pharmaceutically-acceptable salts of the siRNA described herein. A suitable pharmaceutically-acceptable salt of an siRNA described herein can be a sodium salt. In another example, the salt can be an acid-addition salt of an siRNA which is sufficiently basic or an acid-addition salt with an inorganic or organic acid
15 such as hydrochloric, hydrobromic, sulphuric, phosphoric, trifluoroacetic, citric, maleic, tartaric, fumaric, hemifumaric, succinic, hemisuccinic, mandelic, methanesulphonic, dimethanesulphonic, ethane-1,2-sulphonic, benzenesulphonic, salicylic or 4-toluenesulphonic acid.

20 *siRNA Design:* An siRNA of the present invention comprises two strands of a nucleic acid, a first, antisense strand and a second, sense strand. The nucleic acid normally consists of ribonucleotides or modified ribonucleotides however; the nucleic acid may comprise deoxynucleotides (DNA) as described herein. The siRNA further comprises a double-stranded nucleic acid portion or duplex region formed by all or a portion of the antisense strand and all or a portion of the sense strand. The portion of the antisense strand forming the
25 duplex region with the sense strand is the antisense strand duplex region or simply, the antisense duplex region, and the portion of the sense strand forming the duplex region with the antisense strand is the sense strand duplex region or simply, the sense duplex region. The duplex region is defined as beginning with the first base pair formed between the antisense strand and the sense strand and ending with the last base pair formed between the antisense
30 strand and the sense strand, inclusive. The portions of the siRNA on either side of the duplex region are the flanking regions. The portion of the antisense strand on either side of the antisense duplex region is the antisense flanking regions. The portion of the antisense strand 5' to the antisense duplex region is the antisense 5' flanking region. The portion of the

antisense strand 3' to the antisense duplex region is the antisense 3' flanking region. The portion of the sense strand on either side of the sense duplex region is the sense flanking regions. The portion of the sense strand 5' to the sense duplex region is the sense 5' flanking region. The portion of the sense strand 3' to the sense duplex region is the sense 3' flanking
5 region.

Complementarity: In one aspect, the antisense duplex region and the sense duplex region may be fully complementary and in another aspect are at least partially complementary to each other. Such complementarity is based on Watson-Crick base pairing (i.e., A:U and G:C base pairing). Depending on the length of a siRNA a perfect match in
10 terms of base complementarity between the antisense and sense duplex regions is not necessarily required however, the antisense and sense strands must be able to hybridize under physiological conditions.

In one embodiment, the complementarity between the antisense strand and sense strand is perfect (no nucleotide mismatches or additional/deleted nucleotides in either strand).

15 In one embodiment, the complementarity between the antisense duplex region and sense duplex region is perfect (no nucleotide mismatches or additional/deleted nucleotides in the duplex region of either strand).

In another embodiment, the complementarity between the antisense duplex region and the sense duplex region is not perfect. In one embodiment, the identity between the antisense
20 duplex region and the complementary sequence of the sense duplex region is selected from the group consisting of at least 75%, 80%, 85%, 90% and 95%; wherein a siRNA comprising the antisense duplex region and the sense duplex region is suitable for reducing expression of ICAM-1. In another embodiment, the siRNA, wherein the identity between the antisense duplex region and complementary sequence of the sense duplex region is selected from the group consisting of at least 75%, 80%, 85%, 90% and 95%, is able to reduce expression of
25 ICAM-1 by at least 25%, 50% or 75% of a comparative siRNA having a duplex region with perfect identity between the antisense duplex region and the sense duplex region. As used herein the term “comparative siRNA” is a siRNA that is identical to the siRNA to which it is being compared, except for the specified difference, and which is tested under identical
30 conditions.

RNAi using siRNA involves the formation of a duplex region between all or a portion of the antisense strand and a portion of the target nucleic acid. The portion of the target nucleic acid that forms a duplex region with the antisense strand, defined as beginning with

the first base pair formed between the antisense strand and the target sequence and ending with the last base pair formed between the antisense strand and the target sequence, inclusive, is the target nucleic acid sequence or simply, target sequence. The duplex region formed between the antisense strand and the sense strand may, but need not be the same as the duplex 5 region formed between the antisense strand and the target sequence. That is, the sense strand may have a sequence different from the target sequence however; the antisense strand must be able to form a duplex structure both with the sense strand and the target sequence.

Generally a target sequence is selected from the cDNA sequence corresponding to the target mRNA. Examples of suitable target sequences for ICAM-1 include those target 10 sequences shown in Table 1.

In particular the target sequence is one that is homologous to another mammalian ICAM-1 species. In one example, ICAM1-hc-3 and ICAM1-hc-6 all have a homologous sequence in cynomolgous.

In one embodiment, the complementarity between the antisense strand and the target 15 sequence is perfect (no nucleotide mismatches or additional/deleted nucleotides in either nucleic acid).

In one embodiment, the complementarity between the antisense duplex region (the portion of the antisense strand forming a duplex region with the sense strand) and the target sequence is perfect (no nucleotide mismatches or additional/deleted nucleotides in either 20 nucleic acid).

In another embodiment, the complementarity between the antisense duplex region and the target sequence is not perfect. In one embodiment the identity between the antisense duplex region and the complementary sequence of the target sequence is selected from the group consisting of at least 75%, 80%, 85%, 90% or 95% complementarity, wherein a siRNA 25 comprising the antisense duplex region is suitable for reducing expression of ICAM-1. In another embodiment the siRNA, wherein the identity between the antisense duplex region and complementary sequence of the target sequence is selected from the group consisting of at least 75%, 80%, 85%, 90% and 95% complementarity, is able to reduce expression of ICAM-1 by at least 25%, 50% or 75% of a comparative siRNA with perfect identity to the 30 antisense strand and target sequence.

In another embodiment, the siRNA of the invention comprises a duplex region wherein the antisense duplex region has a number of nucleotides selected from the group consisting of 1, 2, 3, 4 and 5 that are not base-paired to a nucleotide in the sense duplex

region, and wherein said siRNA is suitable for reducing expression of ICAM-1. Lack of base-pairing is due to either lack of complementarity between bases (i.e., no Watson-Crick base pairing) or because there is no corresponding nucleotide on either the antisense duplex region or the sense duplex region such that a bulge is created. In one embodiment a siRNA comprising an antisense duplex region having a number of nucleotides selected from the group consisting of 1, 2, 3, 4 and 5 that are not base-paired to the sense duplex region, is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA wherein all nucleotides of said antisense duplex region are base paired with all nucleotides of said sense duplex region.

In another embodiment, the antisense strand has a number of nucleotides selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 that do not base-pair to the sense strand, and wherein a siRNA comprising said antisense strand is suitable for reducing expression of ICAM-1. Lack of complementarity is due to either lack of complementarity between bases or because there is no corresponding nucleotide on either the antisense strand or the sense strand. The lack of a corresponding nucleotide results in either a single-stranded overhang or a bulge (if in the duplex region), in either the antisense strand or the sense strand. In one embodiment a siRNA comprising an antisense strand having a number of nucleotides selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 that do not base pair to the sense strand, is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA wherein all nucleotides of said antisense strand are complementary to all nucleotides of the sense strand. In one embodiment a siRNA comprising an antisense strand having a number of nucleotides selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 that are mismatched to the target sequence, is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA wherein all nucleotides of said antisense strand are complementary to all nucleotides of said sense strand. In another embodiment, all of the mismatched nucleotides are outside the duplex region.

In another embodiment, the sense duplex region has a number of nucleotides selected from 1, 2, 3, 4 or 5 that do not base-pair to the antisense duplex region, and wherein a siRNA comprising said antisense duplex region is suitable for reducing expression of ICAM-1. Lack of complementarity is due to either lack of complementarity between bases or because there is no corresponding nucleotide on either the antisense duplex region or the sense duplex region such that a bulge is created in either the antisense duplex region or the sense duplex region. In one embodiment, a siRNA comprising an sense duplex region having a number of nucleotides selected from 1, 2, 3, 4 and 5 that do not base pair to the antisense duplex region,

is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA wherein all nucleotides of said sense duplex region are complementary to all of the nucleotides of said antisense duplex region.

In another embodiment, the antisense strand has a number of nucleotides selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 that do not base-pair to the target sequence, and wherein a siRNA comprising said antisense strand is suitable for reducing expression of ICAM-1. Lack of complementarity is due to either lack of complementarity between bases or because there is no corresponding nucleotide on either the antisense strand or the target sequence. The lack of a corresponding nucleotide results in a bulge in either the antisense strand or the target sequence. In one embodiment, a siRNA comprising an antisense strand having a number of nucleotides selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 do not base pair to the target sequence, is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA wherein all nucleotides of said antisense strand are complementary to all nucleotides of said target sequence. In one embodiment, a siRNA comprising an antisense strand having a number of nucleotides selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 that are mismatched to the target sequence, is able to reduce expression of ICAM-1 by at least 25%, 50% or 75% of a comparative siRNA wherein all nucleotides of said antisense strand are complementary to all nucleotides of said target sequence.

In another embodiment, the complementarity between an antisense duplex region and both a sense duplex region and a target sequence of an siRNA is such that the antisense duplex region and the sense duplex region or the target sequence hybridize to one another under physiological conditions (37°C in a physiological buffer) and the siRNA is suitable for reducing expression of ICAM-1. In one embodiment, the siRNA comprising an antisense duplex region that hybridizes to a sense duplex region and a target sequence under physiological conditions, is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA with perfect complementarity between the antisense strand and target sequence.

In another embodiment, the complementarity between an antisense duplex region and a sense duplex region of a siRNA is such that the antisense duplex region and sense duplex region may hybridize under the following conditions: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 70°C, and is suitable for reducing expression of ICAM-1. In one embodiment, the siRNA comprising an antisense duplex region and a sense duplex region that hybridize to one another under the conditions 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 70°C,

is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA with perfect complementarity between the antisense duplex region and sense duplex region.

In another embodiment, the complementarity between an antisense strand of a siRNA and a target sequence is such that the antisense strand and target sequence hybridize under the following conditions: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 70°C and wherein the siRNA is suitable for reducing expression of ICAM-1. In one embodiment, the siRNA comprising an antisense strand that hybridizes to the target sequence under the following conditions: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 70°C, is able to reduce expression of ICAM-1 by at least 25%, 50%, 75% of a comparative siRNA with perfect complementarity between the antisense strand and the target sequence.

Length: Another aspect relates to the length of the nucleic acid and particular regions that make up the siRNA.

In one embodiment, the length of the siRNA duplex region is 16 base pairs. In another embodiment, the length of the siRNA duplex region is 17 base pairs. In another embodiment, the length of the siRNA duplex region is 18 base pairs. In another embodiment, the length of the siRNA duplex region is 19 base pairs. In another embodiment, the length of the siRNA duplex region is 20 base pairs. In another embodiment, the length of the siRNA duplex region is 21 base pairs. In another embodiment, the length of the siRNA duplex region is 22 base pairs. In another embodiment, the length of the siRNA duplex region is 23 base pairs. In another embodiment, the length of the siRNA duplex region is 24 base pairs. In another embodiment, the length of the siRNA duplex region is 25 base pairs. In another embodiment, the length of the siRNA duplex region is 26 base pairs. In another embodiment, the length of the siRNA duplex region is 27 base pairs. In another embodiment, the length of the siRNA duplex region is 28 base pairs. In another embodiment, the length of the siRNA duplex region is 29 base pairs. In another embodiment, the length of the siRNA duplex region is 30 base pairs.

In one embodiment the length of the siRNA duplex region is about 17 to about 25 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 10. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 10. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 10. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the

sense duplex region comprises the nucleotide sequence of SEQ ID NO: 10. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 10.

In one embodiment the length of the siRNA duplex region is about 19 to about 25
5 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 12. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 12. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs
10 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 12. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 12. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the
sense duplex region comprises the nucleotide sequence of SEQ ID NO: 12.

In one embodiment the length of the siRNA duplex region is about 19 to about 25
15 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 14. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 14. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs
20 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 14. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 14. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the
sense duplex region comprises the nucleotide sequence of SEQ ID NO: 14.

In one embodiment the length of the siRNA duplex region is about 19 to about 25
25 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 16. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 16. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs
30 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 16. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 16. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the
sense duplex region comprises the nucleotide sequence of SEQ ID NO: 16.

In one embodiment the length of the siRNA duplex region is about 17 to about 25 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 11. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 11. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 11. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 11. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 11.

In one embodiment the length of the siRNA duplex region is about 17 to about 25 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 13. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 13. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 13. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 13. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 13.

In one embodiment the length of the siRNA duplex region is about 17 to about 25 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 15. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 15. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 15. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 15. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 15.

In one embodiment the length of the siRNA duplex region is about 17 to about 25 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 17. In another embodiment the length of the siRNA duplex region is about 19 to about 24 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 17. In another embodiment the length of the siRNA duplex region is about 19 to about 23 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 17. In another embodiment the length of the siRNA duplex region is about 19 to about 22 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 17. In another embodiment the length of the siRNA duplex region is about 19 to about 21 base pairs and the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 17.

In another embodiment, the length of the siRNA duplex region is 16 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 17 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 18 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 19 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 20 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 21 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 22 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 23 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 24 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 25 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 26 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 27 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 28 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 29 consecutive base pairs. In another embodiment, the length of the siRNA duplex region is 30 consecutive base pairs.

In one embodiment, the length of the siRNA duplex region is 23 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 19 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18. In another embodiment, the length of the siRNA duplex region is 24 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 19 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18. In another embodiment, the length of the siRNA

duplex region is 25 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 19 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18.

In one embodiment, the length of the siRNA duplex region is 23 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20. In another embodiment, the length of the siRNA duplex region is 24 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20. In another embodiment, the length of the siRNA duplex region is 25 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20.

In one embodiment, the length of the siRNA duplex region is 23 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22. In another embodiment, the length of the siRNA duplex region is 24 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22. In another embodiment, the length of the siRNA duplex region is 25 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22.

In one embodiment, the length of the siRNA duplex region is 23 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24. In another embodiment, the length of the siRNA duplex region is 24 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24. In another embodiment, the length of the siRNA duplex region is 25 base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24.

In another embodiment, the length of the siRNA duplex region is 23 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 27 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 26. In another

embodiment, the length of the siRNA duplex region is 24 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 27 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 26. In another embodiment, the length of the siRNA duplex region is 25 consecutive base pairs, the antisense duplex 5 region comprises the nucleotide sequence of SEQ ID NO: 27 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 26.

In one embodiment, the length of the siRNA duplex region is 23 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 29 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 28. In another embodiment, 10 the length of the siRNA duplex region is 24 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 29 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 28. In another embodiment, the length of the siRNA duplex region is 25 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 29 and the sense duplex region 15 comprises the nucleotide sequence of SEQ ID NO: 28.

In one embodiment, the length of the siRNA duplex region is 23 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 31 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 30. In another embodiment, the length of the siRNA duplex region is 24 consecutive base pairs, the 20 antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 31 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 30. In another embodiment, the length of the siRNA duplex region is 25 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 31 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 30.

In one embodiment, the length of the siRNA duplex region is 23 consecutive base 25 pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 33 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 32. In another embodiment, the length of the siRNA duplex region is 24 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 33 and the sense 30 duplex region comprises the nucleotide sequence of SEQ ID NO: 32. In another embodiment, the length of the siRNA duplex region is 25 consecutive base pairs, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 33 and the sense duplex region comprises the nucleotide sequence of SEQ ID NO: 32.

In one embodiment, the sense strand is 19 nucleotides in length. In another embodiment, the sense strand is 20 nucleotides in length. In another embodiment, the sense strand is 21 nucleotides in length. In another embodiment, the sense strand is 22 nucleotides in length. In another embodiment, the sense strand is 23 nucleotides in length. In another embodiment, the sense strand is 24 nucleotides in length. In another embodiment, the sense strand is 25 nucleotides in length. In another embodiment, the sense strand is 26 nucleotides in length. In another embodiment, the sense strand is 27 nucleotides in length. In another embodiment, the sense strand is 28 nucleotides in length. In another embodiment, the sense strand is 29 nucleotides in length. In another embodiment, the sense strand is 30 nucleotides in length.

In one embodiment the length of the sense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO: 10. In one embodiment the length of the sense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO: 12. In another embodiment the length of the sense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO: 14. In one embodiment the length of the sense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO: 16.

In one embodiment, the antisense strand is 19 nucleotides in length. In another embodiment, the antisense strand is 20 nucleotides in length. In another embodiment, the antisense strand is 21 nucleotides in length. In another embodiment, the antisense strand is 22 nucleotides in length. In another embodiment, the antisense strand is 23 nucleotides in length. In another embodiment, the antisense strand is 24 nucleotides in length. In another embodiment, the antisense strand is 25 nucleotides in length. In another embodiment, the antisense strand is 26 nucleotides in length. In another embodiment, the antisense strand is 27 nucleotides in length. In another embodiment, the antisense strand is 28 nucleotides in length. In another embodiment, the antisense strand is 29 nucleotides in length. In another embodiment, the antisense strand is 30 nucleotides in length.

In one embodiment the length of the antisense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO:11. In one embodiment the length of the antisense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO: 13. In another embodiment the length of the antisense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID

NO: 15. In another embodiment the length of the antisense strand is about 23 to about 25 nucleotides and comprises the nucleotide sequence of SEQ ID NO: 17.

In another embodiment, the antisense strand and the sense stand are both 23 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 24 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 25 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 26 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 27 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 28 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 29 nucleotides in length. In another embodiment, the antisense strand and the sense stand are both 30 nucleotides in length.

In one embodiment, the length of the antisense strand and the length of the sense strand are each independently about 23 to about 26 nucleotides.

