(12) STANDARD PATENT (11) Application No. **AU 2009324534 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
GNAQ targeted dsRNA compositions and methods for inhibiting expression

(51) International Patent Classification(s)
C12N 15/113 (2010.01) ***A61P 35/00*** (2006.01)

(21) Application No: **2009324534** (22) Date of Filing: **2009.12.10**

(87) WIPO No: **WO10/068816**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/185,543	**2009.06.09**	**US**
61/121,253	**2008.12.10**	**US**
61/244,780	**2009.09.22**	**US**

(43) Publication Date: **2010.06.17**
(44) Accepted Journal Date: **2015.07.30**

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(56) Related Art
WO2006/040409
WO2008/098208
US7687616
TABOUBI, S. et al., FASEB Journal, 2007, vol. 21, pages 4047-4058

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 June 2010 (17.06.2010)

PCT

(10) International Publication Number
WO 2010/068816 A1

(51) International Patent Classification:
A61P 35/00 (2006.01) *C12N 15/113* (2010.01)

(21) International Application Number:
PCT/US2009/067581

(22) International Filing Date:
10 December 2009 (10.12.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/121,253 10 December 2008 (10.12.2008) US
61/185,543 9 June 2009 (09.06.2009) US
61/244,780 22 September 2009 (22.09.2009) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2010/068816 A1

(54) Title: GNAQ TARGETED DSRNA COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION

(57) Abstract: The invention relates to a double-stranded ribonucleic acid (dsRNA) targeting a G-alpha q subunit (GNAQ) of a heterotrimeric G gene, and methods of using the dsRNA to inhibit expression of GNAQ.

GNAQ TARGETED dsRNA COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/121,253, filed Dec 10, 2008, and U.S. Provisional Application No. 61/185,543, filed Jun 9, 2009 , and U.S. Provisional Application No. 61/244,780, filed Sep 22, 2009 , which are hereby incorporated in their entirety by reference.

REFERENCE TO A SEQUENCE LISTING

This application includes a Sequence Listing submitted electronically as a text file named 16267US_sequencelisting.txt, created on DECEMBER 10, 2010, with a size of 560,474 bytes. The sequence listing is incorporated by reference.

Field of the Invention

The invention relates to a double-stranded ribonucleic acid (dsRNA) targeting a G-alpha q subunit (GNAQ) of a heterotrimeric G gene, and methods of using the dsRNA to inhibit expression of GNAQ.

Background of the Invention

Guanine nucleotide-binding proteins (G proteins) are a family of heterotrimeric proteins that couple cell surface, 7-transmembrane domain receptors to intracellular signaling pathways. G proteins are composed of alpha, beta and gamma subunits. The G-alpha q subunit (GNAQ) is one of the G-alpha subunits. GNAQ mediates stimulation of phospholipase C-beta and hydrolysis of GTP.

Mice with GNAQ mutations leading to overexpression of GNAQ exhibit dermal hyperpigmentation. A point mutation in human GNAQ was reported in a melanoma sample (Bamford et al (2004) Br J Cancer, 91:355-358).. In WO/2008/098208 (PCT/US2008/053484), the Applicant's described the presence of mutations that constitutively activate GNAQ in melanocytic neoplasms, e.g., uveal melanomas.

Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire *et al.*) disclosed the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, *e.g.*, WO 99/53050, Waterhouse *et al.*; and WO 99/61631, Heifetz *et al.*), *Drosophila* (see, *e.g.*, Yang, D., *et al.*, *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer *et al.*).

Summary of the Invention

Disclosed herein are dsRNAs targeted to GNAQ for inhibiting expression of GNAQ in a cell. Also disclosed are methods of using the GNAQ dsRNA for siRNA inhibition of GNAQ expression and treatment of disease associated with expression and/or over expression of GNAQ, e.g., uveal melanoma.

Accordingly one aspect of the invention is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of a G-alpha q subunit (GNAQ) of a heterotrimeric G gene, having a sense strand and an antisense strand having a region of complementarity complementary to an mRNA encoding GNAQ, wherein each strand is at least 15 nucleotides in length. In one embodiment the dsRNA is AD-20057, e.g., sense strand is SEQ ID NO:1579 and the antisense strand is SEQ ID NO:1580. In another embodiment, the antisense strand is complementary to at least 15 contiguous nucleotides of SEQ ID NO:1421 or is complementary to at least the first 11 nucleotides of SEQ ID NO:1421. The sense strand can include 15 or more contiguous nucleotides of SEQ ID NO:1421 or SEQ ID NO:1579 and/or the antisense strand can include 15 or more contiguous nucleotides of SEQ ID NO:1422 or SEQ ID NO:1580. In some embodiments the sense strand nucleotide sequence includes SEQ ID NO:1421 and the antisense strand nucleotide sequence includes SEQ ID NO:1422.

In some embodiments the dsRNA of the invention results in the following: administration of 0.1 nM of the dsRNA to a A375 cell results in about 66% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of 1 nM of the dsRNA to a A375 cell results in about 61% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of 1nM of the dsRNA to a A579 cell results in about 82% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of 10nM of the dsRNA to a OMM1.3 cell results in about 42% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of the dsRNA to a UMEL202 cell results in about 81% inhibition of GNAQ mRNA expression as measured by a real time PCR assay.

In another embodiment, the dsRNA is AD-20051 and the sense strand is SEQ ID NO:1565 and the antisense strand is SEQ ID NO:1566. The dsRNA can be complementary to at least the first 11 nucleotides of SEQ ID NO:1407 and/or complementary to at least 15 contiguous nucleotides of SEQ ID NO:1407. In some embodiments the sense strand includes 15 or more contiguous nucleotides of SEQ ID NO: 1407 or SEQ ID NO:1565 and/or the antisense strand includes 15 or more contiguous nucleotides of SEQ ID NO:1408 or SEQ ID NO:1566 . The sense strand nucleotide sequence can include SEQ ID NO:1407 and the antisense strand nucleotide sequence can include SEQ ID NO:1408.

In some embodiments the dsRNA of the invention results in the following: administration of 0.1 nM of the dsRNA to a A375 cell results in about 49% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of 1nM of the dsRNA to a A375 cell results in about 55% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of 1nM of the dsRNA to a A579 cell results in about 83% inhibition of GNAQ mRNA expression as measured by a real time PCR assay or administration of 10nM of the dsRNA to a OMM1.3 cell results in about 42% inhibition of GNAQ mRNA expression as measured by a real time PCR assay.

In other embodiments the dsRNA is AD-20052 or AD-20069.

The antisense strand of the dsRNA is partially or completely complementary to an mRNA encoding a GNAQ, e.g., to a human GNAQ mRNA (e.g., NM_002072) or to a rat GNAQ mRNA (e.g., NM_031036). The region complementary is at least 15 nucleotides in length, e.g., between 19 and 21 nucleotides in length, e.g., 19 nucleotides in length. The region of complementarity can include at least 15 contiguous nucleotides of one of the antisense sequences listed in Tables 2a, 3a, or 4a. In other embodiments, the region of complementarity is one of the antisense sequences listed in Tables 2a, 3a, or 4a.

Additional exemplary dsRNA are provided in the tables herein. In some embodiments, the dsRNA of the invention includes a sense strand and antisense strand are selected from Tables 2b, 3b, 4b or Tables 2c, 3c, or 4c or Tables 2d, 3d, or 4d.

In one aspect, each strand of the dsRNA is no more than 30 nucleotides in length. At least one strand can include a 3' overhang of at least 1 nucleotide, e.g., 2 nucleotides, e.g., dTdT.