In one embodiment, the sense strand is 23 nucleotides in length, said nucleotides consisting of the nucleotide sequence of SEQ ID NO: 18. In another embodiment, the sense strand is 23 nucleotides in length, said nucleotides consisting of the nucleotide sequence of SEQ ID NO: 20. In another embodiment, the sense strand is 23 nucleotides in length, said nucleotides consisting of the nucleotide sequence of SEQ ID NO: 22. In another embodiment, the sense strand is 23 nucleotides in length, said nucleotides consisting of the nucleotide sequence of SEQ ID NO: 24.

Ends (overhangs and blunt ends): Another aspect relates to the end design of the siRNA. The siRNA of the present invention may comprise an overhang or be blunt ended. An “overhang” as used herein has its normal and customary meaning in the art, i.e., a single stranded portion of a nucleic acid that extends beyond the terminal nucleotide of a complementary strand in a double strand nucleic acid. The term “blunt end” includes double stranded nucleic acid whereby both strands terminate at the same position, regardless of whether the terminal nucleotide(s) are base paired. In one embodiment, the terminal nucleotides of a blunt end are base paired. In another embodiment, the terminal nucleotides of a blunt end are not paired.

In one embodiment, the siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides at one end and a blunt end at the other. In another embodiment, the siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides at both ends. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18, the antisense duplex region comprises

the nucleotide sequence of SEQ ID NO: 19, one end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides and the other end is blunt-ended. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21, one end of said siRNA 5 has an overhang of 1, 2, 3, 4 or 5 nucleotides and the other end is blunt-ended. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23, one end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides and the other end of said 10 siRNA is blunt-ended. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25, one end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides and the other end of said siRNA is blunt-ended.

In one embodiment, the siRNA is blunt ended at both ends. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18, the 15 antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 19, and both ends of said siRNA are blunt-ended. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21, and both ends of said siRNA are blunt-ended. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23, and both ends of said siRNA are blunt-ended. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25, and both ends of said 20 siRNA are blunt-ended.

In another embodiment, the siRNA is blunt ended at the end defined by the 5'-end of the sense strand and the 3'-end of the antisense strand. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 19, the end defined by the 25 5'-end of the sense strand and the 3'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21, the end defined by the 30 5'-end of the sense strand and the 3'-end of the antisense strand is blunt-

ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23, the end defined by the 5'-end of the sense strand and the 3'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25, the end defined by the 5'-end of the sense strand and the 3'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides.

In another embodiment, the siRNA is blunt ended at the end defined by the 3'-end of the sense strand and the 5'-end of the antisense strand. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 18, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 19, the end defined by the 3'-end of the sense strand and the 5'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 20, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 21, the end defined by the 3'-end of the sense strand and the 5'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 22, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 23, the end defined by the 3'-end of the sense strand and the 5'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense duplex region comprises the nucleotide sequence of SEQ ID NO: 24, the antisense duplex region comprises the nucleotide sequence of SEQ ID NO: 25, the end defined by the 3'-end of the sense strand and the 5'-end of the antisense strand is blunt-ended, and the other end of said siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides.

In another embodiment, the siRNA comprises a overhang of 1, 2, 3, 4 or 5 nucleotides at a 3'- or 5'-end on either or both the sense and antisense strands. In one embodiment, the siRNA has a 3'-overhang of 1, 2, 3, 4 or 5 nucleotides on the sense strand and is blunt ended at the other end. In another embodiment, the siRNA has a 3'-overhang of 1, 2, 3, 4 or 5

nucleotides on the antisense strand and is blunt ended at the other end. In another embodiment, the siRNA has a 5'-overhang of 1, 2, 3, 4 or 5 nucleotides on the sense strand and is blunt ended at the other end. In another embodiment, the siRNA has a 5'-overhang of 1, 2, 3, 4 or 5 nucleotides on the antisense strand and is blunt ended at the other end. In 5 another embodiment, the siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides at both the 5'-end and 3'-end of the sense stand. In another embodiment, the siRNA has an overhang of 1, 2, 3, 4 or 5 nucleotides at both the 5'-end and 3'-end of the antisense stand. In another embodiment, the siRNA has a 5' overhang of 1, 2, 3, 4 or 5 nucleotides on the sense stand and a 3' overhang of 1, 2, 3, 4 or 5 nucleotides on the antisense strand. In another 10 embodiment, the siRNA has a 3' overhang of 1, 2, 3, 4 or 5 nucleotides on the sense stand and a 5' overhang of 1, 2, 3, 4 or 5 nucleotides on the antisense strand. In another embodiment, the siRNA has a 3' overhang of 1, 2, 3, 4 or 5 nucleotides on the sense stand and a 3' overhang of 1, 2, 3, 4 or 5 nucleotides on the antisense strand. In another 15 embodiment, the siRNA has a 5' overhang of 1, 2, 3, 4 or 5 nucleotides on the sense stand and a 5' overhang of 1, 2, 3, 4 or 5 nucleotides on the antisense strand.

In one embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 10, said sense strand comprising a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 12, said sense strand comprising a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In one embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 14, said sense strand comprising a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 24, said sense strand comprising a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In one embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 18, said sense 20 strand comprising a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 18, said sense strand comprising a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 20, and the antisense strand comprises a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA 25 sense strand comprises the nucleotide sequence of SEQ ID NO: 20, the antisense strand comprises a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 22, said sense strand comprising a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA 30 sense strand comprises the nucleotide sequence of SEQ ID NO: 22, the antisense strand comprises a 3' overhang of 1, 2, 3, 4 or 5 nucleotides.

sense strand comprises the nucleotide sequence of SEQ ID NO: 22, said sense strand comprising a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 24, and the antisense strand comprises a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA
5 sense strand comprises the nucleotide sequence of SEQ ID NO: 24, the antisense strand comprises a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 26, and the antisense strand comprises a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 26, the antisense strand comprises a 3' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA
10 sense strand comprises the nucleotide sequence of SEQ ID NO: 28, and the antisense strand comprises a 5' overhang of 1, 2, 3, 4 or 5 nucleotides. In another embodiment, the siRNA sense strand comprises the nucleotide sequence of SEQ ID NO: 28, the antisense strand comprises a 3' overhang of 1, 2, 3, 4 or 5 nucleotides.

15 *Modification:* Another aspect relates to modifications of the siRNA. The siRNA according to the invention are a ribonucleic acid or a modified ribonucleic acid. Chemical modifications of the siRNA of the present invention provides a powerful tool in overcoming potential limitations including, but not limited to, *in vitro* and *in vivo* stability and bioavailability inherent to native RNA molecules. Chemically-modified siRNA can also
20 minimize the possibility of activating interferon activity and microRNA gene suppression mechanisms in humans. Chemical modification can further enhance the functional delivery of a siRNA to a target cell. The modified siRNA of the present invention may comprise one or more chemically modified ribonucleotides of either or both of the antisense strand or the sense strand. A ribonucleotide may comprise a chemical modification of the base, sugar or
25 phosphate moieties.

Modifications to base moiety: A secondary aspect relates to modifications to a base moiety. One or more nucleotides of a siRNA of the present invention may comprise a modified base. A "modified base" means a nucleotide base other than an adenine, guanine, cytosine or uracil at the 1' position.

30 In one embodiment, the siRNA comprises at least one nucleotide comprising a modified base. In one embodiment, the modified base is on the antisense strand. In another embodiment, the modified base is on the sense strand. In another embodiment, the modified base is in the duplex region. In another embodiment, the modified base is outside the duplex

region, i.e., in a single stranded region. In another embodiment, the modified base is on the antisense strand and is outside the duplex region. In another embodiment, the modified base is on the sense strand and is outside the duplex region. In another embodiment, the 3'-terminal nucleotide of the antisense strand is a nucleotide with a modified base. In another 5 embodiment, the 3'-terminal nucleotide of the sense strand is nucleotide with a modified base. In another embodiment, the 5'-terminal nucleotide of the antisense strand is nucleotide with a modified base. In another embodiment, the 5'-terminal nucleotide of the sense strand is nucleotide with a modified base.

In one embodiment, a siRNA has 1 modified base. In another embodiment, a siRNA has about 2-4 modified bases. In another embodiment, a siRNA has about 4-6 modified bases. In another embodiment, a siRNA has about 6-8 modified bases. In another embodiment, a siRNA has about 8-10 modified bases. In another embodiment, a siRNA has about 10-12 modified bases. In another embodiment, a siRNA has about 12-14 modified bases. In another embodiment, a siRNA has about 14-16 modified bases. In another 10 embodiment, a siRNA has about 16-18 modified bases. In another embodiment, a siRNA has about 18-20 modified bases. In another embodiment, a siRNA has about 20-22 modified bases. In another embodiment, a siRNA has about 22-24 modified bases. In another embodiment, a siRNA has about 24-26 modified bases. In another embodiment, a siRNA has about 26-28 modified bases. In each case the siRNA comprising said modified bases retains 15 about 50% of its activity as compared to the same siRNA but without said modified bases. In another embodiment, a siRNA has about 30 modified bases. In each case the siRNA comprising said modified bases retains about 50% of its activity as compared to the same siRNA but without said modified bases.

In one embodiment, the modified base is a purine. In another embodiment, the modified base is a pyrimidine. In another embodiment, at least one, at least two, at least three, at least half of the purines are modified. In another embodiment, at least one, at least two, at least three, at least half of the pyrimidines are modified. In another embodiment, all of the 25 purines are modified. In another embodiment, all of the pyrimidines are modified.

In another embodiment, the siRNA comprises a nucleotide comprising a modified base, wherein the base is selected from the group consisting of 2-aminoadenosine, 2,6-diaminopurine, inosine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidine (e.g., 5-methylcytidine), 5-alkyluridine (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine), 6-azapyrimidine, 6-alkylpyrimidine (e.g. 6-methyluridine), propyne, quenosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-

carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methoxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid and 2-thiocytidine.

In another aspect, a siRNA of the present invention comprises an abasic nucleotide. The term "abasic" as used herein, refers to moieties lacking a base or having other chemical groups in place of a base at the 1' position, for example a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative. As used herein, a nucleotide with a modified base does not include abasic nucleotides. In one embodiment, the siRNA comprises at least one abasic nucleotide. In one embodiment, the abasic nucleotide is on the antisense strand. In another embodiment, the abasic nucleotide is on the sense strand. In another embodiment, the abasic nucleotide is in the duplex region. In another embodiment, the abasic nucleotide is outside the duplex region. In another embodiment, the abasic nucleotide is on the antisense strand and is outside the duplex region. In another embodiment, the abasic nucleotide is on the sense strand and is outside the duplex region. In another embodiment, the 3'-terminal nucleotide of the antisense strand is an abasic nucleotide. In another embodiment, the 3'-terminal nucleotide of the sense strand is an abasic nucleotide. In another embodiment, the 5'-terminal nucleotide of the antisense strand is an abasic nucleotide. In another embodiment, the 5'-terminal nucleotide of the sense strand is an abasic nucleotide. In another embodiment, a siRNA has a number of abasic nucleotides selected from the group consisting of 1, 2, 3, 4, 5 and 6.

Modifications to sugar moiety: Another secondary aspect relates to modifications to a sugar moiety. One or more nucleotides of an siRNA of the present invention may comprise a modified ribose moiety.

Modifications at the 2'-position wherein the 2'-OH is substituted include the non-limiting examples selected from the group consisting of alkyl, substituted alkyl, alkaryl-, aralkyl-, -F, -Cl, -Br, -CN, -CF₃, -OCF₃, -OCN, -O-alkyl, -S-alkyl, HS-alkyl-O, -O-alkenyl, -S-alkenyl, -N-alkenyl, -SO-alkyl, -alkyl-OSH, -alkyl-OH, -O-alkyl-OH, -O-alkyl-SH, -S-alkyl-OH, -S-alkyl-SH, -alkyl-S-alkyl, -alkyl-O-alkyl, -ONO₂, -NO₂, -N₃, -NH₂, alkylamino, dialkylamino-, aminoalkyl-, aminoalkoxy, aminoacid, aminoacyl-, -ONH₂, -O-aminoalkyl, -O-aminoacid, -O-aminoacyl, heterocycloalkyl-, heterocycloalkaryl-,

aminoalkylamino-, polyalkylamino-, substituted silyl-, methoxyethyl- (MOE), alkenyl and alkynyl. "Locked" nucleic acids (LNA) in which the 2' hydroxyl is connected, e.g., by a methylene bridge, to the 4' carbon of the same ribose sugar is further included as a 2' modification of the present invention. Acyclic ribose sugar analogues where the ring is 5 opened (unlocked nucleic acids) are also included in the present invention.

Preferred substitutents are 2'-methoxyethyl, 2'-OCH₃, 2'-O-allyl, 2'-C-allyl, and 2'-fluoro.

In one embodiment, the siRNA comprises 1-5 2'-modified nucleotides. In another embodiment, the siRNA comprises 5-10 2'-modified nucleotides. In another embodiment, the 10 siRNA comprises 15-20 2'-modified nucleotides. In another embodiment, the siRNA comprises 20-25 2'-modified nucleotides. In another embodiment, the siRNA comprises 25-30 2'-modified nucleotides.

In one embodiment, the antisense strand comprises 1-2 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 2-4 2'-modified nucleotides. In one 15 embodiment, the antisense strand comprises about 4-6 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 6-8 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 8-10 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 10-12 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 12-14 2'-modified nucleotides. In one 20 embodiment, the antisense strand comprises about 14-16 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 16-18 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 18-20 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 22-24 2'-modified nucleotides. In one embodiment, the antisense strand comprises about 24-26 2'-modified nucleotides.

In one embodiment, the sense strand comprises 1-2 2'-modified nucleotides. In one embodiment, the sense strand comprises about 2-4 2'-modified nucleotides. In one 25 embodiment, the sense strand comprises about 4-6 2'-modified nucleotides. In one embodiment, the sense strand comprises about 6-8 2'-modified nucleotides. In one embodiment, the sense strand comprises about 8-10 2'-modified nucleotides. In one embodiment, the sense strand comprises about 10-12 2'-modified nucleotides. In one embodiment, the sense strand comprises about 12-14 2'-modified nucleotides. In one 30 embodiment, the sense strand comprises about 14-16 2'-modified nucleotides. In one embodiment, the sense strand comprises about 16-18 2'-modified nucleotides. In one

embodiment, the sense strand comprises about 18-20 2'-modified nucleotides. In one embodiment, the sense strand comprises about 22-24 2'-modified nucleotides. In one embodiment, the sense strand comprises about 24-26 2'-modified nucleotides.

In one embodiment, the siRNA comprises 1-5 2'-OCH₃ modified nucleotides. In 5 another embodiment, the siRNA comprises 5-10 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA comprises 15-20 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA comprises 20-25 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA comprises 25-30 2'-OCH₃ modified nucleotides.

In one embodiment, the antisense strand comprises 1-2 2'-OCH₃ modified 10 nucleotides. In one embodiment, the antisense strand comprises about 2-4 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 4-6 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 6-8 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 8-10 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 10-12 2'- 15 OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 12-14 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 14-16 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 16-18 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 18-20 2'-OCH₃ modified nucleotides. In one embodiment, the antisense strand comprises about 22-24 2'-OCH₃ modified 20 nucleotides. In one embodiment, the antisense strand comprises about 24-26 2'-OCH₃ modified nucleotides.

In one embodiment, the sense strand comprises 1-2 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 2-4 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 4-6 2'-OCH₃ modified nucleotides. In one 25 embodiment, the sense strand comprises about 6-8 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 8-10 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 10-12 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 12-14 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 14-16 2'-OCH₃ modified nucleotides. In one 30 embodiment, the sense strand comprises about 16-18 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 18-20 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 22-24 2'-OCH₃ modified nucleotides. In one embodiment, the sense strand comprises about 24-26 2'-OCH₃ modified nucleotides.

In one embodiment, the siRNA duplex region comprises 1-5 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA duplex region comprises 5-10 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA duplex region comprises 15-20 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA duplex region comprises 20-25 2'-OCH₃ modified nucleotides. In another embodiment, the siRNA duplex region comprises 25-30 2'-OCH₃ modified nucleotides.

In one embodiment, the antisense duplex region comprises 1-2 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 2-4 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 4-6 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 6-8 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 8-10 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 10-12 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 12-14 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 14-16 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 16-18 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 18-20 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 22-24 2'-OCH₃ modified nucleotides. In one embodiment, the antisense duplex region comprises about 24-26 2'-OCH₃ modified nucleotides.

In one embodiment, the sense duplex region comprises 1-2 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 2-4 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 4-6 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 6-8 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 8-10 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 10-12 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 12-14 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 14-16 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 16-18 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 18-20 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region

comprises about 22-24 2'-OCH₃ modified nucleotides. In another embodiment, the sense duplex region comprises about 24-26 2'-OCH₃ modified nucleotides.

In one embodiment, the siRNA comprises an antisense strand 19 nucleotides in length and a sense strand 19 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 2, 4, 6, 8, 10, 12 ,14, 16 and 18, wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 20 nucleotides in length and a sense strand 20 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 2, 4, 6, 8, 10, 12 ,14, 16, 18 and 20 wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 21 nucleotides in length and a sense strand 21 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 2, 4, 6, 8, 10, 12 ,14, 16, 18 and 20, wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 22 nucleotides in length and a sense strand 22 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 2, 4, 6, 8, 10, 12 ,14, 16, 18, 20 and 22, wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 23 nucleotides in length and a sense strand 23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 2, 4, 6, 8, 10, 12 ,14, 16, 18, 20 and 22 wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'.

In another embodiment, the siRNA comprises an antisense strand 18-23 nucleotides in length and a sense strand 18-23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 3, 5, 7, 9, 11, 13, 15 and 17, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 4, 6, 8, 10, 12 ,14 and 16, wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from

3'-5'. In another embodiment, the siRNA comprises an antisense strand 18-23 nucleotides in length and a sense strand 18-23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 5, 7, 9, 11, 13 and 15, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 6, 8, 10, 12 and 14, wherein
5 said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 18-23 nucleotides in length and a sense strand 18-23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 7, 9, 11, 13 and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 8, 10 and 12, wherein said antisense strand is numbered
10 from 5'-3' and said sense strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 18-23 nucleotides in length and a sense strand 18-23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 7, 9 and 11, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 8, 10 and 12, wherein said antisense strand is numbered from 5'-3' and said sense
15 strand is numbered from 3'-5'. In another embodiment, the siRNA comprises an antisense strand 18-23 nucleotides in length and a sense strand 18-23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 7 and 9, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 8 and 10, wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'. In
20 another embodiment, the siRNA comprises an antisense strand 18-23 nucleotides in length and a sense strand 18-23 nucleotides in length, wherein said antisense strand comprises 2'-OCH₃ modifications at nucleotides 9 and 11, and wherein said sense strand comprises 2'-OCH₃ modifications at nucleotides 8 and 10, wherein said antisense strand is numbered from 5'-3' and said sense strand is numbered from 3'-5'.
25

In another embodiment the antisense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 2'-deoxy nucleotides.

In another embodiment the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 2'-deoxy nucleotides.

In another embodiment the antisense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 2'-fluoro nucleotides.
30

In another embodiment the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 2'-fluoro nucleotides.

In another embodiment the pyrimidine nucleotides in the antisense strand are 2'-O-methyl pyrimidine nucleotides.

In another embodiment of the purine nucleotides in the antisense strand are 2'-O-methyl purine nucleotides.

5 In another embodiment the pyrimidine nucleotides in the antisense strand are 2'-deoxy pyrimidine nucleotides.

In another embodiment the purine nucleotides in the antisense strand are 2'-deoxy purine nucleotides.

10 In another embodiment the pyrimidine nucleotides in the antisense strand are 2'-fluoro pyrimidine nucleotides.

In another embodiment the purine nucleotides in the antisense strand are 2'-fluoro purine nucleotides.

In another embodiment the pyrimidine nucleotides in the sense strand are 2'-O-methyl pyrimidine nucleotides.

15 In another embodiment of the purine nucleotides in the sense strand are 2'-O-methyl purine nucleotides.

In another embodiment the pyrimidine nucleotides in the sense strand are 2'-deoxy pyrimidine nucleotides.

20 In another embodiment the purine nucleotides in the sense strand are 2'-deoxy purine nucleotides.

In another embodiment the pyrimidine nucleotides in the sense strand are 2'-fluoro pyrimidine nucleotides.

In another embodiment the purine nucleotides in the sense strand are 2'-fluoro purine nucleotides.

25 In another embodiment the pyrimidine nucleotides in the antisense duplex region are 2'-O-methyl pyrimidine nucleotides.

In another embodiment of the purine nucleotides in the antisense duplex region are 2'-O-methyl purine nucleotides.

30 In another embodiment the pyrimidine nucleotides in the antisense duplex region are 2'-deoxy pyrimidine nucleotides.

In another embodiment the purine nucleotides in the antisense duplex region are 2'-deoxy purine nucleotides.

In another embodiment the pyrimidine nucleotides in the antisense duplex region are 2'-fluoro pyrimidine nucleotides.

In another embodiment the purine nucleotides in the antisense duplex region are 2'-fluoro purine nucleotides.

5 In another embodiment the pyrimidine nucleotides in the sense duplex region are 2'-O-methyl pyrimidine nucleotides.

In another embodiment the purine nucleotides in the sense duplex region are 2'-O-methyl purine nucleotides.

10 In another embodiment the pyrimidine nucleotides in the sense duplex region are 2'-deoxy pyrimidine nucleotides.

In another embodiment the purine nucleotides in the sense duplex region are 2'-deoxy purine nucleotides.

In another embodiment the pyrimidine nucleotides in the sense duplex region are 2'-fluoro pyrimidine nucleotides.

15 In another embodiment the purine nucleotides in the sense duplex region are 2'-fluoro purine nucleotides.

In another embodiment the pyrimidine nucleotides in the antisense duplex flanking regions are 2'-O-methyl pyrimidine nucleotides.

20 In another embodiment the purine nucleotides in the antisense duplex flanking regions are 2'-O-methyl purine nucleotides.

In another embodiment the pyrimidine nucleotides in the antisense duplex flanking regions are 2'-deoxy pyrimidine nucleotides.

In another embodiment the purine nucleotides in the antisense duplex flanking regions are 2'-deoxy purine nucleotides.

25 In another embodiment the pyrimidine nucleotides in the antisense duplex flanking regions are 2'-fluoro pyrimidine nucleotides.

In another embodiment the purine nucleotides in the antisense duplex flanking regions are 2'-fluoro purine nucleotides.

30 In another embodiment the pyrimidine nucleotides in the sense duplex flanking regions are 2'-O-methyl pyrimidine nucleotides.

In another embodiment the purine nucleotides in the sense duplex flanking regions are 2'-O-methyl purine nucleotides.

In another embodiment the pyrimidine nucleotides in the sense duplex flanking regions are 2'-deoxy pyrimidine nucleotides.

In another embodiment the purine nucleotides in the sense duplex flanking regions are 2'-deoxy purine nucleotides.

5 In another embodiment the pyrimidine nucleotides in the sense duplex flanking regions are 2'-fluoro pyrimidine nucleotides.

In another embodiment the purine nucleotides in the sense duplex flanking regions are 2'-fluoro purine nucleotides.

10 *Pattern:* In one aspect, the antisense duplex region comprises a plurality of groups of modified nucleotides, referred to herein as “modified groups”, wherein each modified group consists of one or more identically modified nucleotides, wherein each modified group is flanked on one or both sides by a second group of nucleotides, referred to herein as “flanking groups”, wherein each said flanking group consists of one or more nucleotides that are either unmodified or modified in a manner different from the nucleotides of said modified group. In one embodiment, each modified group in the antisense duplex region is identical, i.e., each modified group consists of an equal number of identically modified nucleotides. In another embodiment, each flanking group has an equal number of nucleotide. In another embodiment, each flanking group is identical. In another embodiment, the nucleotides of said modified groups in the antisense duplex region comprise a modified base. In another embodiment, the nucleotides of said modified groups comprise a modified phosphate backbone. In another embodiment, the nucleotides of said modified groups comprise a modified 2' position.

25 In another aspect, the sense duplex region comprises a plurality of groups of modified groups, wherein each modified group consists of one or more identically modified nucleotides, wherein each modified group is flanked on one or both sides by a flanking group, wherein each said flanking group consists of one or more nucleotides that are either unmodified or modified in a manner different from the nucleotides of said modified group. In one embodiment, each modified group in the sense duplex region is identical. In another embodiment, each flanking group has an equal number of nucleotides. In another embodiment, each flanking group is identical. In another embodiment, the nucleotides of said modified groups in the sense duplex region comprise a modified base. In another embodiment, the nucleotides of said modified groups comprise a modified phosphate

backbone. In another embodiment, the nucleotides of said modified groups comprise a modified 2' position.

In another aspect, the antisense duplex region and the sense duplex region each comprise a plurality of modified groups, wherein each modified group consists of one or more identically modified nucleotides, wherein each modified group is flanked on one or both sides by a flanking group, wherein each said flanking group consists of one or more nucleotides that are either unmodified or modified in a manner different from the nucleotides of said modified group. In one embodiment, each modified group in the antisense duplex region and the sense duplex region are identical. In another embodiment, each flanking group in the antisense duplex region and the sense duplex region each have an equal number of nucleotides. In another embodiment, each flanking group in the antisense duplex region and in the sense duplex region are identical. In another embodiment, the nucleotides of said modified groups in the antisense duplex region and the sense duplex region each comprise the same modified groups and the same flanking groups. In another embodiment, the nucleotides of said modified groups in the antisense duplex region and the sense duplex region each comprise a modified base. In another embodiment, the nucleotides of said modified groups in the antisense duplex region and the sense duplex region each comprise a modified phosphate backbone. In another embodiment, the nucleotides of said modified groups in the antisense duplex region and the sense duplex region each comprise a modified 2' position.

In one aspect, the antisense strand comprises a plurality of groups of modified nucleotides, referred to herein as "modified groups", wherein each modified group consists of one or more identically modified nucleotides, wherein each modified group is flanked on one or both sides by a second group of nucleotides, referred to herein as "flanking groups", wherein each said flanking group consists of one or more nucleotides that are either unmodified or modified in a manner different from the nucleotides of said modified group. In one embodiment, each modified group in the antisense strand is identical, i.e., each modified group consists of an equal number of identically modified nucleotides. In another embodiment, each flanking group has an equal number of nucleotide. In another embodiment, each flanking group is identical. In another embodiment, the nucleotides of said modified groups in the antisense strand comprise a modified base. In another embodiment, the nucleotides of said modified groups comprise a modified phosphate backbone. In another embodiment, the nucleotides of said modified groups comprise a modified 2' position.

In another aspect, the sense strand comprises a plurality of groups of modified groups, wherein each modified group consists of one or more identically modified nucleotides, wherein each modified group is flanked on one or both sides by a flanking group, wherein each said flanking group consists of one or more nucleotides that are either unmodified or modified in a manner different from the nucleotides of said modified group. In one embodiment, each modified group in the sense strand is identical. In another embodiment, each flanking group has an equal number of nucleotides. In another embodiment, each flanking group is identical. In another embodiment, the nucleotides of said modified groups in the sense strand comprise a modified base. In another embodiment, the nucleotides of said modified groups comprise a modified phosphate backbone. In another embodiment, the nucleotides of said modified groups comprise a modified 2' position.

In another aspect, the antisense strand and the sense strand each comprise a plurality of modified groups, wherein each modified group consists of one or more identically modified nucleotides, wherein each modified group is flanked on one or both sides by a flanking group, wherein each said flanking group consists of one or more nucleotides that are either unmodified or modified in a manner different from the nucleotides of said modified group. In one embodiment, each modified group in the antisense strand and the sense strand are identical. In another embodiment, each flanking group in the antisense strand and the sense strand each have an equal number of nucleotides. In another embodiment, each flanking group in the antisense strand and in the sense strand are identical. In another embodiment, the nucleotides of said modified groups in the antisense strand and the sense strand each comprise the same modified groups and the same flanking groups. In another embodiment, the nucleotides of said modified groups in the antisense strand and the sense strand each comprise a modified base. In another embodiment, the nucleotides of said modified groups in the antisense strand and the sense strand each comprise a modified phosphate backbone. In another embodiment, the nucleotides of said modified groups in the antisense strand and the sense strand each comprise a modified 2' position.

In another aspect, the modified groups and the flanking groups form a regular pattern on the antisense stand. In another embodiment, the modified groups and the flanking groups form a regular pattern on the sense strand. In one embodiment, the modified groups and the flanking groups form a regular pattern on the both the antisense strand and the sense strand. In another embodiment, the modified groups and the flanking groups form a regular pattern on the antisense duplex region. In another embodiment, the modified groups and the flanking

groups form a regular pattern on the sense duplex region. In one embodiment, the modified groups and the flanking groups form a regular pattern on the both the antisense duplex region and the sense duplex region.

In another aspect, the pattern is a spatial or positional pattern. A spatial or positional pattern means that (a) nucleotide(s) are modified depending on their position within the nucleotide sequence of a double-stranded portion. Accordingly, it does not matter whether the nucleotide to be modified is a pyrimidine or a purine. Rather the position of a modified nucleotide is dependent upon: (a) its numbered position on a strand of nucleic acid, wherein the nucleotides are numbered from the 5'-end to the 3'-end with the 5'-end nucleotide of the strand being position one, or (b) the position of the modified group relative to a flanking group. Thus, according to this embodiment, the modification pattern will always be the same, regardless of the sequence which is to be modified.

In another embodiment, the number of modified groups on the antisense strand is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of modified groups on the sense strand is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of flanking groups on the antisense strand of nucleic acid is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of flanking groups on the sense strand of nucleic acid is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In one embodiment, the number of modified groups and the number of flanking groups on either or both the antisense strand and the sense strand are the same.

In another embodiment, the number of modified groups on the antisense duplex region is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of modified groups on the sense duplex region is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of flanking groups on the antisense duplex region of nucleic acid is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of flanking groups on the sense duplex region of nucleic acid is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In one embodiment, the number of modified groups and the number of flanking groups on either or both the antisense duplex region and the sense duplex region are the same.

In one embodiment, the number of modified groups and the number of flanking groups on a strand or on a duplex region are the same. In another embodiment, the number of modified groups and the number of flanking groups on a strand or on a duplex region are the same, wherein the number is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13.

In another embodiment, the number of nucleotides in a modified group is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13. In another embodiment, the number of nucleotides in a flanking group is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13.

5 In one embodiment, each modified group on both the antisense strand and the sense strand is identical. In one embodiment, each modified group on both the antisense duplex region and the sense duplex region is identical. In another embodiment, each modified group and each flanking group on both the antisense strand and the sense strand are identical. In one embodiment, each modified group and each flanking group on both the antisense duplex region and the sense duplex region are identical.

10 In one embodiment, each modified group, each modified group position, each flanking group and each flanking group position on both the antisense strand and the sense strand are identical. In one embodiment, each modified group, each modified group position, each flanking group and each flanking group position on both the antisense duplex region and the sense duplex region are identical. In another embodiment, the modified groups on the antisense strand are complementary with the modified groups on the sense strand (the modified groups on the antisense strand and the sense strand are perfectly aligned across from one another). In another embodiment, there are no mismatches in the modified groups such that each modified group on the antisense strand is base paired with each modified group on the sense strand. In another embodiment, each modified group on the sense strand is shifted
15 by 1, 2, 3, 4 or 5 nucleotides relative to the modified groups on the antisense strand. For example, if each modified group on the sense strand is shifted by one nucleotide and a modified group started at position one on the antisense strand (numbering 5' to 3'), a modified group on the sense strand would begin at position two (numbering 3' to 5'). In another embodiment, the modified groups on the antisense strand do not overlap the modified groups of the sense strand, i.e., no nucleotide of a modified group on the antisense strand is base paired with a nucleotide of a modified group on the sense strand.
20

25 In one embodiment, deoxyribonucleotides at an end of a strand of nucleic acid are not considered when determining a position of a modified group, i.e., the positional numbering begins with the first ribonucleotide or modified ribonucleotide. In another embodiment, abasic nucleotides at an end of a strand of nucleic acid are not considered when determining a position of a modified group.
30

In one embodiment, a modified group comprises a 5'-end nucleotide of either or both of the antisense strand and the sense strand. In another embodiment, a flanking group

comprises the 5'-end nucleotide of either or both of the antisense strand and the sense strand. In another embodiment, the 5'-end nucleotide of either or both of the antisense strand and the sense strand is unmodified. In another embodiment, a modified group comprises the 5'-most nucleotide of either or both of the antisense duplex region and sense duplex region. In 5 another embodiment, a flanking group comprises the 5'-most nucleotide of either or both of the antisense duplex region or the sense duplex region. In another embodiment, the 5'-most nucleotide of either or both of the antisense duplex region or the sense duplex region is unmodified. In another embodiment, the nucleotide at position 10 of the antisense strand is unmodified. In another embodiment, the nucleotide at position 10 of the sense strand is 10 modified. In another embodiment, a modified group comprises the nucleotide at position 10 of the sense strand.

In one embodiment, the modification at the 2' position is selected from the group comprising amino, fluoro, methoxy, alkoxy and C₁-C₃-alkyl. In another embodiment, the modification is 2'-O-methyl.

15 In another embodiment, each modified group consists of one nucleotide and each flanking group consists of one nucleotide. In one embodiment, each modified group on the antisense strand is aligned with a flanking group on the sense strand.

In another embodiment, each modified group consists of one 2'-O-methyl modified 20 nucleotide and each flanking group consists of one nucleotide. In one embodiment, each flanking group consists of one unmodified nucleotide. In one embodiment, each flanking group consists of one 2'-F modified nucleotide. In another embodiment, each modified group on both the antisense strand and the sense strand consists of one 2'-O-methyl modified nucleotide and each flanking group on both the antisense strand and the sense strand consists 25 of one nucleotide, wherein no modified group on one strand is either aligned or both aligned and base paired with another modified group on the other strand and no flanking group on one strand is either aligned or both aligned and base paired with a flanking group on the other strand. In another embodiment, excluding any optional overhangs, each modified group on each strand is either aligned or both aligned and based paired with a flanking group on the other strand. In one embodiment, the flanking group is unmodified. In another embodiment, 30 the nucleotide of position one on the antisense strand is 2'-O-methyl modified. In another embodiment, the 5'-most nucleotide of the antisense duplex region is 2'-O-methyl modified.

Positional modification schemes are described in international patent application WO 2004/015107, incorporated by reference in its entirety.

In another embodiment, the antisense and sense strand of the siRNAs of the invention are 2'-O-methyl modified. In one example, the 2'-O-methyl modification on the sense strand can start at position 2 and the 2'-O-methyl modification on the antisense strand can start at position 1. By way of example, for ICAM1-hc-3 the 2'-O-methyl modification is shown in 5 upper case in the sense and antisense strands below:

Sense	5'	cCcCaAcCcUuGaUgAuAuGuAu	3' (SEQ ID NO: 20)
Antisense	3'	GgGgUuGgGaAcUaCuAuAcAuA	5' (SEQ ID NO: 21)

10 Alternatively, the 2'-O-methyl modification on the sense strand starts at position 1 and the 2'-O-methyl modification on the antisense strand starts at position 2.