In some embodiments, the dsRNA is modified. For example, the dsRNA can include a modification that causes the dsRNA to have increased stability in a biological sample. In one embodiment, the dsRNA includes at least one modified nucleotide, e.g., a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, or a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. In other embodiments the modified nucleotide is a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide. The dsRNA of the invention can include at least one 2'-O-methyl modified nucleotide and at least one 2'-deoxythymidine-3'-phosphate nucleotide comprising a 5'-phosphorothioate group.

Any of the dsRNA of the invention can be modified according to a set of rules, e.g., the sense strand includes all 2'-O-methyl modified pyrimidines and the antisense strand comprises 2'-O-methyl modified pyrimidines when the pyrimidine is adjacent to A and each strand comprises dTdT at the 3' end or the sense strand comprises all 2'-O-methyl modified pyrimidines and the antisense strand comprises 2'-O-methyl modified pyrimidines when the pyrimidine is adjacent to A and each strand comprises dTsdT at the 3' end or the sense strand comprises all 2'-O-methyl modified pyrimidines and the antisense strand comprises 2'-O-methyl modified pyrimidines when a) the pyrimidine is adjacent to A or b) the pyrimidine is a uracil adjacent to a U or a G, and each strand comprises dTsdT at the 3' end.

In some embodiments the dsRNA include a ligand. The ligand can be conjugated to the 3'-end of the sense strand of the dsRNA.

Another aspect of the invention is a composition for inhibiting expression of a GNAQ gene including a dsRNA targeting GNAQ and a pharmaceutical formulation. In one embodiment, the pharmaceutical formulation is a lipid formulation. Exemplary formulations are described herein and include, for example, a LNP formulation, a LNP01 formulation, a XTC-SNALP formulation, a SNALP formulation, or a LNP11 formulation.

Also included herein is an isolated cell containing a dsRNA of the invention, a vector including the nucleotide sequence that encodes at least one strand of the dsRNA of the invention, and a cell including said vector.

A dsRNA of the invention, upon contact with a cell expressing said GNAQ, inhibits expression of said GNAQ gene by at least 40% compared to a cell not so contacted. In some embodiments, a dsRNA of the invention has a pM IC50, e.g., an IC50 of less than 10 pM.

Another aspect of the invention is method of inhibiting GNAQ expression in a cell, the method including introducing into the cell any of the dsRNA of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a GNAQ gene, thereby inhibiting expression of the GNAQ gene in the cell. In some embodiments, expression is inhibited by at least 20%, 40%, 60%, or at least 80%. Also included is a method of treating a disorder mediated by GNAQ expression by administering to a human in need of such treatment a therapeutically effective amount of any of the dsRNA of the invention. Examples of said disorders include uveal melanoma, cutaneous melanoma, Blue nevi, Nevi of Ota, a small lung tumor, or a neuroendocrine tumors. The method of treatment can include administering an addition composition, e.g., a second dsRNA.

Description of the Drawings

FIG. 1 is a graph showing IFN-alpha cytokine induction in human PBMCs following transfection with a set of GNAQ targeted dsRNA.

FIG. 2 shows TNF-alpha cytokine induction in human PBMCs following transfection with a set of GNAQ targeted dsRNA.

FIG. 3 shows cell viability of OMM1.3 and MEL285 cells following transfection with 1nM of dsRNAs. The Y-axis is viability normalized to control AD-1955.

FIG. 4 shows cell viability of MEL202 and MEL285 cells following transfection with 1nM of dsRNAs. The Y-axis is viability normalized to control AD-1955.

FIG. 5 shows cell viability of OMM1.3 and MEL285 cells following transfection with 0.01nM of dsRNAs. The Y-axis is viability normalized to control AD-1955.

FIG. 6 shows cell viability of MEL202 and MEL285 cells following transfection with 0.01nM of dsRNAs. The Y-axis is viability normalized to control AD-1955.

FIG. 7 shows day 7 cell viability of OMM1.3, MEL202, and MEL285 cells following transfection with AD-20057 and AD-20051 dsRNAs

FIG. 8 shows day 7 cell viability of OMM1.3, MEL202, and MEL285 cells following transfection with AD-20069 and AD-20093 dsRNAs.

Detailed Description of the Invention

The invention provides dsRNAs and methods of using the dsRNAs for inhibiting the expression of a G-alpha q subunit (GNAQ) of a heterotrimeric G gene in a cell or a mammal where the dsRNA targets a GNAQ gene. The invention also provides compositions and methods for treating pathological conditions and diseases, such as uveal melanoma in a mammal caused by the over-expression of a GNAQ gene. A dsRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi).

The dsRNAs of the compositions featured herein include an antisense strand having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is complementary to at least part of an mRNA transcript of a GNAQ gene. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in pathologies associated with GNAQ expression in mammals. Very low dosages of GNAQ dsRNAs in particular can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of a GNAQ gene. Using cell-based assays, the present inventors demonstrate that dsRNAs targeting GNAQ can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of a GNAQ gene. Thus, methods and compositions including these dsRNAs are useful for treating pathological processes that can be mediated by down regulating GNAQ over-expression, such as, e.g., treatment of uveal melanoma.

The following detailed description discloses how to make and use the compositions containing dsRNAs to inhibit the expression of a GNAQ gene, as well as compositions (e.g., pharmaceutical compositions) and methods for treating diseases and disorders caused by the expression of this gene.

Accordingly, in some aspects, pharmaceutical compositions containing a GNAQ dsRNA and a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of a GNAQ gene, and methods of using the pharmaceutical compositions to treat diseases caused by expression of a GNAQ gene are featured in the invention.

Definitions

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

"G," "C," "A" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. “T” and “dT” are used interchangeably herein and refer to a deoxyribonucleotide wherein the nucleobase is thymine, e.g., deoxyribothymine. However, it will be understood that the term “ribonucleotide” or “nucleotide” or “deoxyribonucleotide” can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

As used herein, “GNAQ” refers to a G-alpha q subunit (GNAQ) of a heterotrimeric G gene. GNAQ is also known as guanine nucleotide binding protein (G protein), q polypeptide and G-ALPHA-q, GAQ. The sequence of a human GNAQ mRNA transcript can be found at NM_002072.2. The sequence of rat GNAQ mRNA can be found at NM_031036.

A used herein “target” or “target gene” refers to a GNAQ gene.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a GNAQ gene, including mRNA that is a product of RNA processing of a primary transcription product.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but generally not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs includes, but not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (e.g., a target gene, e.g., an mRNA encoding GNAQ) including a 5' UTR, an open reading frame (ORF), or a 3' UTR. For example, a polynucleotide is complementary to at least a part of a GNAQ mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding GNAQ.

The term "double-stranded RNA" or "dsRNA," as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands. In general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include at least one non-ribonucleotide, e.g., a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, "dsRNA" may include chemical modifications to ribonucleotides, including substantial modifications at multiple nucleotides and including all types of modifications disclosed herein or known in the art. Any such modifications, as used in an siRNA type molecule, are encompassed by "dsRNA" for the purposes of this specification and claims

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs. The term "siRNA" is also used herein to refer to a dsRNA as described above.

As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule.

The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, e.g., within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand.

As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP represents a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as an iRNA agent or a plasmid from which an iRNA agent is transcribed. SNALP are described, e.g., in U.S. Patent Application Publication Nos. 20060240093, 20070135372, and USSN 61/045,228 filed on April 15, 2008. These applications are hereby incorporated by reference.