15 *Modifications to phosphate backbone:* Another secondary aspect relates to modifications to a phosphate backbone. All or a portion of the nucleotides of the siRNA of the invention may be linked through phosphodiester bonds, as found in unmodified nucleic acid. A siRNA of the present invention however, may comprise a modified phosphodiester linkage. The phosphodiester linkages of either the antisense stand or the sense strand may be modified to independently include at least one heteroatom selected from the group consisting of nitrogen and sulfur. In one embodiment, a phosphoester group connecting a ribonucleotide 20 to an adjacent ribonucleotide is replaced by a modified group. In one embodiment, the modified group replacing the phosphoester group is selected from the group consisting of phosphorothioate, methylphosphonate or phosphoramidate group.

25 In one embodiment, all of the nucleotides of the antisense strand are linked through phosphodiester bonds. In another embodiment, all of the nucleotides of the antisense duplex region are linked through phosphodiester bonds. In another embodiment, all of the nucleotides of the sense strand are linked through phosphodiester bonds. In another embodiment, all of the nucleotides of the sense duplex region are linked through phosphodiester bonds. In another embodiment, the antisense strand comprises a number of modified phosphoester groups selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 , 16, 30 17, 18, 19, 20, 21, 22, 23 or 24. In another embodiment, the antisense duplex region comprises a number of modified phosphoester groups selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 , 16, 17, 18, 19, 20, 21, 22, 23 or 24. In another embodiment, the sense strand comprises a number of modified phosphoester groups selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 , 16, 17, 18, 19, 20, 21, 22, 23 or 24. In another embodiment, the

sense duplex region comprises a number of modified phosphoester groups selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24.

5' and 3' end modifications: Another secondary aspect relates to 5' and 3' modifications. The siRNA of the present invention may include nucleic acid molecules comprising one or more modified nucleotides, abasic nucleotides, acyclic or deoxyribonucleotide at the terminal 5'- or 3'-end on either or both of the sense or antisense strands. In one embodiment, the 5'- and 3'-end nucleotides of both the sense and antisense strands are unmodified. In another embodiment, the 5'-end nucleotide of the antisense strand is modified. In another embodiment, the 5'-end nucleotide of the sense strand is modified. In another embodiment, the 3'-end nucleotide of the antisense strand is modified. In another embodiment, the 3'-end nucleotide of the sense strand is modified. In another embodiment, the 5'-end nucleotide of the antisense strand and the 5'-end nucleotide of the sense strand are modified. In another embodiment, the 3'-end nucleotide of the antisense strand and the 3'-end nucleotide of the sense strand are modified. In another embodiment, the 5'-end nucleotide of the antisense strand and the 3'-end nucleotide of the sense strand are modified. In another embodiment, the 3'-end nucleotide of the antisense strand and the 5'-end nucleotide of the sense strand are modified. In another embodiment, the 3'-end nucleotide of the antisense strand and both the 5'- and 3'-end nucleotides of the sense strand are modified. In another embodiment, both the 5'- and 3'-end nucleotides of the antisense strand are modified. In another embodiment, both the 5'- and 3'-end nucleotides of the sense strand are modified.

In another embodiment, the 5'-end nucleotide of the antisense strand is phosphorylated. In another embodiment, the 5'-end nucleotide of the sense strand is phosphorylated. In another embodiment, the 5'-end nucleotides of both the antisense strand and the sense strand are phosphorylated. In another embodiment, the 5'-end nucleotide of the antisense strand is phosphorylated and the 5'-end nucleotide of the sense strand has a free hydroxyl group (5'-OH). In another embodiment, the 5'-end nucleotide of the antisense strand is phosphorylated and the 5'-end nucleotide of the sense strand is modified.

Modifications to the 5'- and 3'-end nucleotides are not limited to the 5' and 3' positions on these terminal nucleotides. Examples of modifications to end nucleotides include, but are not limited to, biotin, inverted (deoxy) abasics, amino, fluoro, chloro, bromo, CN, CF, methoxy, imidazole, carboxylate, thioate, C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl or aralkyl, OCF₃, OCN, O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH₃;

SO₂CH₃; ONO₂; NO₂; N₃; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino or substituted silyl, as, among others, described, e.g., in PCT patent application WO 99/54459, European patents EP 0 586 520 B1 or EP 0 618 925 B1, incorporated by reference in their entireties. As used herein, “alkyl” means C₁-C₁₂-alkyl and “lower alkyl” means C₁-C₆-alkyl, including C₁-, C₂-, C₃-, C₄-, C₅- and C₆-alkyl.

5

In another aspect, the 5'-end of the antisense strand, the 5'- end of the sense strand, the 3'-end of the antisense strand or the 3'-end of the sense strand is covalently connected to a prodrug moiety. In one embodiment, the moiety is cleaved in an endosome. In another the moiety is cleaved in the cytoplasm.

10 Various possible non-limiting embodiments of the siRNA of the present invention having different kinds of end modification(s) are presented in the following Table 3.

Table 3: Various embodiments of the interfering ribonucleic acid according to the present invention		
	Antisense strand	Sense strand
1.) 5'-end	free OH	free OH
	3'-end	free OH
2.) 5'-end	free OH	free OH
	3'-end	end modification
3.) 5'-end	free OH	free OH
	3'-end	free OH
4.) 5'-end	free OH	free OH
	3'-end	end modification
5.) 5'-end	free OH	end modification
	3'-end	free OH
6.) 5'-end	free OH	end modification
	3'-end	free OH
7.) 5'-end	free OH	end modification
	3'-end	end modification
8.) 5'-end	free OH	end modification
	3'-end	end modification

In another embodiment the terminal 3' nucleotide or two terminal 3'-nucleotides on either or both of the antisense strand or sense strand is a 2'-deoxynucleotide. In another embodiment the 2'-deoxynucleotide is a 2'-deoxy-pyrimidine. In another embodiment the 2'-deoxynucleotide is a 2' deoxy-thymidine.

5 *shRNA and linked siRNA:* Another aspect relates to shRNA and linked siRNA. It is within the present invention that the double-stranded structure is formed by two separate strands, i.e. the antisense strand and the sense strand. However, it is also within the present invention that the antisense strand and the sense strand are covalently linked to each other. Such linkage may occur between any of the nucleotides forming the antisense strand and 10 sense strand, respectively. Such linkage can be formed by covalent or non-covalent linkages. Covalent linkage may be formed by linking both strands one or several times and at one or several positions, respectively, by a compound preferably selected from the group comprising methylene blue and bifunctional groups. Such bifunctional groups are preferably selected from the group comprising bis(2-chloroethyl)amine, N-acetyl-N'-(p-glyoxylbenzoyl)cystamine, 4-thiouracile and psoralene.

15

In one aspect, the antisense strand and the sense strand are linked by a loop structure. In another embodiment, of the loop structure is comprised of a non-nucleic acid polymer. In another embodiment, the non-nucleic acid polymer is polyethylene glycol. In another embodiment, the 5'-end of the antisense strand is linked to the 3'-terminus of the sense 20 strand. In another embodiment, the 3'-end of the antisense strand is linked to the 5'-end of the sense strand.

In another embodiment, the loop consists of a nucleic acid. As used herein, locked nucleic acid (LNA) (Elayadi and Corey (2001) Curr Opin Investig Drugs. 2(4):558-61) and peptide nucleic acid (PNA) (reviewed in Faseb J. (2000) 14:1041-1060) are regarded as 25 nucleic acids and may also be used as loop forming polymers. In one embodiment the nucleic acid is ribonucleic acid. In one embodiment the 5'-terminus of the antisense strand is linked to the 3'-terminus of the sense strand. In another embodiment the 3'-end of the antisense strand is linked to the 5'-terminus of the sense strand. The loop consists of a minimum length of four nucleotides or nucleotide analogues. In one embodiment the loop consists of a length 30 of nucleotides or nucleotide analogs selected from 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In one embodiment the length of the loop is sufficient for linking the two strands covalently in a manner that a back folding can occur through a loop structure or similar structure. The ribonucleic acid constructs may be incorporated into suitable vector systems. Preferably the

vector comprises a promoter for the expression of RNAi. Preferably the respective promoter is pol III and more preferably the promoters are the U6, H1, 7SK promoter as described in Good et al. (1997) *Gene Ther.*, **4**, 45-54.

In another embodiment, the nucleic acid according to the present invention comprise a phosphorothioate internucleotide linkage. In one embodiment, a phosphorothioate internucleotide linkage is within 5 nucleotides from the 3'-end or the 5'-end of either or both of the antisense strand and the sense strand. The antisense strand can comprise about one to about five phosphorothioate internucleotide linkages.

In various aspects of the invention, siRNA molecules can have an overhang at the 3'-end of the sense strand and/or antisense strand that is 1, 2, 3, 4 or 5 nucleotides in length. In another embodiment,, an overhang at the 5'-end of the sense and/or antisense stand that is 1, 2, 3, 4 and 5 nucleotides in length can be present. Yet another embodiment provides for a siRNA molecule that is blunt-ended on both ends and has a length selected from the group consisting of 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 consecutive nucleotides.

Other aspects of the invention provide a siRNA molecule that is blunt-ended on one end, has an overhang at the other end of the molecule and the double stranded portion of the siRNA molecule has a length selected from the group consisting of 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 consecutive nucleotides.

Yet other aspects of the invention provide a siRNA molecule that:

a) has overhangs on both ends and the double stranded portion of the siRNA molecule has a length selected from the group consisting of 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 consecutive nucleotides;

b) comprises an overhang, said overhang comprising at least one deoxyribonucleotide;

c) comprises an overhang, said overhang comprising or consisting of two deoxyribonucleotides;

d) comprises overhangs on the 3'-end of the antisense strand and at the 3'-end of the sense strand, said overhangs comprising at least one deoxyribonucleotide;

e) comprises overhangs on the 3'-end of the antisense strand and at the 3'-end of the sense strand, said overhangs consisting two deoxyribonucleotides.

The nucleotide(s) forming the overhang may be (a) deoxyribonucleotide(s), (a) ribonucleotide(s) or a combination thereof. In one embodiment, the antisense strand and/or the sense strand comprise a TT dinucleotide at the 3' end. Furthermore, the duplexed/double

stranded region of the siRNA molecules discussed in this section can also have mismatches within said duplexed/souble stranded region as discussed above.

Processes of making: The nucleic acid of the present invention can be produced using routine methods in the art including chemically synthesis or expressing the nucleic acid either in vitro (e.g., run off transcription) or in vivo. In one embodiment, the siRNA is produced using solid phase chemical synthesis. In another embodiment, the nucleic acid is produced using an expression vector. In one embodiment, the expression vector produced the nucleic acid of the invention in the target cell. Accordingly, such vector can be used for the manufacture of a medicament. Methods for the synthesis of the nucleic acid molecule described herein are known to the ones skilled in the art. Such methods are, among others, described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 15 33-45, and Brennan, U.S. Pat. No. 6,001,311 (each incorporated herein by reference in their entireties).

Delivery/formulations: siRNA can be delivered to cells, both in vitro and in vivo, by a variety of methods known to those of skill in the art, including direct contact with cells (“naked” siRNA) or by in combination with one or more agents that facilitate targeting or delivery into cells. Such agents and methods include lipoplexes, liposomes, iontophoresis, hydrogels, cyclodextrins, nanocapsules, micro- and nanospheres and proteinaceous vectors (e.g., Bioconjugate Chem. (1999) 10:1068-1074 and WO 00/53722). The nucleic acid/vehicle combination may be locally delivered in vivo by direct injection or by use of an infusion pump. The siRNA of the invention can be delivered in vivo by various means including intravenous, subcutaneous, intramuscular or intradermal injection or inhalation. The molecules of the instant invention can be used as pharmaceutical agents. Preferably, pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject. In the case of treating cancer, the treatment reduces tumor burden or tumor mass in the subject.

There is also provided the use of a composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing stability of

a liposome or lipoplex solutions by preventing their aggregation and fusion. The formulations also have the added benefit *in vivo* of resisting opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug. Such liposomes have been
5 shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic et al., Science 1995, 267, 1275-1276; Oku et al., 1995, Biochim. Biophys. Acta, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu et
10 al., J. Biol. Chem. 1995, 267, 1275-1276; Choi et al., International PCT Publication No. WO 96/10391; Ansell et al., International PCT Publication No. WO 96/10390; Holland et al., International PCT Publication No. WO 96/10392). Long-circulating liposomes also protect the siRNA from nuclease degradation.

The siRNA of the present invention may be formulated as pharmaceutical compositions. The pharmaceutical compositions may be used as medicaments or as diagnostic agents, alone or in combination with other agents. For example, one or more siRNAs of the invention can be combined with a delivery vehicle (e.g., liposomes) and excipients, such as carriers, diluents. Other agents such as preservatives and stabilizers can also be added. Methods for the delivery of nucleic acid molecules are known in the art and described, e.g., in Akhtar et al., 1992, Trends Cell Bio., 2, 139; Delivery Strategies for Antisense Oligonucleotide Therapeutics, ed. Akhtar, 1995, Maurer et al., 1999, Mol. Memb. Biol., 16, 129-140; Hofland and Huang, 1999, Handb. Exp. Pharmacol., 137, 165-192; and Lee et al., 2000, ACS Symp. Ser., 752, 184-192, U.S. Pat. No. 6,395,713 and PCT WO 94/02595 (each of which are incorporated herein by reference in their entireties). The siRNA of the present invention can also be administered in combination with other therapeutic compounds, either administrated separately or simultaneously, e.g., as a combined unit dose. In one embodiment, the invention includes a pharmaceutical composition comprising one or more siRNA according to the present invention in a physiologically/pharmaceutically acceptable excipient, such as a stabilizer, preservative, diluent, buffer, and the like.
25

Dosage levels for the medicament and pharmaceutical compositions of the invention can be determined by those skilled in the art by routine experimentation. In one embodiment, a unit dose contains between about 0.01 mg/kg and about 100 mg/kg body weight of siRNA. In one embodiment, the dose of siRNA is about 10 mg/kg and about 25 mg/kg body weight.
30

In one embodiment, the dose of siRNA is about 1 mg/kg and about 10 mg/kg body weight. In one embodiment, the dose of siRNA is about 0.05 mg/kg and about 5 mg/kg body weight. In another embodiment, the dose of siRNA is about 0.1 mg/kg and about 5 mg/kg body weight. In another embodiment, the dose of siRNA is about 0.1 mg/kg and about 1 mg/kg body weight. In another embodiment, the dose of siRNA is about 0.1 mg/kg and about 0.5 mg/kg body weight. In another embodiment, the dose of siRNA is about 0.5 mg/kg and about 1 mg/kg body weight.

5 In one embodiment, the routes of administration for the pharmaceutical compositions includes topically to the lung by nebulisation, eye drops, intravenous, oral, subcutaneous, dry powder delivery devices or topically to skin as ointments or patches. In one embodiment the pharmaceutical compositions is a sterile injectable aqueous suspension or solution. In one embodiment the pharmaceutical composition is in lyophilized form. In one embodiment, the pharmaceutical composition comprises lyophilized lipoplexes, wherein the lipoplexes comprises a siRNA of the present invention. In another embodiment, the pharmaceutical 10 composition comprises an aqueous suspension of lipoplexes, wherein the lipoplexes comprises a siRNA of the present invention.

15

The pharmaceutical compositions and medicaments of the present invention may be administered to mammal. In one embodiment, the mammal is selected from the group consisting humans, dogs, cats, horses, cattle, pig, goat, sheep, mouse, rat, hamster and guinea 20 pig. In one embodiment, the mammal is a human. In another embodiment, the mammal is a non-human mammal.

25 The nucleic acid molecules (e.g., siRNA molecules) as disclosed herein and the medicaments and pharmaceutical compositions containing the same may be used for the treatment of a variety of diseases or disorders including inflammatory disorders, respiratory diseases and disorders, and proliferative cell disorders.

In one example, the invention can be used to treat inflammatory or obstructive airways diseases, resulting, for example, in reduction of tissue damage, airways inflammation, bronchial hyperreactivity, remodelling or disease progression. Inflammatory or obstructive airways diseases and conditions include acute lung injury (ALI), adult/acute 30 respiratory distress syndrome (ARDS), chronic obstructive pulmonary, airways or lung disease (COPD, COAD or COLD).

In one example, the siRNAs of the invention can be used to treat asthma. Asthma refers to a periodic condition involving the respiratory system in which airways constrict

and/or produce mucus. Asthma may be in response to one or more environmental triggers. An asthma attack or episode may be triggered by such things as exposure to an environmental stimulant such as an allergen, environmental tobacco smoke, perfume, pet dander, moist air, exercise or exertion, or emotional stress. In children, triggers include, but are not limited to, 5 viral illnesses such the common cold. In children and adults viral infection, such as those causing exacerbations resulting in hospitalisation, can be treated by the pharmaceutical compositions. This airway narrowing causes symptoms such as wheezing, shortness of breath, chest tightness, and coughing. The siRNAs of the invention can further be used to treat COPD which refers to chronic lung disease typically diagnosed by the presence of 10 chronic bronchitis and emphysema. COPD is commonly caused by smoking. Severe exacerbations can be caused by infections treatable by this invention such as human rhinovirus. Chronic bronchitis is typically diagnosed when the lining of the bronchial tubes get reddish, swollen and/or the presence of mucus. Alveoli are irritated when one has emphysema.

15 The invention is also applicable to the treatment of bronchitis of whatever type or genesis including, e.g., acute, arachidic, catarrhal, croupus, chronic or phthinoid bronchitis.

In another example, the disease is an inflammatory disorder such as inflammatory bowel disease; systemic lupus erythematosus; rheumatoid arthritis; juvenile chronic arthritis; spondyloarthropathies; systemic sclerosis, for example, scleroderma; idiopathic inflammatory 20 myopathies for example, dermatomyositis, polymyositis; Sjogren's syndrome; systemic vaculitis; sarcoidosis; thyroiditis, for example, Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis; immune-mediated renal disease, for example, glomerulonephritis, tubulointerstitial nephritis; demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic polyneuropathy; 25 hepatobiliary diseases such as infectious hepatitis such as hepatitis A, B, C, D, E and other nonhepatotropic viruses; autoimmune chronic active hepatitis; primary biliary cirrhosis; granulomatous hepatitis; and sclerosing cholangitis; inflammatory and fibrotic lung diseases (e.g., cystic fibrosis); gluten-sensitive enteropathy; autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, 30 psoriasis; allergic diseases of the lung such as eosinophilic pneumonia, idiopathic pulmonary fibrosis, allergic conjunctivitis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus host disease, also acute organ damage e.g.

lung and kidney following ischaemia, infection and other trauma and sub-acute cardiac damage following myocardial infarction.

In another example, the siRNAs of the invention can be used to treat cardiovascular and cerebrovascular disorders such as congestive heart failure, acute heart failure, myocardial infarction, the formation of atherosclerotic plaques, hypertension, platelet aggregation, 5 angina, stroke, reperfusion injury, vascular injury including restenosis and peripheral vascular disease.

In yet another example, the fibrosis such as kidney or lung fibrosis. In yet another example, the aVb6-related disease is associated with dysregulated TGF- β include cancer and 10 connective tissue (fibrotic) disorders.

"Proliferative disorder" are defined as any cellular disorder in which the cells within an tissues/organ proliferate more rapidly than the surrounding normal tissue growth. The proliferative disorder, includes but is not limited to neoplasms. A "neoplasm" is an abnormal tissue growth, generally forming a distinct mass, that grows by cellular proliferation more 15 rapidly than normal tissue growth. Neoplasms show partial or total lack of structural organization and functional coordination with normal tissue.

These can be broadly classified into three major types. Malignant neoplasms arising from epithelial structures are called carcinomas, malignant neoplasms that originate from connective tissues such as muscle, cartilage, fat or bone are called sarcomas and malignant tumors affecting hematopoietic structures (structures pertaining to the formation of blood 20 cells) including components of the immune system, are called leukemias and lymphomas. A tumor is the neoplastic growth of the disease cancer. As used herein, a neoplasm, also referred to as a "tumor", is intended to encompass hematopoietic neoplasms as well as solid neoplasms, as outlined below. These also include all common forms of cancer such as 25 Bladder Cancer, Melanoma, Breast Cancer, Non-Hodgkin Lymphoma, Colon and Rectal Cancer, Pancreatic Cancer, Endometrial Cancer, Prostate Cancer, Kidney (Renal Cell) Cancer, Skin Cancer (Nonmelanoma), Leukemia, Thyroid Cancer, Lung Cancer. Further proliferative disorders include, but are not limited to neurofibromatosis, all diseases related to 30 vascular proliferation including non-physiological endothelial proliferation (angiogenesis), and atherosclerosis.

Non-limiting examples of malignant neoplasms that can be treated according to the instant disclosure include carcinomas, sarcomas, hematopoietic malignancies, and germ cell tumors, and including bladder cancer, melanoma, breast cancer, non-hodgkin lymphoma,

colon and rectal cancer, pancreatic cancer, endometrial cancer, prostate cancer, kidney or renal cell cancer, non-melanoma skin cancer, leukemia, thyroid cancer, lung cancer, neurofibromatosis, all diseases related to vascular proliferation including non-physiological endothelial proliferation, atherosclerosis, adenoma, angiomyoma, arachnoid cysts, 5 astrocytoma, bone neoplasms, Bowen's disease, breast cyst, breast neoplasms, breast neoplasms, male, Burkitt lymphoma, carcinoid tumor, carcinoma, carcinoma, Merkel cell carcinoma, non-small-cell lung carcinoma, small cell lung carcinoma, cementoma, choledochal cyst, chondroma, chondrosarcoma, chordoma, craniopharyngioma, cysts, dermoid cyst, digestive system neoplasms, ear neoplasms, endocrine gland neoplasms, 10 endometrial neoplasms, ependymoma, epidermal cyst, fibromatosis, aggressive, fibromatosis, juvenile hyaline, gastrointestinal neoplasms, gastrointestinal stromal tumors, genital neoplasms, female, genital neoplasms, male, glioblastoma, glioma, hamartoma, hamartoma syndrome, multiple, head and neck neoplasms, hemangioma, histiocytoma, benign fibrous, 15 histiocytoma, malignant fibrous, Hodgkin disease, Hutchinson's melanotic freckle, insulinoma, Krukenberg tumor, laryngeal neoplasms, leiomyoma, leiomyosarcoma, leukemia, lipoma, lung neoplasms, lymphangioma, lymphoma, lymphoma, non-hodgkin, mediastinal cyst, medulloblastoma, melanoma, melanoma, amelanotic, meningioma, mesothelioma, mouth neoplasms, multiple myeloma, myoma, myxoma, neoplasm metastasis, neoplasm, residual, neoplasms, neoplasms, connective and soft tissue, nervous system neoplasms, 20 neurilemmoma, neuroblastoma, neuroendocrine tumors, neuroma, acoustic, nevus, odontogenic tumors, osteosarcoma, otorhinolaryngologic neoplasms, ovarian cysts, ovarian neoplasms, Paget's disease, mammary, papilloma, paraganglioma, paraneoplastic syndromes, nervous system, pheochromocytoma, pilonidal sinus, popliteal cyst, precancerous conditions, 25 pseudomyxoma peritonei, ranula, rectal neoplasms, respiratory tract neoplasms, retinoblastoma, rhabdoid tumor, rhabdomyosarcoma, sarcoma, sarcoma, Ewing's, Sezary syndrome, skin neoplasms, Tarlov cysts, teratoma, thymoma, tonsillar neoplasms, tuberous sclerosis, urologic neoplasms, uterine cervical dysplasia, uterine cervical neoplasms, Wilms tumor, vulvar neoplasms, benign prostatic hyperplasia, breast hyperplasia including atypical usual ductal hyperplasia, compensatory liver hyperplasia, congenital adrenal hyperplasia, 30 endometrial hyperplasia including polycystic ovary syndrome and endometrial adenocarcinoma, focal epithelial hyperplasia, Heck's disease, and sebaceous hyperplasia.

Other diseases that can be treated with siRNA molecules, as disclosed herein, include: Bell palsy, Burkitt lymphoma, chickenpox, cytomegalovirus infections, ecthyma, contagious,

encephalitis, herpes simplex, Epstein-Barr virus infections, erythema infectiosum, exanthema subitum, herpes labialis, herpes simplex, herpes zoster, herpes zoster oticus,, Herpesviridae infections, infectious mononucleosis, molluscum contagiosum, polyomavirus infections, smallpox, warts, human papillomavirus HPV, infectious mononucleosis, EBV-associated 5 malignancies including but not limited to nasopharyngeal carcinoma and chronic fatigue syndrome; and, for KSHV Kaposi's sarcoma.

In one embodiment, the present invention is related to lipoplexes comprising a siRNA according to the present invention. Such lipoplexes consist siRNA and liposomes. Such lipoplexes may be used to deliver the siRNA of the invention to a target cell either in vitro or 10 in vivo.

In one embodiment the lipoplex has a zeta-potential of about 40 to 55 mV, preferably about 45 to 50 mV. The size of the lipoplex according to the present invention is about 80 to 200 nm, about 100 to 140 nm or about 110 nm to 130 nm, as determined by dynamic light scattering (QELS) such as, e. g., by using an N5 submicron particle size analyzer from 15 Beckman Coulter according to the manufacturer's recommendation.

In one embodiment the liposome as forming part of the lipoplex is a positively charged liposome consisting of:

- a) about 50 mol% β -arginyl-2,3-diaminopropionic acid-N-palmityl-N-oleyl-amide trihydrochloride, preferably β -(L-arginyl)-2,3-L-diaminopropionic acid-N-palmityl-N-oleyl-amide tri-hydrochloride,
- b) about 48 to 49 mol% 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE), and
- c) about 1 to 2 mol% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polyethylen-glycole, preferably N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.

The lipoplex and lipid composition forming the liposomes is preferably in a carrier however, the lipoplex can also be present in a lyophilised form. The lipid composition contained in a carrier usually forms a dispersion. More preferably, the carrier is an aqueous medium or aqueous solution as also further characterised herein. The lipid composition typically forms a liposome in the carrier, whereby such liposome preferably also contains the 30 carrier inside.

The lipid composition contained in the carrier and the carrier, respectively, preferably has an osmolarity of about 50 to 600 mosmole/kg, preferably about 250 – 350 mosmole/kg, and more preferably about 280 to 320 mosmole/kg.

5 The liposomes preferably are formed by the first lipid component and optionally also by the first helper lipid, preferably in combination with the first lipid component, preferably exhibit a particle size of about 20 to 200 nm, preferably about 30 to 100 nm, and more preferably about 40 to 80 nm. It is noted that the size of the particles follows a certain statistical distribution.

10 A further optional feature of the lipid composition in accordance with the present invention is that the pH of the carrier is preferably from about 4.0 to 6.0. However, also other pH ranges such as from 4.5 to 8.0, preferably from about 5.5 to 7.5 and more preferably about 6.0 to 7.0 are within the present invention.

15 For realizing these particular features various measures may be taken. For adjusting the osmolarity, for example, a sugar or a combination of sugars is particularly useful. Insofar, the lipid composition of the present invention may comprise one or several of the following sugars: sucrose, trehalose, glucose, galactose, mannose, maltose, lactulose, inulin and raffinose, whereby glucose, sucrose, trehalose, inulin and raffinose are particularly preferred. In a particularly preferred embodiment the osmolarity mostly adjusted by the addition of sugar is about 300 mosmole/kg which corresponds to a sucrose solution of 270 mM or a 20 glucose solution of 280 mM. Preferably the carrier is isotonic to the body fluid into which such lipid composition is to be administered. As used herein the term that the osmolarity is mostly adjusted by the addition of sugar means that at least about 80 %, preferably at least about 90 % of the osmolarity is provided by said sugar or a combination of said sugars.

25 If the pH of the lipid composition of the present invention is adjusted, this is done by using buffer substances which, as such, are basically known to the one skilled in the art. Preferably, basic substances are used which are suitable to compensate for the basic characteristics of the cationic lipids and more specifically of the ammonium group of the cationic head group. When adding basic substances such as basic amino acids and weak bases, respectively, the above osmolarity is to be taken into consideration. The particle size of 30 such lipid composition and the liposomes formed by such lipid composition is preferably determined by dynamic light scattering such as by using an N5 submicron particle size analyzer from Beckman Coulter according to the manufacturer's recommendation.

If the lipid composition contains one or several nucleic acid(s), such lipid composition usually forms a lipoplex (liposome-nucleic acid complex). The more preferred concentration of the overall lipid content in the lipoplex in preferably isotonic 270 mM sucrose or 280 mM glucose is from about 0.01 to 100 mg/ml, preferably 0.01 to 40 mg/ml and more preferably 5 0.01 to 25 mg/ml. It is to be acknowledged that this concentration can be increased so as to prepare a reasonable stock, typically by a factor of 2 to 3. It is also within the present invention that based on this, a dilution is prepared, whereby such dilution is typically made such that the osmolarity is within the range specified above. More preferably, the dilution is 10 prepared in a carrier which is identical or in terms of function and more specifically osmolarity similar to the carrier used in connection with the lipid composition or in which the lipid composition is contained. In the embodiment of the lipid composition of the present invention whereby the lipid composition also comprises a nucleic acid, preferably a functional nucleic acid such as, but not limited to, a siRNA, the concentration of the functional nucleic acid, preferably of siRNA in the lipid composition is about 0.2 to 0.4 15 mg/ml, preferably 0.28 mg/ml, and the total lipid concentration is about 1.5 to 2.7 mg/ml, preferably 2.17 mg/ml. It is to be acknowledged that this mass ratio between the nucleic acid fraction and the lipid fraction is particularly preferred, also with regard to the charge ratio thus realized. In connection with any further concentration or dilution of the lipid composition of the present invention, it is preferred that the mass ratio and the charge ratio, 20 respectively, realized in this particular embodiment is preferably maintained despite such concentration or dilution.

Such concentration as used in, for example, a pharmaceutical composition, can be either obtained by dispersing the lipid in a suitable amount of medium, preferably a physiologically acceptable buffer or any carrier described herein, or can be concentrated by 25 appropriate means. Such appropriate means are, for example, ultra filtration methods including cross-flow ultra-filtration. The filter membrane may exhibit a pore width of 1,000 to 300,000 Da molecular weight cut-off (MWCO) or 5 nm to 1 µm. Preferred is a pore width of about 10,000 to 100,000 Da MWCO. It will also be acknowledged by the one skilled in the art that the lipid composition more specifically the lipoplexes in accordance with the present 30 invention may be present in a lyophilized form. Such lyophilized form is typically suitable to increase the shelf life of a lipoplex. The sugar added, among others, to provide for the appropriate osmolarity, is used in connection therewith as a cryo-protectant. In connection therewith it is to be acknowledged that the aforementioned characteristics of osmolarity, pH

as well as lipoplex concentration refers to the dissolved, suspended or dispersed form of the lipid composition in a carrier, whereby such carrier is in principle any carrier described herein and typically an aqueous carrier such as water or a physiologically acceptable buffer, preferably an isotonic buffer or isotonic solution.

5

EXAMPLES

Example 1: The effect of various ICAM-1 siRNAs on mRNA knockdown, protein knockdown and cell viability

The effect of various ICAM-1 siRNAs on mRNA knockdown, protein knockdown and cell viability was determined as follows.

Materials:

siRNA duplexes (ICAM1-h-2, ICAM1-hc-3, ICAM1-hc-6, ICAM1-h-26 and the luciferase control) were synthesised at BioSpring Frankfurt Germany. Table 4 below shows the sequences in 5'-3' direction with modification pattern for each strand.

Table 4:

ICAM1-h-2 (SEQ ID NO: 18) AGACAUAGAUUGAUGGAUGUUAAA (sense) with 2' OMethyl modification of the ribose on even numbered nucleotides
ICAM1-h-2 (SEQ ID NO: 19) UUUUACAUCCAUCAUCAUGUCU (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3 (SEQ ID NO: 20) CCCCAACCCUUGAUGAU AUGUAU (sense) with 2' OMethyl modification of the ribose on even numbered nucleotides
ICAM1-hc-3 (SEQ ID NO: 21) AUACAUAUCAUCAAGGGUUGGGG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-6 (SEQ ID NO: 22) CUGACCCCACCCUUGAUGAU AU (sense) with 2' OMethyl modification of the ribose on even numbered nucleotides
ICAM1-hc-6 (SEQ ID NO: 23) AUAUCAUCAAGGGUUGGGGUCAG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-h-26 (SEQ ID NO: 24) GGUACCUGCACACCUAAAACACU (sense) with 2' OMethyl modification of the ribose on even numbered nucleotides
ICAM1-h-26 (SEQ ID NO: 25) AGUGUUUUAGGUGUGCAGGUACC (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
Luciferase (sense) (SEQ ID NO: 34) AUCACGUACGCGGAAUACUUCGA with 2'OMethyl modiciation of the ribose on even numbered nucleotides
Luciferase (antisense) (SEQ ID NO: 35) UCGAAAUAUUCCCGGUACGUGAU with 2'OMethyl modification of the ribose on odd numbered nucleotides

RPMI 1640 (Sigma), L-Glutamine (Invitrogen), Foetal Calf Serum, Flat bottomed culture plates (Costar), fully supplemented airways media (Promocell C21160), Pronase (Sigma P5147), Ultrapure water, Atufect01 lipid (provided by Silence Therapeutics Santel, A *et. al.*, (2006). A novel siRNA-lipoplex technology for RNAi in the mouse vascular endothelium.

Gene Ther, 13, 1222-1234), RNeasy96 (Qiagen 74182) Rnase-free Dnase (Qiagen 79254), Ribogreen (Invitrogen), WST1 (Roche-Diagnostics), supported nitrocellulose membranes (Amersham RPN 303E), anti human ICAM-1 antibody (Santa Cruz G5, sc8439), anti mouse HRP conjugate (Cell Signalling, #7076), anti GapDH antibody (R&D Systems #2275-pc-1).

5 All other chemicals were purchased from Sigma, at a purity suitable for the final use.

Cell Lines:

10 H292 cells were purchased from ECACC (91091815 passage 81) and were maintained in RPMI 1640 supplemented with 5mM L-Glutamine and 10% foetal Calf Serum. Cells were seeded at a density of 5,000 cells/well/100µl into 96 well flat-bottomed culture plates 24 hours prior to siRNA transfection in complete cell media.

15 *Epithelial Cell Cultures:*

Frozen aliquots of Normal Human Bronchial Epithelial (NHBE) cells were purchased from Promocell. Cells were revived following the manufacturer's instructions and maintained at a density of 30-80% confluence in fully supplemented airways media. Cells were used at passages 4 to 5.

20 Airway Epithelial Cells (AEC) from COPD patients were isolated from lung transplant tissue, obtained with informed ethical consent. Large airways were dissected from the lung and digested in 0.1% Pronase at 4°C for 2-3 days. The tissue was agitated and the resultant cell suspension pelleted by centrifugation, and washed with fully supplemented airways epithelial cell media. The cells were grown in fully supplemented airways epithelial cell media, maintained at 30-70% confluence. Cells were used at passages 4 to 5. Cells were seeded at a density of 10,000 cells/well/100µl into 96 well flat-bottomed culture plates 24 hours prior to siRNA transfection in complete cell media.