"Introducing into a cell," when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; a dsRNA may also be "introduced into a cell," wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, dsRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection.

The terms "silence," "inhibit the expression of," "down-regulate the expression of," "suppress the expression of" and the like, in as far as they refer to a target gene, herein refer to the at least partial suppression of the expression of a GNAQ gene, as manifested by a reduction of the amount of mRNA which may be isolated or detected from a first cell or group of cells in which a GNAQ gene is transcribed and which has or have been treated such that the expression of a GNAQ gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to GNAQ gene transcription, e.g., the amount of protein encoded by a GNAQ gene which is secreted by a cell, or the number of cells displaying a certain phenotype, e.g., apoptosis. In principle, GNAQ gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of a GNAQ gene by a certain degree and therefore is encompassed by the instant invention, the assays provided in the Examples below shall serve as such reference.

For example, in certain instances, expression of a GNAQ gene is suppressed by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of the double-stranded oligonucleotide featured in the invention. In some embodiments, a GNAQ gene is suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide featured in the invention. In some embodiments, a GNAQ gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide featured in the invention.

As used herein in the context of GNAQ expression, the terms "treat," "treatment," and the like, refer to relief from or alleviation of pathological processes mediated by GNAQ expression. In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes mediated by GNAQ expression), the terms "treat," "treatment," and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition, such as tumor reduction in uveal melanoma.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes mediated by GNAQ expression or an overt symptom of pathological processes mediated by GNAQ expression. The specific amount that is therapeutically effective can be readily determined by an ordinary medical practitioner, and may vary depending on factors known in the art, such as, for example, the type of pathological processes mediated by GNAQ expression, the patient's history and age, the stage of pathological processes mediated by GNAQ expression, and the administration of other anti-pathological processes mediated by GNAQ expression agents.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

Double-stranded ribonucleic acid (dsRNA)

As described in more detail herein, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a GNAQ gene in a cell or mammal, where the dsRNA includes a sense strand having a first sequence and an antisense strand comprising a second sequence complementary to mRNA encoding GNAQ, wherein said first sequence is complementary to said second sequence at a region of complementarity and wherein each strand is 15 to 30 base pairs in length. In some embodiments, the dsRNA of the invention inhibits the expression of said GNAQ gene by at least 40% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by Western blot. Expression of a GNAQ gene can be reduced by at least 30% when measured by an assay as described in the Examples below. For example, expression of a GNAQ gene in cell culture, such as in HepB3 cells, can be assayed by measuring GNAQ mRNA levels, such as by bDNA or TaqMan assay, or by measuring protein levels, such as by ELISA assay.

The dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. The dsRNA includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure.

One strand of the dsRNA (the antisense strand) includes a region of complementarity that is complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of a target gene, the other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. The region of complementarity is generally at least 15 nucleotides in length, or between 19 and 21 nucleotides in length, or 19, 20, or 21 nucleotides in length. In some embodiments the region of complementarity includes at least 15 contiguous nucleotides of one of the antisense sequences listed in Tables 2a, 3a, or 4a. In other embodiments the region of complementarity includes one of the antisense sequences listed in Tables 2a, 3a, or 4a.

Generally, the duplex structure is between 15 and 30, or between 25 and 30, or between 18 and 25, or between 19 and 24, or between 19 and 21, or 19, 20, or 21 base pairs in length. In one embodiment the duplex is 19 base pairs in length. In another embodiment the duplex is 21 base pairs in length. When two different dsRNAs are used in combination, the duplex lengths can be identical or can differ..

Each strand of the dsRNA of invention is generally between 15 and 30, or between 18 and 25, or 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In other embodiments, each is strand is 25-30 nucleotides in length. Each strand of the duplex can be the same length or of different lengths. When two different siRNAs are used in combination, the lengths of each strand of each siRNA can be identical or can differ.

The dsRNA of the invention can include one or more single-stranded overhang(s) of one or more nucleotides. In one embodiment, at least one end of the dsRNA has a single-stranded nucleotide overhang of 1 to 4, or 1, 2, 3, or 4 nucleotides. In another embodiment, the overhang include dTdT. In another embodiment, the antisense strand of the dsRNA has 1-10 nucleotides overhangs each at the 3' end and the 5' end over the sense strand. In further embodiments, the sense strand of the dsRNA has 1-10 nucleotides overhangs each at the 3' end and the 5' end over the antisense strand.

A dsRNAs having at least one nucleotide overhang can have unexpectedly superior inhibitory properties than the blunt-ended counterpart. In some embodiments the presence of only one nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its overall stability. A dsRNA having only one overhang has proven particularly stable and effective *in vivo*, as well as in a variety of cells, cell culture mediums, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3'-terminal end of the sense strand. The dsRNA can also have a blunt end, generally located at the 5'-end of the antisense strand. Such dsRNAs can have improved stability and inhibitory activity, thus allowing administration at low dosages, *i.e.*, less than 5 mg/kg body weight of the recipient per day. Generally, the antisense strand of the dsRNA has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In one embodiment, a GNAQ gene is a human GNAQ gene, e.g., the sequence identified by GenBank accession number NM_002072.2.

In specific embodiments, the sense strand of the dsRNA is one of the a sense sequences from Tables 2-4, and the antisense strand is one of the antisense sequences of Tables 2-4. Alternative antisense agents that target elsewhere in the target sequence provided in Tables 2-4 can readily be determined using the target sequence and the flanking GNAQ sequence.

The skilled person is well aware that dsRNAs having a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 2-4, the dsRNAs featured in the invention can include at least one strand of a length described therein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Tables 2-4 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, 21, or 22, or more contiguous nucleotides from one of the sequences of Tables 2-4, and differing in their ability to inhibit the expression of a GNAQ gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired GNAQ target sequence can readily be made using the corresponding GNAQ antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Tables 2-4 identify a site in a GNAQ that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Tables 2-4 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a GNAQ gene.

Additional dsRNA of the invention include those that cleave a target mRNA at the same location as a dsRNA described in any of the tables. In general, a RISC complex will cleave a target mRNA between the nucleotides complementary to nucleotides 10 and 11 of the antisense strand of a dsRNA, e.g., siRNA, of the invention. Cleavage e sites can be assayed using, e.g., a 5' RACE assay.

For example, the duplex AD-20057 includes the sense and antisense strands below. Treatment of a cell with this duplex results in cleavage of human GNAQ mRNA at the nucleotides complementary to nucleotides 10 and 11 of the antisense strand, e.g., nucleotides 1211 and 1212. Therefore, also included in the invention are those dsRNA that cleave at that location.

The dsRNA featured in the invention can contain one or more mismatches to the target sequence. In one embodiment, the dsRNA featured in the invention contains no more than 3 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5' or 3' end of the region of complementarity. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of a target gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of a target gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of a target gene is important, especially if the particular region of complementarity in a target gene is known to have polymorphic sequence variation within the population.

Modifications

In yet another embodiment, the dsRNA is chemically modified to enhance stability. The nucleic acids featured in the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Specific examples of dsRNA compounds useful in this invention include dsRNAs containing modified backbones or no natural internucleoside linkages. As defined in this specification, dsRNAs having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified dsRNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Modified dsRNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference

Modified dsRNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or ore or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones;

methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, each of which is herein incorporated by reference.