25 *Treatment of the cell cultures with the siRNA described above:*

30 siRNA stocks were prepared in Ultrapure water to a final concentration of 100µM. These stocks were diluted to 400nM with either Optimem (Invitrogen for H292 experiments) or unsupplemented airways media (for primary epithelial cell experiments). This was complexed with an equal volume of 20µg/ml Atufect01 (Santel, A et. Al., (2006). A novel

siRNA-lipoplex technology for RNAi in the mouse vascular endothelium. Gene Ther, 13, 1222-1234.) lipid, made up from a 1mg/ml stock (supplied by Silence Therapeutics), diluted with either Optimem (for H292 experiments) or unsupplemented airways media (for primary epithelial cell experiments). The complex was incubated for 30mins at 37°C, 90% humidity,
5 5% CO₂. Meanwhile the media was aspirated from the plated cells and replaced with 100µl fresh fully supplemented growth media. The siRNA:lipid complex was diluted to give a series of siRNA/lipid concentrations at fixed ratio. 25µl was added to each well, and the cells incubated with complex for 24 hours at 37°C, 90% humidity, 5% CO₂. The complex was aspirated to waste and the cells were either analysed for ICAM-1 mRNA levels or 100µl of
10 growth media added, and the cells left for a further 48 hours. Cells were then assessed for cell viability and ICAM-1& GapDH protein levels. Two controls were included – an addition of complex media alone, or no addition at all.

Real-Time RT-PCR:

15 RNA was isolated from the cell monolayers using RNeasy 96, with the inclusion of an on-column DNase I step to ensure removal of genomic DNA. Total relative levels of RNA from each sample were measured using a fluorescent dye (Ribogreen), and this was used to normalise the levels of ICAM-1 mRNA. Relative levels of ICAM-1 mRNA were measured using one step quantitative RT-PCR with Quantitect Probe RT-PCR, a Stratagene MX3000p
20 real-time thermocycler and primers and probe designed against ICAM-1. An RNA standard curve was included on each plate to measure primer/probe efficiency. See Fig. 1a, 2a, 3a, 4a, 6a, 7a, 8a, 9a and 10a

Assessment of Cell Viability:

25 Cell viability was assessed by measuring the reduction of the dye WST1 by viable cells using a Spectramax M5 plate reader. The conversion was measured over a range of timepoints to ensure the conversion was in the linear range. Wells containing no cells were included to measure background breakdown of the dye. See Fig. 1c, 2c, 3c, 4c, 6c, 7c, 8c, 9c and 10c.

30

Western Blotting for ICAM-1:

After measurement of cell viability the cells were lysed in ice-cold sample application buffer (62.5mM Tris.HCL, pH 6.8, 2% SDS, 10% Glycerol, 140mM 2-Mercaptoethanol,

0.1mg/ml bromophenol blue) and mixed. The lysates were heated at 95°c for 5mins and then separated by SDS-PAGE. The proteins were transferred to supported nitrocellulose membranes and these were then blocked with 5% non-fat milk for 1hr. The membranes were probed with 1/1000 anti human ICAM-1 overnight at room temperature, washed, and probed with anti mouse-HRP conjugated secondary antibody for 1 hour at room temperature. After washing the resulting bands were visualised with West Dura chemiluminescent substrate, and captured using a 16-bit CCD camera system (Syngene Genegnome), ensuring the signals were within the linear range of the camera. Each blot was normalised for loading variations by re-probing the same blot for GapDH and measuring the signal in a similar way. See Fig. 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b 9b and 10b.

Summary of results:

For each siRNA concentration, ICAM-1 mRNA levels (panel (a) in Figs 1-10), ICAM-1 protein levels (panel (b) in Figs 1-10) and the cell viability (as measured by the WST-1 assay; panel (c) in Figs 1-10) are normalised against the media control (=1) and the mean +/- SEM calculated. These are shown graphically for the mean of four independent experiments for the H292 human epithelial cell line (Fig1-5). All four ICAM-1 directed reagents give dose related knockdown of both ICAM-1 mRNA and protein with no loss in cell viability (Fig 1-4). Fig 5 demonstrates that there is no ICAM-1 knockdown by the luciferase control. Fig 6-10 shows similar data generated in human primary airway epithelial cells. Data are the mean of 5 experiments in three COPD donors and two individual experiments in one normal donor unless otherwise indicated in the graph title. The ICAM-1 specific siRNA knock down both protein and message (Fig 6-9) while the luciferase control does not (Fig 10).

25

Example 2: The effect of varying the length of the ICAM-hc-3 siRNA sequence on ICAM mRNA knockdown

30

The effect of reducing the length of ICAM1-hc3 (between 17 and 23 nucleotides) on mRNA knockdown was measured after 24 hour transfection of the H292 human lung epithelial cell line. For this purpose, cells were transfected with a range of eight concentrations of siRNA complexed with a fixed concentration of 1μg/ml Atufect 01 lipid.

Materials

siRNA duplexes (ICAM1-hc-3-17, ICAM1-hc-3-18, ICAM1-hc-3-19, ICAM1-hc-3-20, ICAM1-hc-3-21, ICAM1-hc-3-22 and ICAM1-hc-3 (the 23mer) were synthesized by

5 Integrated DNA Technologies (IDT), Leuven, Belgium. The antisense sequences of the siRNA duplexes are summarized in Table 5 below and the sequences are shown in 5'-3' direction with modification pattern for each strand. All other reagents were as specified under example 1.

10

Table 5:

ICAM1-hc-3 (SEQ ID NO: 21) AUACAUUAUCAUCAAGGGUUGGGG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3-22 (SEQ ID NO: 36) AUACAUUAUCAUCAAGGGUUGGG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3-21 (SEQ ID NO: 37) AUACAUUAUCAUCAAGGGUUGG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3-20 (SEQ ID NO: 38) AUACAUUAUCAUCAAGGGUUG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3-19 (SEQ ID NO: 39) AUACAUUAUCAUCAAGGGUU (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3-18 (SEQ ID NO: 40) AUACAUUAUCAUCAAGGGU (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides
ICAM1-hc-3-17 (SEQ ID NO: 13) AUACAUUAUCAUCAAGGG (antisense) with 2' OMethyl modification of the ribose on odd numbered nucleotides

Sense strands for ICAM1-hc-3-18, 19, 20, 21 and 22 are fully complementary to the antisense strand shown in Table 5 and of matching (length 18 to 22 nucleotides) giving fully base-paired, blunt end structures in the same manner as SEQ ID NO 13 and 12 for ICAM1-hc-3-17 and SEQ ID NO 21 and 20 for ICAM1-hc-3.

15

Treatment of H292 cells with the siRNA described above.

Cells were cultured as in example 1

20

siRNA stocks were prepared in Ultrapure water to a final concentration of 20 μ M. These stocks were diluted to 316nM with Optimem medium (Invitrogen) then serially diluted in half log increments with Optimem. Each dilution was complexed with an equal volume of 10 μ g/ml Atufect01 lipid (supplied by Silence Therapeutics, Berlin: Santel, A *et. al.*, (2006). A novel 5 siRNA-lipoplex technology for RNAi in the mouse vascular endothelium (Gene Ther, 13, 1222-1234.), made up from a 1mg/ml stock diluted with Optimem. The complexes were incubated for 30mins at 37°C, 90% humidity, 5% CO₂. Meanwhile the media was aspirated from the plated cells and replaced with 100 μ l fresh fully supplemented growth media. 25 μ l of the siRNA complex of the appropriate concentration was added to each well, and the cells incubated with 10 complex for 24 hours at 37°C, 90% humidity, 5% CO₂. The complex was aspirated to waste and the cells were either analysed for ICAM-1 mRNA or 100 μ l growth medium was added and the cells left for a further 48 hours then assessed for cell viability.

mRNA was measured by real time RT-PCR and cell viability was assessed by WST-1 15 assay as described under example 1. Determinations were carried out on duplicate wells and the results normalised against the medium only (Optimem) control. The error bar represents the range of duplicates.

Summary of results

20 All the siRNA tested, based on ICAM1-hc-3, of length range 17-23 nucleotides gave dose-related knock-down of ICAM-1 mRNA after 24 hours (Fig 11). Cytotoxicity data from this experiment, based on the WST-1 assay, is summarised in Fig 12. All siRNA were free of cytotoxicity (Fig 12) except for ICAM1-hc-3-20 which showed a slight suppression of the WST-1 25 signal at higher concentrations (Fig 12d). In a repeat experiment, similar knockdown of ICAM-1 mRNA was observed (Fig. 13). Again in this second experiment, all siRNA were free of cytotoxicity except for ICAM1-hc-3-20 where a small effect was observed at higher concentrations (data not shown).

CLAIMS

1. An isolated siRNA comprising a sense sequence and an antisense sequence, wherein the sense and the antisense sequence are substantially complementary to each other to form a double-stranded structure that is 17-25 base pairs in length, and wherein the antisense sequence comprises a nucleotide sequence sufficiently complementary to a target human ICAM-1 DNA nucleotide sequence selected from the group of sequences shown in Table 1 to support RNA interference.

Table 1:

SEQ ID NO:2	GATTGATGGATGTTAAA
SEQ ID NO:3	CCCTTGATGATATGTAT
SEQ ID NO:4	CCAACCCTTGATGATAT
SEQ ID NO:5	TGCACACCTAAACACT

2. The siRNA of claim 1, wherein the target ICAM-1 nucleotide sequence is selected from the group of sequences shown in Table 2.

Table 2

SEQ ID NO:6	AGACATGATTGATGGATGTTAAA
SEQ ID NO:7	CCCCAACCCCTTGATGATATGTAT
SEQ ID NO:8	CTGACCCCCAACCTTGATGATAT
SEQ ID NO:9	GGTACCTGCACACCTAAACACT

3. The siRNA of claim 1, wherein the antisense strand sequence is 17 to 25 nucleotides in length and comprises the sequence of SEQ ID NO:11, wherein the first nucleotide of the antisense sequence begins at nucleotide position 1 of SEQ ID NO: 11.

4. The siRNA of claim 1, wherein the antisense strand sequence is 17 to 25 nucleotides in length and comprises the sequence of SEQ ID NO:13, wherein the first nucleotide of the antisense sequence begins at nucleotide position 1 of SEQ ID NO: 13.

5. The siRNA of claim 1, wherein the antisense strand sequence is 17 to 25 nucleotides in length and comprises the sequence of SEQ ID NO:15, wherein the first nucleotide of the antisense sequence begins at nucleotide position 1 of SEQ ID NO: 15.

6. The siRNA of claim 1, wherein the antisense strand sequence is 17 to 25 nucleotides in length and comprises the sequence of SEQ ID NO:17, wherein the first nucleotide of the antisense sequence begins at nucleotide position 1 of SEQ ID NO: 17.
7. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:18, and the antisense strand sequence comprises the sequence of SEQ ID NO:19.
8. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:20, and the antisense strand sequence comprises the sequence of SEQ ID NO:21.
9. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:22, and the antisense strand sequence comprises the sequence of SEQ ID NO:23.
10. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:24, and the antisense strand sequence comprises the sequence of SEQ ID NO:25.
11. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:26, and the antisense strand sequence comprises the sequence of SEQ ID NO:27.
12. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:28, and the antisense strand sequence comprises the sequence of SEQ ID NO:29.
13. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:30, and the antisense strand sequence comprises the sequence of SEQ ID NO:31.
14. The siRNA of claim 1, wherein the sense strand sequence comprises the sequence of SEQ ID NO:32, and the antisense strand sequence comprises the sequence of SEQ ID NO:33.
15. The siRNA of claim 1, wherein the antisense strand and the sense strand are each 17 to 25 nucleotides in length.
16. The siRNA of claim 1, wherein the double stranded structure is from 17 to 25 nucleotides.

17. The siRNA of claim 1, wherein the double stranded structure is 23 nucleotides.

18. The siRNA of any of the proceeding claims, wherein the siRNA:

- a) is blunt ended at both ends;
- b) has an overhang at one end and a blunt end at the other; or
- c) has an overhang at both ends.

19. The siRNA of any of the proceeding claims, wherein one or more nucleotides on the sense and/or antisense strands are modified.

20. The siRNA of any of the proceeding claims, wherein alternating nucleotides on the sense and/or antisense strands are modified.

21. The siRNA of claim 20, wherein the alternating nucleotides on both the sense and antisense strands are modified at the 2'-hydroxyl group of the ribose ring.

22. The siRNA according to any of the preceding claims, wherein the modification at the 2'-hydroxyl group of ribose is selected from the group consisting of amino, fluoro, O-methyl, hydrogen, alkoxy and alkyl.

23. The siRNA of any of claim 21, wherein the alternating nucleotides on both the sense and antisense strands are modified with an O-methyl group at the 2'-position of the ribose.

24. The siRNA of claim 23, wherein each of the odd numbered nucleotides are modified in the antisense strand and each of the even numbered nucleotides are modified in the sense strand.

25. The siRNA of any of the proceeding claims, wherein a first stretch of the sense strand and a second stretch of the antisense strand each consist of contiguous alternating single 2'-O-methyl modified and single unmodified ribonucleotides, wherein each modified ribonucleotide in the first stretch is base paired with the unmodified ribonucleotide in the second stretch.

26. A delivery vector comprising a siRNA according to any of claims 1-24
27. A method of generating a dicer substrate, comprising contacting a chain of ribose nucleotides with Dicer such that the products of Dicer activity are siRNAs of any of the preceding claims 3-14.
28. An siRNA delivery agent comprising a siRNA according to any preceding claim.
29. The siRNA delivery agent according to claim 28, wherein the delivery system is one, several, or a combination of the following: RNA binding proteins, cell penetrating peptide (e.g. antennapedia homeodomain peptide), targeting agents (e.g., peptides, antibodies or aptamers) endosomal release agents (e.g., certain viral coat peptides) cationic polymers and other polycations e.g., poly(L-lysine), polyethylenimine, polymethacrylate or carbohydrate-based polymers e.g. chitosan and β -cyclodextrin , lipids, , polyethylene glycol or other substances known to assist oligonucleotide delivery such as carbon nanotubes, Gold or silica nanoparticles and quantum dots and where the siRNA is covalently bonded or non-covalently complexed with the delivery system and exists in solution or in the form of oligomeric complexes or nanoparticles, dendrimers, liposomes, lipoplexes, biodegradable polymeric vectors or polymeric hydrogels.
30. The siRNA delivery agent of claim 29, wherein the liposome consists of
 - a) about 50 mol% β -arginyl-2,3-diaminopropionic acid-N-palmityl-N-oleyl-amide trihydrochloride, preferably (β -(L-arginyl)-2,3-L-diaminopropionic acid-N-palmityl-N-oleyl-amide tri-hydrochloride);
 - b) about 48 to 49 mol% 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE); and
 - c) about 1 to 2 mol% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polyethyleneglycole, preferably N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt.
31. A cell comprising an siRNA according to any of the preceding claims.

32. A composition comprising the siRNA of any of the proceeding claims and a physiologically acceptable excipient.
33. Use of a siRNA of any of the proceeding claims for the manufacture of a medicament.
34. Use of a siRNA of any of the proceeding claims for the treatment of a disease or disorder where the modulation of ICAM-1 expression is desired.
35. A method of treating a disease or disorder comprising the administration of a composition comprising an siRNA according to any of the proceeding claims to an individual having a disease or disorder where the modulation of ICAM-1 expression is desired.
36. The use or method of claims 34 or claim 35, wherein said disease or disorder is a respiratory disease or disorder, an inflammatory disease or disorder, a proliferative cell disorder, or a cardiovascular disease or disorder.
37. The use or method of claim 36, wherein the respiratory disease or disorder is asthma, COPD, Acute Lung Injury (ALI), or acute respiratory distress syndrome (ARDS).
38. The use or method of claim 36, wherein the respiratory disease or disorder is human rhinovirus infection.
39. The use or method of claim 36, wherein the inflammatory disorder is rheumatoid arthritis, psoriasis, sepsis, gastrointestinal, Crohn's disease, ulcerative colitis, or irritable bowel disease, infection and acute organ damage e.g., kidney and lung following ischaemia or other trauma and sub-acute cardiac damage following myocardial infarction.
40. The use or method of claim 36, wherein the proliferative cell disorder is severe cancer leukemia.
41. The use or method of claim 36, wherein the disease or disorder is atherosclerosis.