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

Other embodiments of the invention are dsRNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH2--NH--CH2--, --CH2--N(CH3)--O--CH2--[known as a methylene (methylimino) or MMI backbone], --CH2--O--N(CH3)--CH2--, --CH2--N(CH3)--N(CH3)--CH2-- and --N(CH3)--CH2--CH2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH2--] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. Also preferred are dsRNAs having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified dsRNAs may also contain one or more substituted sugar moieties. Preferred dsRNAs comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Particularly preferred are O[(CH2)nO]mCH3, O(CH2)nOCH3, O(CH2)nNH2, O(CH2)nCH3, O(CH2)nONH2, and O(CH2)nON[(CH2)nCH3)]2, where n and m are from 1 to about 10. Other preferred dsRNAs comprise one of the following at the 2' position: C1 to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH3, OCN, Cl, Br, CN, CF3, OCF3, SOCH3, SO2CH3, ONO2, NO2, N3, NH2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an dsRNA, or a group for improving the pharmacodynamic properties of an dsRNA, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--CH2CH2OCH3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a O(CH2)2ON(CH3)2 group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O--CH2--O--CH2--N(CH2)2, also described in examples herein below.

Other preferred modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the dsRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. DsRNAs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

A dsRNA may also include nucleobase (often referred to in the art simply as “base”) modifications or substitutions. As used herein, “unmodified” or “natural” nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-

azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, DsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., DsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, also herein incorporated by reference.

Conjugates

Another modification of the dsRNAs featured in the invention involves chemically linking to the dsRNA one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the dsRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acid. Sci. USA, 1989, 86: 6553-6556), cholic acid (Manoharan et al., Biorg. Med. Chem. Let., 1994, 4:1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306-309; Manoharan et al., Biorg. Med. Chem. Let., 1993, 3:2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J, 1991, 10:1111-1118; Kabanov et al., FEBS Lett., 1990, 259:327-330; Svinarchuk et al., Biochimie, 1993, 75:49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651-3654; Shea et al., Nucl. Acids Res., 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides &

Nucleotides, 1995, 14:969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923-937).

Representative U.S. patents that teach the preparation of such dsRNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within a dsRNA. The present invention also includes dsRNA compounds which are chimeric compounds. “Chimeric” dsRNA compounds or “chimeras,” in the context of this invention, are dsRNA compounds, particularly dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of a dsRNA compound. These dsRNAs typically contain at least one region wherein the dsRNA is modified so as to confer upon the dsRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the dsRNA may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of dsRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter dsRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxydsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the dsRNA may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to dsRNAs in order to enhance the activity, cellular distribution or cellular uptake of the dsRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al., FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such dsRNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of dsRNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the dsRNA still bound to the solid support or following cleavage of the dsRNA in solution phase. Purification of the dsRNA conjugate by HPLC typically affords the pure conjugate.

<u>Vector encoded dsRNAs</u>

In another aspect, dsRNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), **12**:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) **92**:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

The recombinant dsRNA expression vectors are generally DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, *et al.*, *Curr. Topics Micro. Immunol.* (1992) 158:97-129)); adenovirus (see, for example, Berkner, *et al.*, BioTechniques (1998) 6:616), Rosenfeld *et al.* (1991, Science 252:431-434), and Rosenfeld *et al.* (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, *e.g.*, Eglitis, *et al.*, *Science* (1985) 230:1395-1398; Danos and Mulligan, *Proc. Natl. Acad. Sci. USA* (1998) 85:6460-6464; Wilson *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury *et al.*, 1991, Science 254:1802-1805; van Beusechem. *et al.*, 1992, Proc. Nad. Acad. Sci. USA 89:7640-19 ; Kay *et al.*, 1992, Human Gene Therapy 3:641-647; Dai *et al.*, 1992, Proc. Natl.Acad. Sci. USA 89:10892-10895; Hwu *et al.*, 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette *et al.*, 1991, Human Gene Therapy 2:5-10; Cone *et al.*, 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (*e.g.*, rat, hamster, dog, and chimpanzee) (Hsu *et al.*, 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

Any viral vector capable of accepting the coding sequences for the dsRNA molecule(s) to be expressed can be used, for example vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (e.g., lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

For example, lentiviral vectors featured in the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors featured in the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, e.g., Rabinowitz J E et al. (2002), J Virol 76:791-801, the entire disclosure of which is herein incorporated by reference.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the dsRNA into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A et al., Nat. Genet. 33: 401-406, the entire disclosures of which are herein incorporated by reference.

Viral vectors can be derived from AV and AAV. In one embodiment, the dsRNA featured in the invention is expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter.

A suitable AV vector for expressing the dsRNA featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), J. Virol. 61: 3096-3101; Fisher K J *et al.* (1996), J. Virol, 70: 520-532; Samulski R *et al.* (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

The promoter driving dsRNA expression in either a DNA plasmid or viral vector featured in the invention may be a eukaryotic RNA polymerase I (*e.g.,* ribosomal RNA promoter), RNA polymerase II (*e.g.,* CMV early promoter or actin promoter or U1 snRNA promoter) or generally RNA polymerase III promoter (*e.g.,* U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, *e.g.*, the insulin regulatory sequence for pancreas (Bucchini *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 -thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Generally, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (*e.g.*, Oligofectamine) or non-cationic lipid-based carriers (*e.g.*, Transit-TKO™). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single target gene or multiple target genes over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

Target gene specific dsRNA molecules can also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Pharmaceutical compositions containing dsRNA

In one embodiment, the invention provides pharmaceutical compositions containing a dsRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition containing the dsRNA is useful for treating a disease or disorder associated with the expression or activity of a GNAQ gene, such as pathological processes mediated by GNAQ expression. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV) delivery. Another example is compositions that are formulated for direct delivery into the brain parenchyma, *e.g.*, by infusion into the brain, such as by continuous pump infusion.

The pharmaceutical compositions featured herein are administered in dosages sufficient to inhibit expression of GNAQ genes. In general, a suitable dose of dsRNA will be in the range of 0.01 to 200.0 milligrams siRNA per kilogram body weight of the recipient per day, generally in the range of 1 to 50 mg per kilogram body weight per day. For example, the dsRNA can be administered at 0.0059 mg/kg, 0.01 mg/kg, 0.0295 mg/kg, 0.05 mg/kg, 0.0590 mg/kg, 0.163 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.543 mg/kg, 0.5900 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.628 mg/kg, 2 mg/kg, 3 mg/kg, 5.0 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, or 50 mg/kg per single dose.

In one embodiment, the dosage is between 0.01 and 0.2 mg/kg. For example, the dsRNA can be administered at a dose of 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg 0.08 mg/kg 0.09 mg/kg , 0.10 mg/kg, 0.11 mg/kg, 0.12 mg/kg, 0.13 mg/kg, 0.14 mg/kg, 0.15 mg/kg, 0.16 mg/kg, 0.17 mg/kg, 0.18 mg/kg, 0.19 mg/kg, or 0.20 mg/kg.

In one embodiment, the dosage is between 0.005 mg/kg and 1.628 mg/kg. For example, the dsRNA can be administered at a dose of 0.0059 mg/kg, 0.0295 mg/kg, 0.0590 mg/kg, 0.163 mg/kg, 0.543 mg/kg, 0.5900 mg/kg, or 1.628 mg/kg.

In one embodiment, the dosage is between 0.2 mg/kg and 1.5 mg/kg. For example, the dsRNA can be administered at a dose of 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, or 1.5 mg/kg.

The dsRNA can be administered at a dose of 0.03 mg/kg.

The pharmaceutical composition may be administered once daily, or the dsRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The effect of a single dose on GNAQ levels is long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals, or at not more than 5, 6, 7, 8, 9, or 10 week intervals.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes mediated by GNAQ expression. Such models are used for *in vivo* testing of dsRNA, as well as for determining a therapeutically effective dose. A suitable mouse model is, for example, a mouse containing a plasmid expressing human GNAQ. Another suitable mouse model is a transgenic mouse carrying a transgene that expresses human GNAQ.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays.

A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The dsRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by target gene expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Administration

The present invention also includes pharmaceutical compositions and formulations which include the dsRNA compounds featured in the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.*, intraparenchymal, intrathecal or intraventricular, administration.

The dsRNA can be delivered in a manner to target a particular tissue, such as the liver (*e.g.*, the hepatocytes of the liver).

The present invention includes pharmaceutical compositions that can be delivered by injection directly into the brain. The injection can be by stereotactic injection into a particular region of the brain (*e.g.*, the substantia nigra, cortex, hippocampus, striatum, or globus pallidus), or the dsRNA can be delivered into multiple regions of the central nervous system (*e.g.*, into multiple regions of the brain, and/or into the spinal cord). The dsRNA can also be delivered into diffuse regions of the brain (*e.g.*, diffuse delivery to the cortex of the brain).

In one embodiment, a dsRNA targeting GNAQ can be delivered by way of a cannula or other delivery device having one end implanted in a tissue, *e.g.*, the brain, *e.g.*, the substantia nigra, cortex, hippocampus, striatum, corpus callosum or globus pallidus of the brain. The cannula can be connected to a reservoir of the dsRNA composition. The flow or delivery can be mediated by a pump, *e.g.*, an osmotic pump or minipump, such as an Alzet pump (Durect, Cupertino, CA). In one embodiment, a pump and reservoir are implanted in an area distant from the tissue, *e.g.*, in the abdomen, and delivery is effected by a conduit leading from the pump or reservoir to the site of release. Infusion of the dsRNA composition into the brain can be over several hours or for several days, *e.g.*, for 1, 2, 3, 5, or 7 days or more. Devices for delivery to the brain are described, for example, in U.S. Patent Nos. 6,093,180, and 5,814,014.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful. Suitable topical formulations include those in which the dsRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). DsRNAs featured in the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, dsRNAs may be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-10} alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

Liposomal formulations

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

Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall.

Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*.

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

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

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

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

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

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

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

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

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

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

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, FEBS Letters, 1987, 223, 42; Wu *et al.*, Cancer Research, 1993, 53, 3765).

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

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto *et al.* (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{1215G}$, that contains a PEG moiety. Illum *et al.* (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (*e.g.*, PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov *et al.* (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume *et al.* (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle *et al.* (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin *et al.* (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky *et al.*) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi *et al*). U.S. Pat. No. 5,540,935 (Miyazaki *et al.*) and U.S. Pat. No. 5,556,948 (Tagawa *et al.*) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry *et al.* discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa *et al.* discloses protein-bonded liposomes and asserts that the contents of such liposomes may include a dsRNA. U.S. Pat. No. 5,665,710 to Rahman *et al.* describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love *et al.* discloses liposomes comprising dsRNAs targeted to the raf gene.

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

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

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

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

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

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

Nucleic acid lipid particles

In one embodiment, a GNAQ dsRNA featured in the invention is fully encapsulated in the lipid formulation, e.g., to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). SNALPs and SPLPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. In some embodiments the lipid to dsRNA ratio can be about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, or 11:1.

The cationic lipid may be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid may comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

The non-cationic lipid may be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid may be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles may be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl ($C1_6$), or a PEG- distearyloxypropyl (C_{18}). Other examples of PEG conjugates include PEG-cDMA (N-[(methoxy poly(ethylene glycol)2000)carbamyl]-1,2-dimyristyloxlpropyl-3-amine), mPEG2000-DMG (mPEG-dimyrystylglycerol (with an average molecular weight of 2,000) and PEG-C-DOMG (R-3-[(ω-methoxy-poly(ethylene glycol)2000)carbamoyl)]-1,2-dimyristyloxlpropyl-3-amine). The conjugated lipid that prevents aggregation of particles may be from 0 mol % to about 20 mol % or about 1.0, 1.1., 1.2, .13, 1.4, 1.5, 1.6,1.7, 1.8, 1.9, or 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

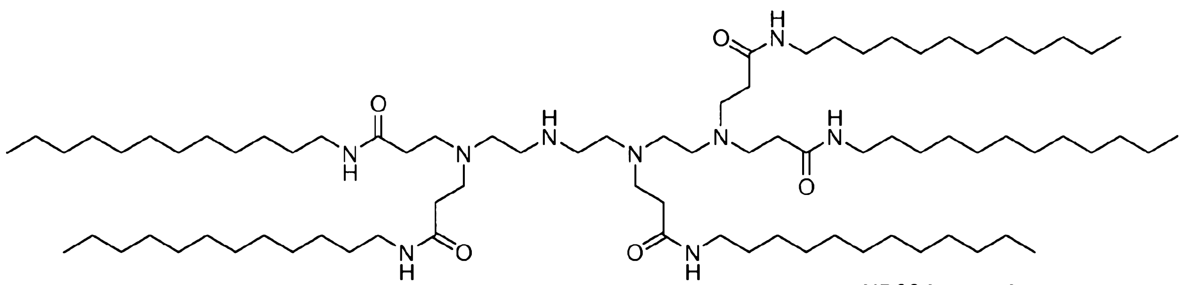
In one embodiment, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

For example, the lipid-siRNA particle can include 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

In still another embodiment, the compound 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1) can be used to prepare lipid-siRNA particles. For example, the dsRNA can be formulated in a lipid formulation comprising Tech-G1, distearoyl phosphatidylcholine (DSPC), cholesterol and mPEG2000-DMG at a molar ratio of 50:10:38.5:1.5 at a total lipid to siRNA ratio of 7:1 (wt:wt).

LNP01

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (Formula 1), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-siRNA nanoparticles (*i.e.,* LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, *e.g.*, 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous siRNA (*e.g.*, in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-siRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (*e.g.*, 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, *e.g.*, about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.



Formula 1

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-siRNA formulations are as follows:

	Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio	Process
SNALP	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1	
SNALP-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1	

LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1	Extrusion
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1	Extrusion
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1	In-line mixing
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1	In-line mixing
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing

LNP09 formulations and XTC comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, which is hereby incorporated by reference.

LNP11 formulations and MC3 comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009, which is hereby incorporated by reference.

LNP12 formulations and TechG1 comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009, which is hereby incorporated by reference.

Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be about 20-300 nm, such as 40-100 nm in size. The particle size distribution should be unimodal. The total siRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated siRNA can be incubated with an RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, *e.g.*, 0.5% Triton-X100. The total siRNA in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" siRNA content (as measured by the signal in the absence of surfactant) from the total siRNA content. Percent entrapped siRNA is typically >85%. For SNALP formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, and at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

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

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

Emulsions

The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

In one embodiment of the present invention, the compositions of dsRNAs and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215).

Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385; Ho *et al.*, J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or dsRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of dsRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of dsRNAs and nucleic acids.

Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the dsRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

Penetration Enhancers

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

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

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

Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C.sub.1-10 alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri *et al.*, J. Pharm. Pharmacol., 1992, 44, 651-654).

Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita *et al.*, J. Pharm. Sci., 1990, 79, 579-583).

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

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

Carriers

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

Excipients

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

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

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

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

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

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

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more dsRNA compounds and (b) one or more anti-cytokine biologic agents which function by a non-RNAi mechanism. Examples of such biologics include, biologics that target IL1β (*e.g.*, anakinra), IL6 (tocilizumab), or TNF (etanercept, infliximab, adlimumab, or certolizumab).