Fig 1: Knockdown of ICAM-1 in the H292 human epithelial cell line by ICAM1-hc-2

Fig 1a: mRNA

Fig 1b: Protein

Fig 1c: Cytotoxicity

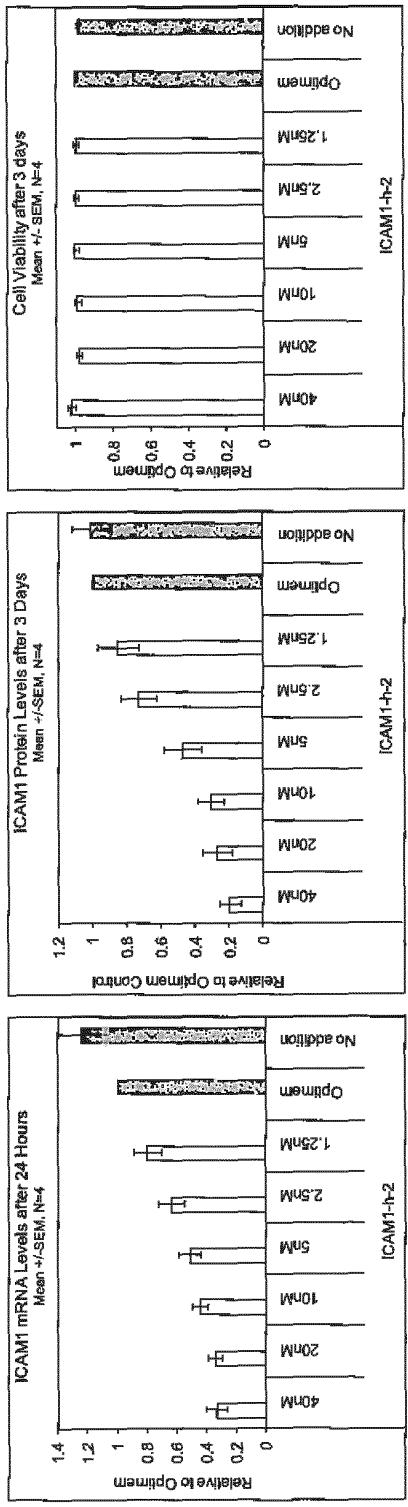


Fig 2: Knockdown of ICAM-1 in the H292 human epithelial cell line by ICAM1-hc-3

Fig 2a: mRNA

Fig 2b: Protein

Fig 2c: Cytotoxicity

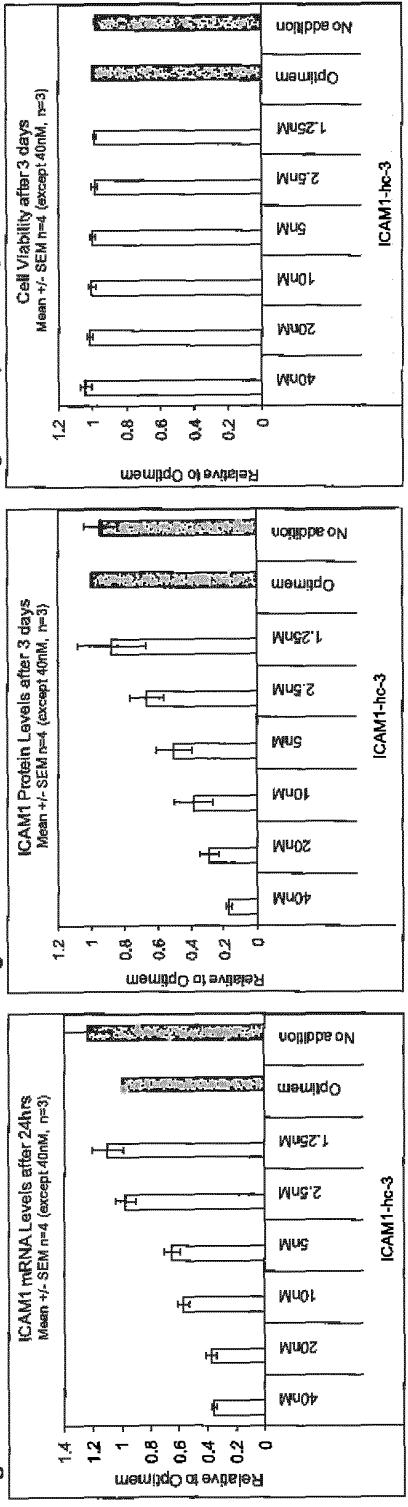


Fig 3: Knockdown of ICAM-1 in the H292 human epithelial cell line by ICAM1-hc-6

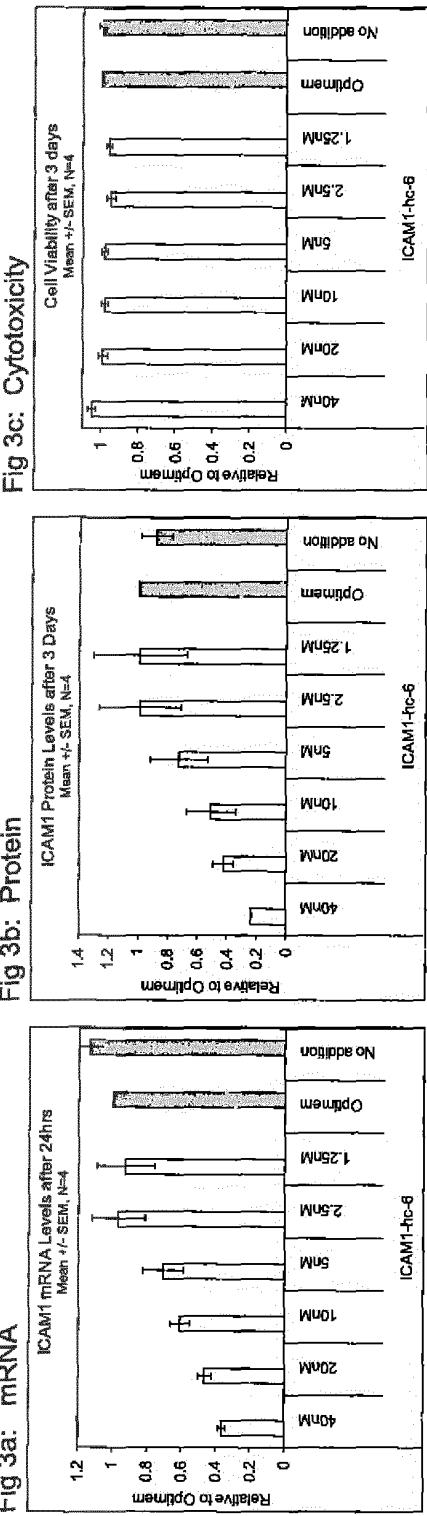


Fig. 3b: Protein

Fig 3c: Cytotoxicity

Fig 3c: Cytotoxicity

Fig 4: Knockdown of ICAM-1 in the H292 human epithelial cell line by lCAM1-h-26

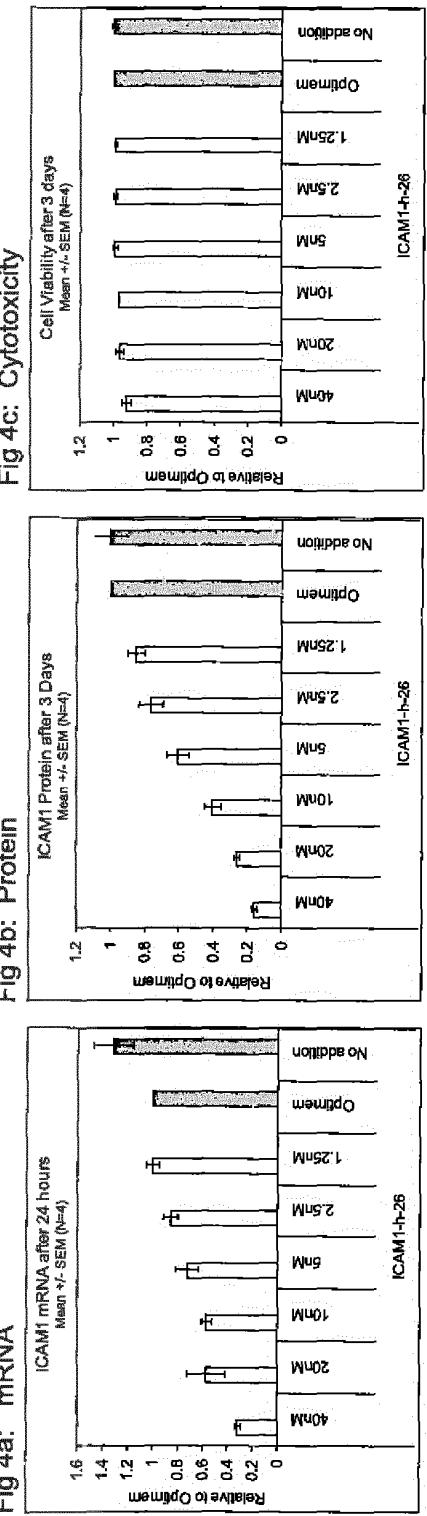


Fig 4b: Protein

Fig 4c: Cytotoxicity

Fig 4c: Cytotoxicity

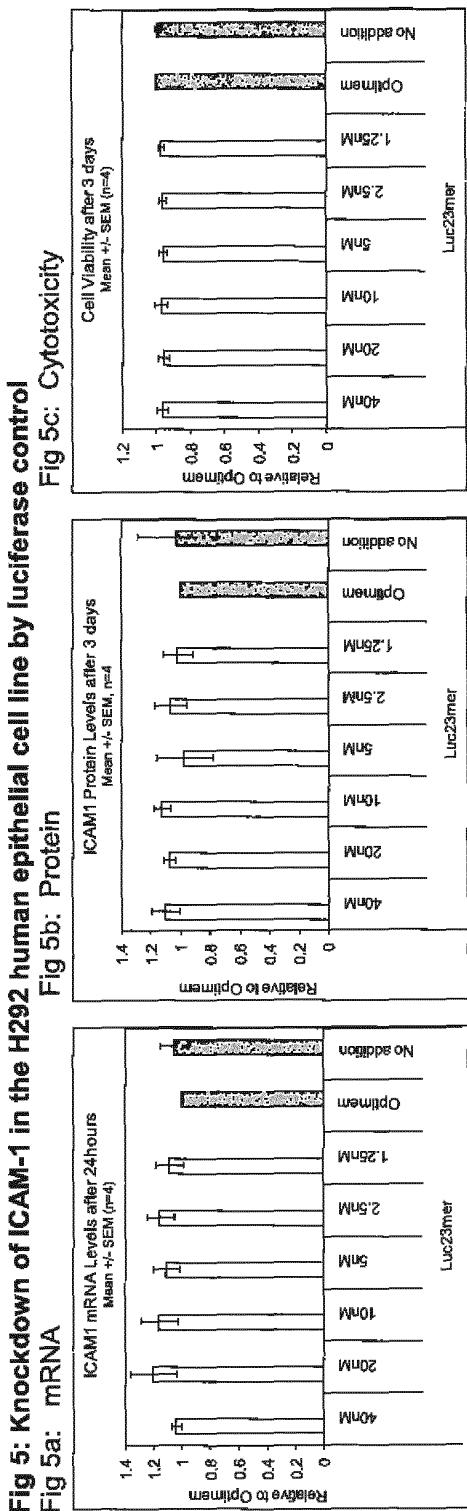


Fig 6: Knockdown of ICAM-1 in primary human epithelial cells by ICAM1-hc-2

Fig 6a: mRNA

Fig 6b: Protein

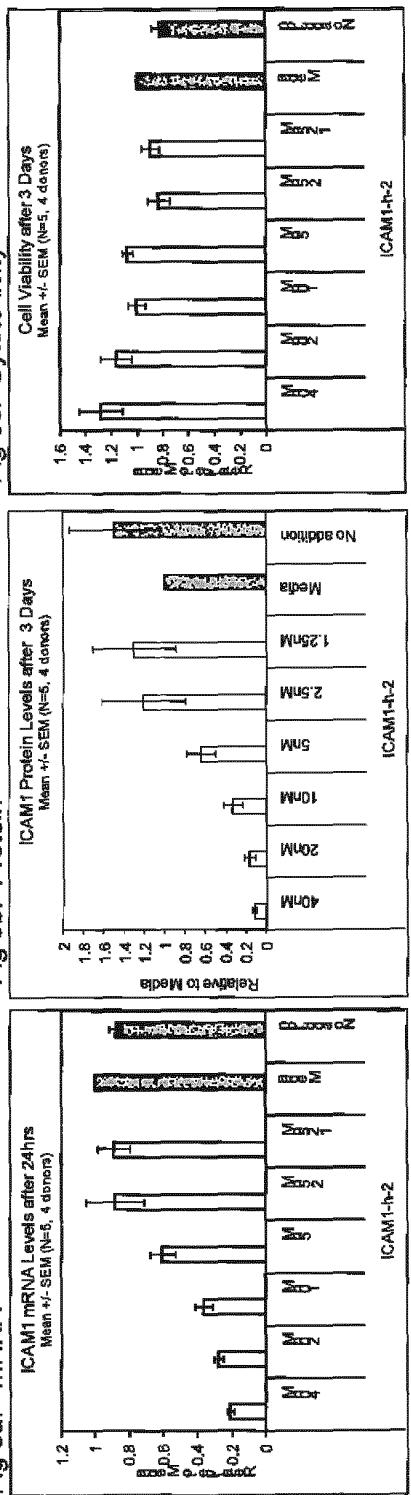


Fig 7: Knockdown of ICAM-1 in primary human epithelial cells by ICAM-M-hc-3

Fig 7a: mRNA

Fig 7b: Protein

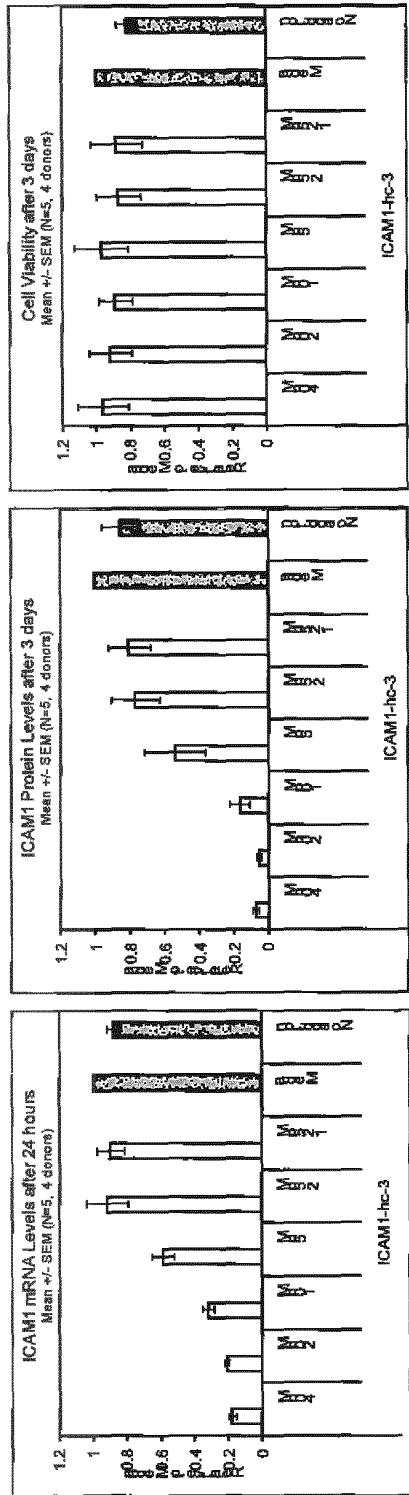
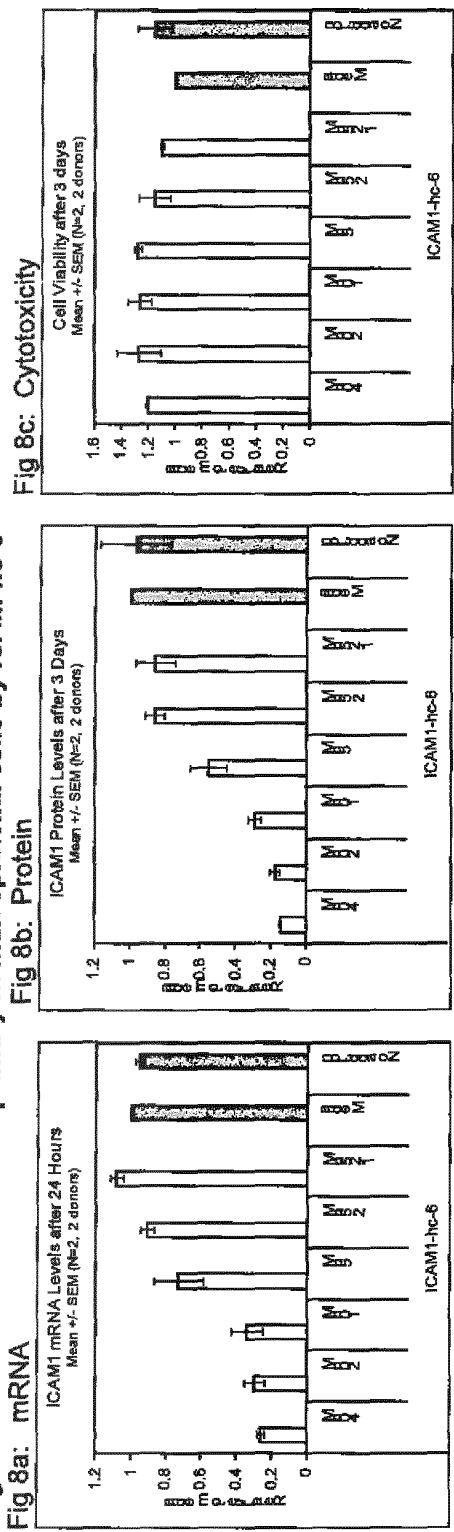
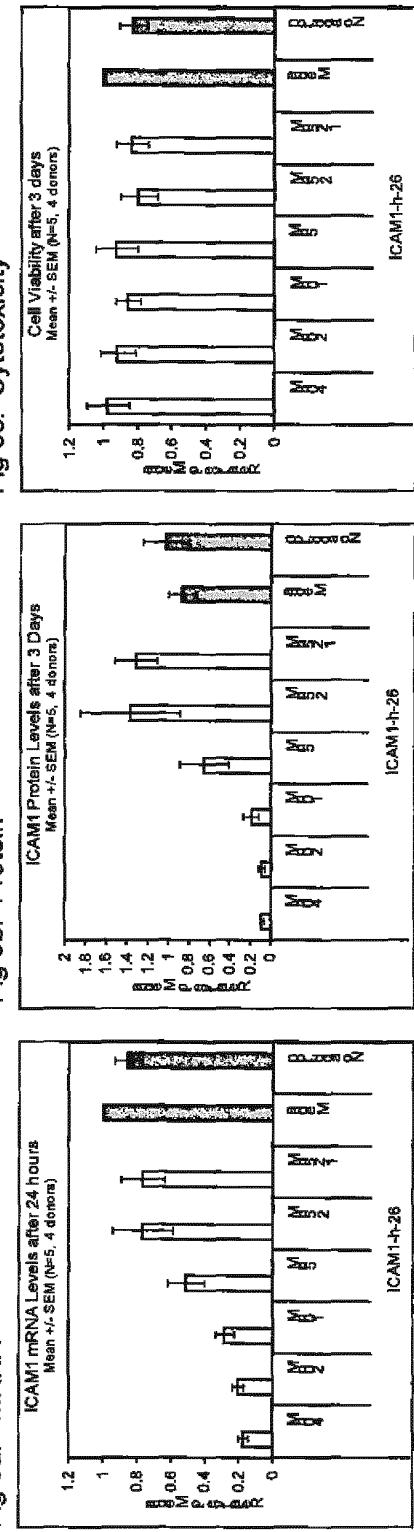