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the dsRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by GNAQ expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Methods for treating diseases caused by expression of a GNAQ gene

The invention relates in particular to the use of a dsRNA targeting GNAQ and compositions containing at least one such dsRNA for the treatment of a GNAQ-mediated disorder or disease. For example, a dsRNA targeting a GNAQ gene can be useful for the treatment of cancers that have either an activating mutation of GNAQ and/or are the result of overexpression of GNAQ. Tumors to be targeted include uveal melanoma, cutaneous melanoma, Blue nevi, Nevi of Ota, and neuroendocrine tumors (including but not limited to carcinoid tumors, large cell lung cancer, and small cell lung cancer).

A dsRNA targeting a GNAQ gene is also used for treatment of symptoms of disorders, such as uveal melanoma. Symptoms associated include, *e.g.*, melanoma progression, increasing eye pressure, pain in the eye, and impaired peripheral vision.

Owing to the inhibitory effects on GNAQ expression, a composition according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

The invention further relates to the use of a dsRNA or a pharmaceutical composition thereof, *e.g.*, for treating a GNAQ mediated disorder or disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. In one example, a dsRNA targeting GNAQ can be administered in combination with radiation therapy. In other examples, a dsRNA targeting GNAQ can be administered in combination with a pharmaceutical or therapeutic method for treating a symptom of a GNAQ disease, such as pain medication.

The dsRNA and an additional therapeutic agent can be administered in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or by another method described herein.

The invention features a method of administering a dsRNA targeting GNAQ to a patient having a disease or disorder mediated by GNAQ expression, such as a uveal melanoma. Administration of the dsRNA can stabilize and improve vision, for example, in a patient with uveal melanoma. Patients can be administered a therapeutic amount of dsRNA, such as 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, or 2.5 mg/kg dsRNA. The dsRNA can be administered by intravenous infusion over a period of time, such as over a 5 minute, 10 minute, 15 minute, 20 minute, or 25 minute period. The administration is repeated, for example, on a regular basis, such as biweekly (i.e., every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration biweekly for three months, administration can be repeated once per month, for six months or a year or longer. Administration of the dsRNA can reduce GNAQ levels in the blood or urine of the patient by at least 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80 % or 90% or more.

Before administration of a full dose of the dsRNA, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction.

Many GNAQ-associated diseases and disorders are hereditary. Therefore, a patient in need of a GNAQ dsRNA can be identified by taking a family history. A healthcare provider, such as a doctor, nurse, or family member, can take a family history before prescribing or administering a GNAQ dsRNA. A DNA test may also be performed on the patient to identify a mutation in the GNAQ gene, before a GNAQ dsRNA is administered to the patient.

Methods for inhibiting expression of a GNAQ gene

In yet another aspect, the invention provides a method for inhibiting the expression of a GNAQ gene in a mammal. The method includes administering a composition featured in the invention to the mammal such that expression of the target GNAQ gene is reduced or silenced.

When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the dsRNAs and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Other embodiments are, for example, in the claims.

The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A.L. Lehninger, Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg Advanced Organic Chemistry 3rd Ed. (Plenum Press) Vols A and B(1992).

Example 1. dsRNA synthesis

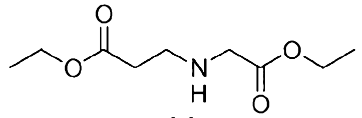
Source of reagents

Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Conjugates

For the synthesis of 3'-cholesterol-conjugated siRNAs (herein referred to as -Chol-3'), an appropriately modified solid support is used for RNA synthesis. The modified solid support is prepared as follows:

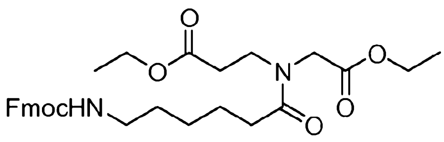
Diethyl-2-azabutane-1,4-dicarboxylate **AA**



AA

A 4.7 M aqueous solution of sodium hydroxide (50 mL) is added into a stirred, ice-cooled solution of ethyl glycinate hydrochloride (32.19 g, 0.23 mole) in water (50 mL). Then, ethyl acrylate (23.1 g, 0.23 mole) isis added and the mixture is stirred at room temperature until completion of the reaction is ascertained by TLC. After 19 h the solution is partitioned with dichloromethane (3 x 100 mL). The organic layer is dried with anhydrous sodium sulfate, filtered and evaporated. The residue is distilled to afford AA (28.8 g, 61%).

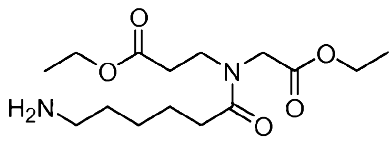
3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester **AB**



AB

Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) is dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimde (3.25 g, 3.99 mL, 25.83 mmol) is added to the solution at 0°C. It is then followed by the addition of Diethyl-azabutane-1,4-dicarboxylate (5 g, 24.6 mmol) and dimethylamino pyridine (0.305 g, 2.5 mmol). The solution is brought to room temperature and stirred further for 6 h. Completion of the reaction is ascertained by TLC. The reaction mixture is concentrated under vacuum and ethyl acetate is added to precipitate diisopropyl urea. The suspension is filtered. The filtrate is washed with 5% aqueous hydrochloric acid, 5% sodium bicarbonate and water. The combined organic layer is dried over sodium sulfate and concentrated to give the crude product which is purified by column chromatography (50 % EtOAC/Hexanes) to yield 11.87 g (88%) of AB.

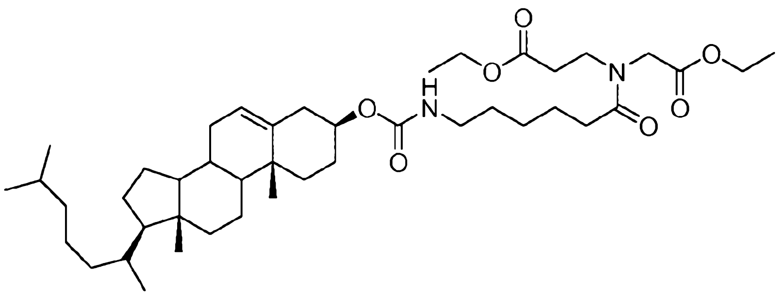
3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester **AC**



AC

3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoyl]-amino}-propionic acid ethyl ester AB (11.5 g, 21.3 mmol) is dissolved in 20% piperidine in dimethylformamide at 0°C. The solution is continued stirring for 1 h. The reaction mixture is concentrated under vacuum, water is added to the residue, and the product is extracted with ethyl acetate. The crude product is purified by conversion into its hydrochloride salt.

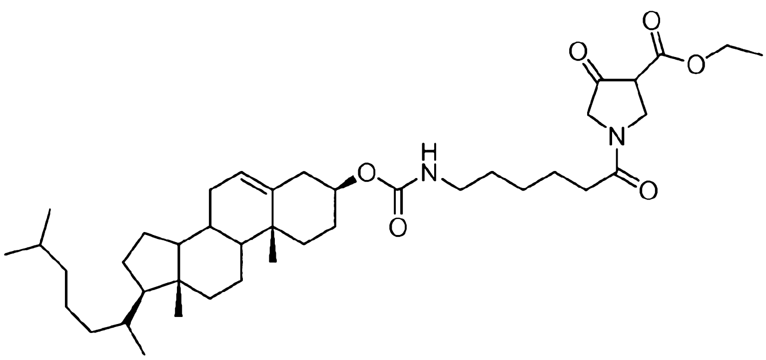
3-({6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}ethoxycarbonylmethyl-amino)-propionic acid ethyl ester **AD**



AD

The hydrochloride salt of 3-[(6-Amino-hexanoyl)-ethoxycarbonylmcthyl-amino]-propionic acid ethyl estcr AC (4.7 g, 14.8 mmol) is taken up in dichloromcthanc. The suspcnsion is cooled to 0°C on icc. To the suspcnsion diisopropylethylamine (3.87 g, 5.2 mL, 30 mmol) is added. To the resulting solution cholesteryl chloroformate (6.675 g, 14.8 mmol) is added. The reaction mixture is stirred overnight. The reaction mixture is diluted with dichloromethane and washed with 10% hydrochloric acid. The product is purified by flash chromatography (10.3 g, 92%).

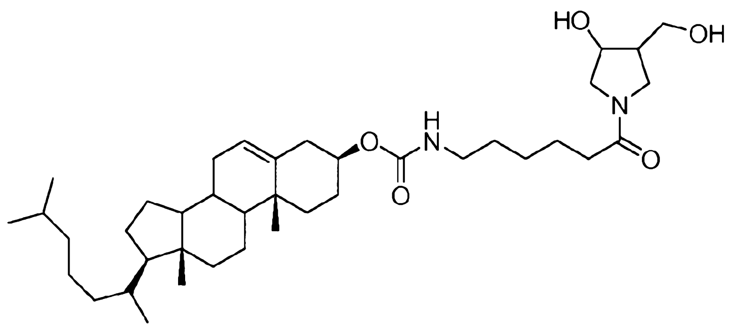
1-{6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-yloxycarbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**



AE

Potassium t-butoxide (1.1 g, 9.8 mmol) is slurried in 30 mL of dry toluene. The mixture is cooled to 0°C on ice and 5 g (6.6 mmol) of diester AD is added slowly with stirring within 20 mins. The temperature is kept below 5°C during the addition. The stirring is continued for 30 mins at 0°C and 1 mL of glacial acetic acid is added, immediately followed by 4 g of $NaH_2PO_4 \cdot H_2O$ in 40 mL of water The resultant mixture is extracted twice with 100 mL of dichloromethane each and the combined organic extracts are washed twice with 10 mL of phosphate buffer each, dried, and evaporated to dryness. The residue is dissolved in 60 mL of toluene, cooled to 0°C and extracted with three 50 mL portions of cold pH 9.5 carbonate buffer. The aqueous extracts are adjusted to pH 3 with phosphoric acid, and extracted with five 40 mL portions of chloroform which are combined, dried and evaporated to dryness. The residue is purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

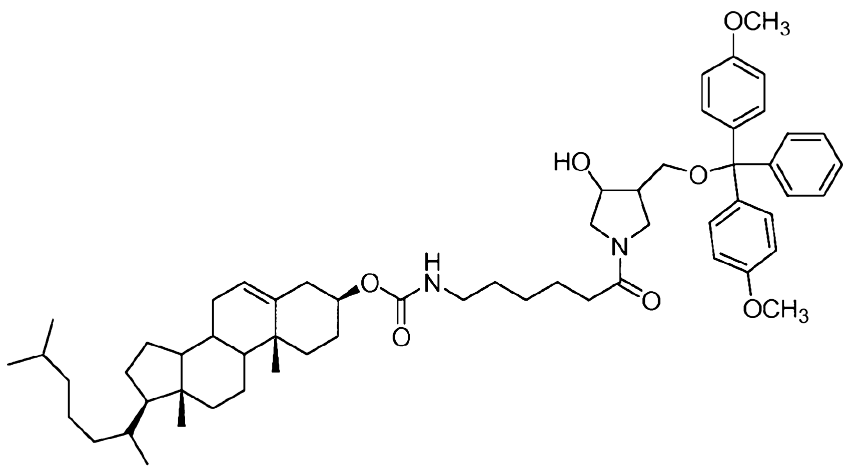
[6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**



AF

Methanol (2 mL) is added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring is continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.5 mL) is added, the mixture is extracted with ethylacetate (3 x 40 mL). The combined ethylacetate layer is dried over anhydrous sodium sulfate and concentrated under vacuum to yield the product which is purified by column chromatography (10% MeOH/$CHCl_3$) (89%).

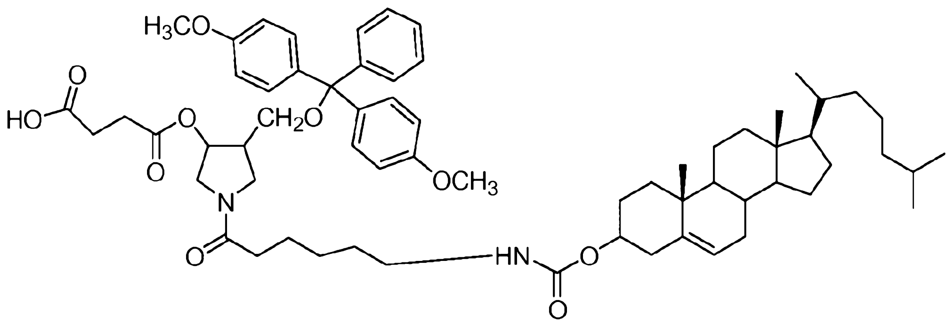
(6-{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-hydroxy-pyrrolidin-1-yl}-6-oxo-hexyl)-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AG**



AG

Diol AF (1.25 gm 1.994 mmol) is dried by evaporating with pyridine (2 x 5 mL) *in vacuo*. Anhydrous pyridine (10 mL) and 4,4'-dimethoxytritylchloride (0.724 g, 2.13 mmol) are added with stirring. The reaction is carried out at room temperature overnight. The reaction is quenched by the addition of methanol. The reaction mixture is concentrated under vacuum and to the residue dichloromethane (50 mL) is added. The organic layer is washed with 1M aqueous sodium bicarbonate. The organic layer is dried over anhydrous sodium sulfate, filtered and concentrated. The residual pyridine is removed by evaporating with toluene. The crude product is purified by column chromatography (2% MeOH/Chloroform, Rf = 0.5 in 5% MeOH/$CHCl_3$) (1.75 g, 95%).

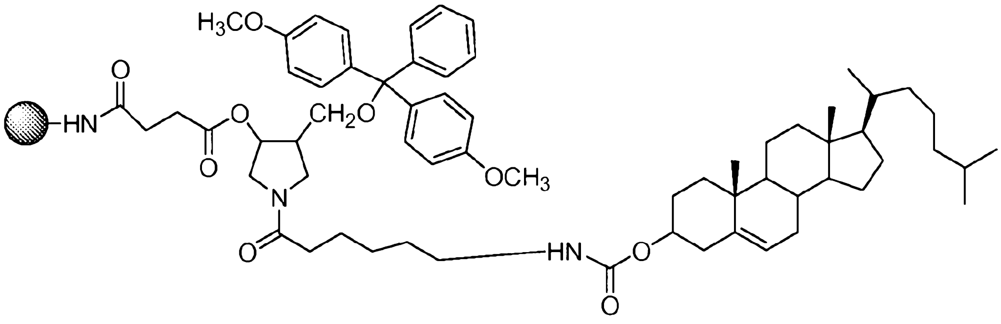
Succinic acid mono-(4-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-1-{6-[17-(1,5-dimethyl-hexyl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}-pyrrolidin-3-yl) ester **AH**



AH

Compound AG (1.0 g, 1.05 mmol) is mixed with succinic anhydride (0.150 g, 1.5 mmol) and DMAP (0.073 g, 0.6 mmol) and dried in a vacuum at 40°C overnight. The mixture is dissolved in anhydrous dichloroethane (3 mL), triethylamine (0.318 g, 0.440 mL, 3.15 mmol) is added and the solution is stirred at room temperature under argon atmosphere for 16 h. It is then diluted with dichloromethane (40 mL) and washed with ice cold aqueous citric acid (5 wt%, 30 mL) and water (2 X 20 mL). The organic phase is dried over anhydrous sodium sulfate and concentrated to dryness. The residue is used as such for the next step.