Fig 8: Knockdown of ICAM-1 in primary human epithelial cells by ICAM-hc-6**Fig 9: Knockdown of ICAM-1 in primary human epithelial cells by ICAM-h26**

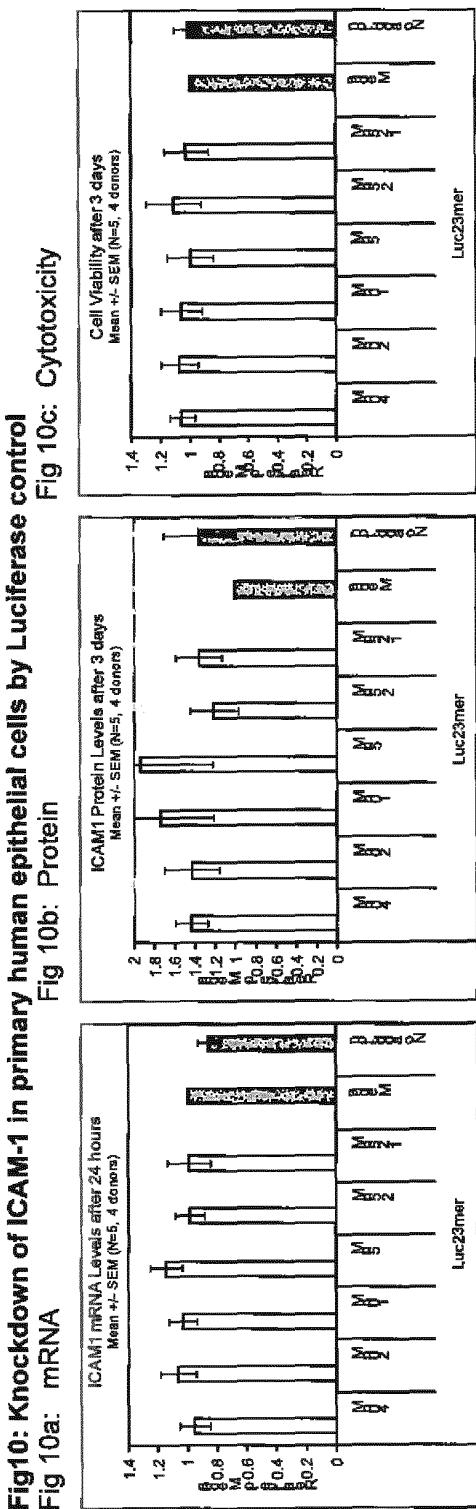


Fig 11: Effect of varying length of ICAM1-hc-3 on ICAM1 mRNA knockdown in H292 cell line 24 hours after transfection

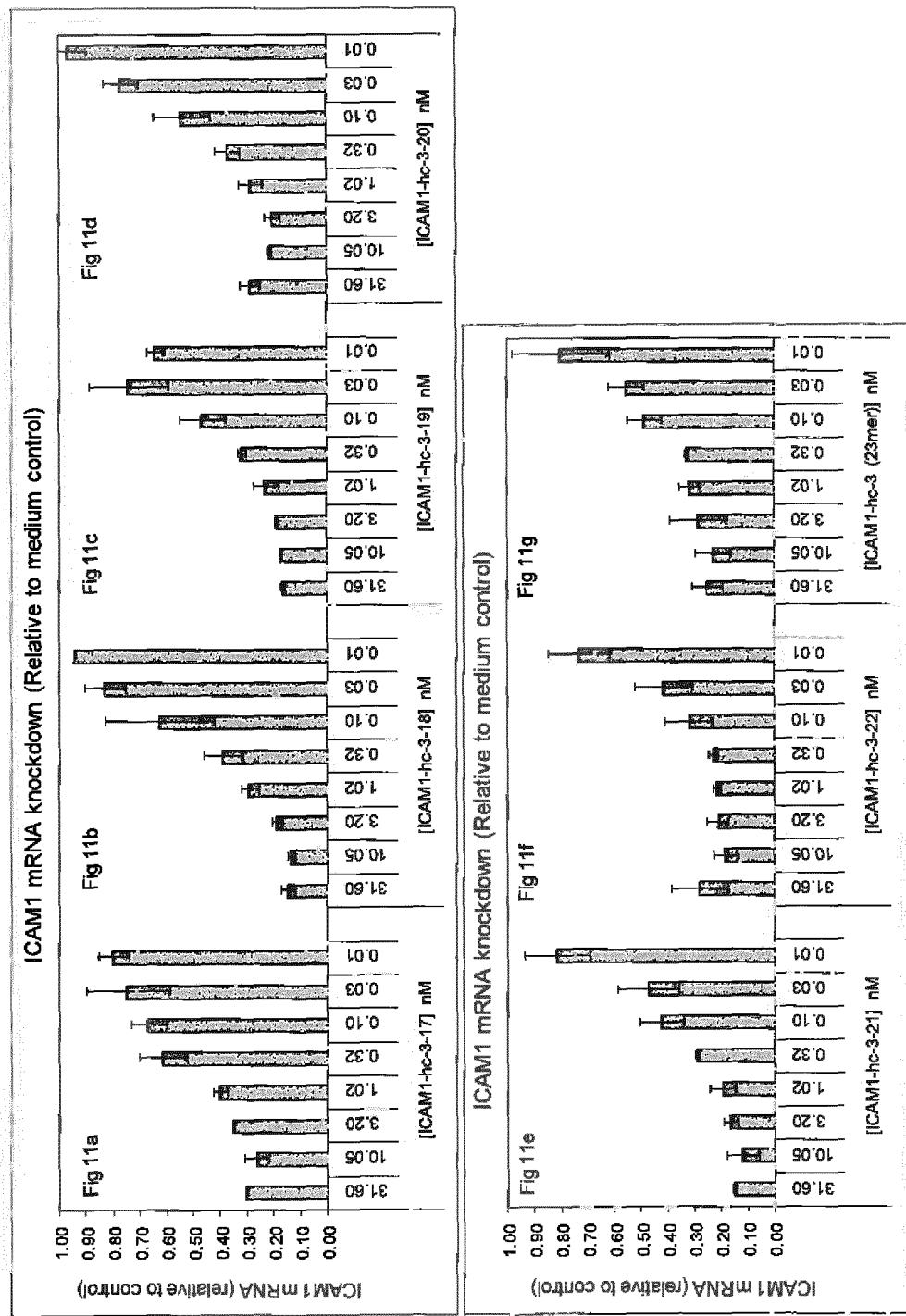


Fig 12: Effect of varying length of ICAM1-hc-3 on cell viability (by WST-1) in H292 cell line 72 hours after transfection

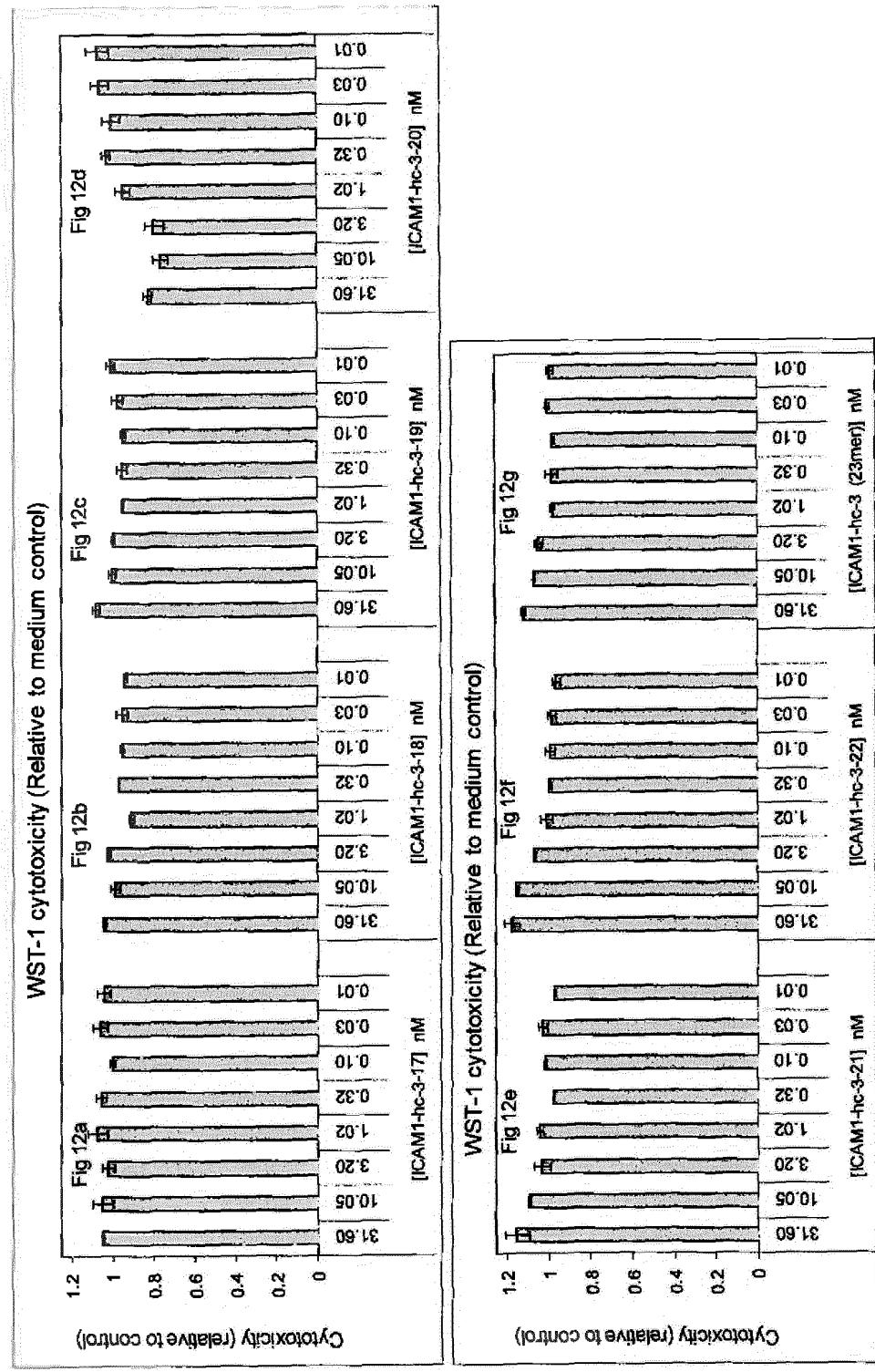
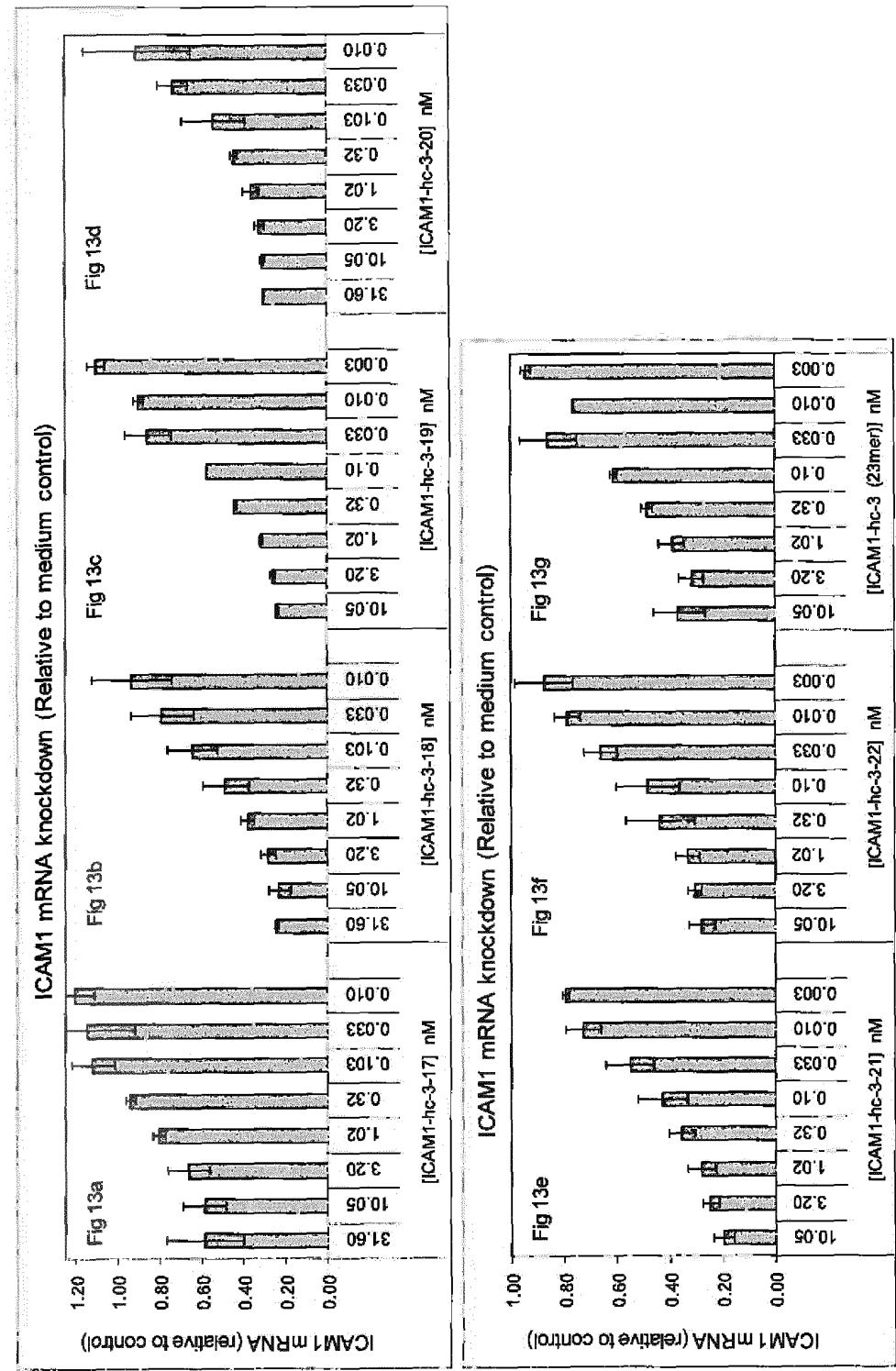


Fig 13: Effect of varying length of ICAM1-hc-3 on ICAM1 mRNA knockdown in H292 cell line 24 hours after transfection



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/053149

A. CLASSIFICATION OF SUBJECT MATTER	INV. C12N15/113	A61K31/712	A61P35/00	A61P11/00	A61P29/00
			A61P9/10		

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

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

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

EPO-Internal, BIOSIS, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/045039 A2 (SIRNA THERAPEUTICS INC [US]; RICHARDS IVAN [US]; MCSWIGGEN JAMES [US]) 19 May 2005 (2005-05-19) the whole document -----	1-3,7, 11,15-41
X	US 2008/086001 A1 (KHVOROVA ANASTASIA [US] ET AL) 10 April 2008 (2008-04-10) paragraph [0030] - paragraph [0037]; claims 1-9; example 17; sequence 442 -----	1-3,7, 11,15-41
X	WO 2010/111497 A2 (MERCK SHARP & DOHME [US]; STRAPPS WALTER [US]; SHAH JYOTI [US]; PICKER) 30 September 2010 (2010-09-30) the whole document ----- -/-	1-3,7, 11,15-41

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2012	30/01/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Spindler, Mark-Peter

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2012/053149

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/065546 A2 (UNIV PENNSYLVANIA [US]; REICH SAMUEL JOTHAM [US]; TOLENTINO MICHAEL J) 5 August 2004 (2004-08-05) the whole document -----	1-3,7, 11,15-41
X	VICKERS T A ET AL: "Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents: A comparative analysis", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 278, no. 9, 28 February 2003 (2003-02-28), pages 7108-7118, XP002281434, ISSN: 0021-9258, DOI: 10.1074/jbc.M210326200 figure 3; table 1 -----	1-3,7, 11,15-41
A	WO 2010/094491 A1 (SILENCE THERAPEUTICS AG [DE]; SANTEL ANSGAR [DE]; KAUFMANN JOEORG [DE]) 26 August 2010 (2010-08-26) claims 1-11; figures 2, 9-11; examples 2-7 -----	19-25, 28-30
A	WO 2004/015107 A2 (ATUGEN AG [DE]; GIESE KLAUS [DE]; KAUFMANN JOERG [DE]; KLIPPEL-GIESE A) 19 February 2004 (2004-02-19) cited in the application claims 1-8; figures 15, 16; examples 9, 11 -----	19-25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2012/053149

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

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

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

3, 7, 11(completely); 1, 2, 15-41(partially)

Remark on Protest

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 3, 7, 11(completely); 1, 2, 15-41(partially)

sirNA having a double-stranded structure of 17-25 base pairs wherein the antisense strand is complementary to a target ICAM-1 sequence defined by SEQ ID NO: 2 or 6; implementations thereof

2. claims: 4, 5, 8, 9, 12, 13(completely); 1, 2, 15-41(partially)

sirNA having a double-stranded structure of 17-25 base pairs wherein the antisense strand is complementary to a target ICAM-1 sequence defined by SEQ ID NO: 3, 4, 7, or 8; implementations thereof

3. claims: 6, 10, 14(completely); 1, 2, 15-41(partially)

sirNA having a double-stranded structure of 17-25 base pairs wherein the antisense strand is complementary to a target ICAM-1 sequence defined by SEQ ID NO: 5 or 9; implementations thereof

INTERNATIONAL SEARCH REPORT

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International application No PCT/EP2012/053149

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