Cholesterol derivatised CPG **AI**



AI

Succinate AH (0.254 g, 0.242 mmol) is dissolved in a mixture of dichloromethane/acetonitrile (3:2, 3 mL). To that solution DMAP (0.0296 g, 0.242 mmol) in acetonitrile (1.25 mL), 2,2'-Dithio-bis(5-nitropyridine) (0.075 g, 0.242 mmol) in acetonitrile/dichloroethane (3:1, 1.25 mL) are added successively. To the resulting solution triphenylphosphine (0.064 g, 0.242 mmol) in acetonitrile (0.6 ml) is added. The reaction mixture turned bright orange in color. The solution is agitated briefly using a wrist-action shaker (5 mins). Long chain alkyl amine-CPG (LCAA-CPG) (1.5 g, 61 mM) is added. The suspension is agitated for 2 h. The CPG is filtered through a sintered funnel and washed with acetonitrile, dichloromethane and ether successively. Unreacted amino groups are masked using acetic anhydride/pyridine. The achieved loading of the CPG is measured by taking UV measurement (37 mM/g).

The synthesis of siRNAs bearing a 5'-12-dodecanoic acid bisdecylamide group (herein referred to as "5'-C32-") or a 5'-cholesteryl derivative group (herein referred to as "5'-Chol-") is performed as described in WO 2004/065601, except that, for the cholesteryl derivative, the oxidation step is performed using the Beaucage reagent in order to introduce a phosphorothioate linkage at the 5'-end of the nucleic acid oligomer.

Nucleic acid sequences are represented herein using standard nomenclature, and specifically the abbreviations of Table 1.

Table 1: Abbreviations of nucleoside monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleoside(s)
A	adenosine
C	cytidine
G	guanosine
U	uridine
N	any nucleotide (G, A, C, U, or dT)

Abbreviation	Nucleoside(s)
a	2'-O-methyladenosine
c	2'-O-methylcytidine
g	2'-O-methylguanosine
u	2'-O-methyluridine
dT	2'-deoxythymidine
s	a phosphorothioate linkage

Example 2. siRNA Design and Synthesis

Transcripts

siRNA design was carried out to identify siRNAs targeting the G-alpha q subunit (GNAQ) of a heterotrimeric G gene. Three sets were designed, each specific for a different set of cross species: 1: human and monkey; 2) human, monkey and mouse; and 3) mouse and rat. GNAQ sequences were obtained from the NCBI Refseq collection on November 24, 2008 as follows:

Species	GNAQ sequence ref
human	NM_002072.2
rat	NM_031036.1
monkey	AB170509.1
mouse	NM_008139.5

siRNA Design and Specificity Prediction

The predicted specificity of all possible 19mers was determined for each sequence. The GNAQ siRNAs were used in a comprehensive search against the human, cynomolgous monkey, mouse and rat transcriptomes (defined as the set of NM_ and XM_ records within the NCBI Refseq set for human, mouse and rat, and the 'core' sequences from the Unigene clusters for Macaca fascicularis) using the FASTA algorithm. The Python script 'offtargetFasta.py' was then used to parse the alignments and generate a score based on the position and number of mismatches between the siRNA and any potential 'off-target' transcript. The off-target score is weighted to emphasize differences in the 'seed' region of siRNAs, in positions 2-9 from the 5' end of the molecule. The off-target score is calculated as follows: mismatches between the oligo and the transcript are given penalties. A mismatch in the seed region in positions 2-9 of the oligo is given a penalty of 2.8; mismatches in the putative cleavage sites 10 and 11 are given a penalty of 1.2, and all other mismatches a penalty of 1. The off-target score for each oligo-transcript pair is then calculated by summing the mismatch penalties. The lowest off-target score from all the oligo-transcript pairs is then determined and used in subsequent sorting of oligos. Both siRNA strands were assigned to a category of specificity according to the calculated scores:

a score above 3 qualifies as highly specific, equal to 3 as specific, and between 2.2 and 2.8 as moderately specific. In picking which oligos to synthesize, off-target score of the antisense strand was sorted from high to low.

Synthesis of dsRNA

The sense and antisense strands of the dsRNA duplexes were synthesized on a MerMade 192 synthesizer at 1µmol scale. For each sense and antisense sequence listed in Tables 2a, 3a, and 4a, sequence were modified as follows and as listed in Tables 2d, 3d, and 4d:

1. In the sense strand, all pyrimidines (U, C) were replaced with corresponding 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U); in the antisense strand, all pyrimidines (U, C) adjacent to A (UA, CA) were replaced with corresponding 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U); a 2 base dTdT extension at the 3' end of both strands was introduced.
2. In the sense strand, all pyrimidines (U, C) are replaced with corresponding 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U); in the antisense strand, all pyrimidines (U, C) adjacent to A (UA, CA) are replaced with corresponding 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U); a 2 base dTsdT (including a phosphorothioate) extension at the 3' end of both strands was introduced.
3. In the sense strand, all pyrimidines (U, C) are replaced with corresponding 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U); in the antisense strand, all pyrimidines (U, C) adjacent to A (UA, CA) and all U adjacent to another U (UU) or G (UG) were replaced with corresponding 2'-O-Methyl bases (2' O-Methyl C and 2'-O-Methyl U); a 2 base dTsdT (including a phosphorothioate) extension at the 3' end of both strands was introduced.

The synthesis of each strand of the dsRNA used solid supported oligonucleotide synthesis using phosphoramidite chemistry.

Synthesis was performed at 1umole scale in 96 well plates. The amidite solutions were prepared at 0.1M concentration and ethyl thio tetrazole (0.6M in Acetonitrile) was used as an activator. The synthesized sequences were cleaved and deprotected in 96 well plates, using methylamine in the first step and triethylamine.3HF in the second step. The crude sequences thus obtained were precipitated using acetone: ethanol mix and the pellet were re-suspended in 0.5M sodium acetate buffer. Samples from each sequence were analyzed by LC-MS and the resulting mass data confirmed the identity of the sequences. A selected set of samples were also analyzed by IEX chromatography.

All sequences were purified on AKTA explorer purification system using Source 15Q column. A single peak corresponding to the full length sequence was collected in the eluent and was subsequently analyzed for purity by ion exchange chromatography.

The purified sequences were desalted on a Sephadex G25 column using AKTA purifier. The desalted sequences were analyzed for concentration and purity. For the preparation of duplexes, equimolar amounts of sense and antisense strand were heated in the required buffer (e.g. 1xPBS) at 95°C for 2-5 minutes and slowly cooled to room temperature. Integrity of the duplex was confirmed by HPLC analysis.

<u>Synthesis and Duplex Annealing for *in vivo* studies</u>

Step 1. Oligonucleotide Synthesis

Oligonucleotides for *in vivo* studies were synthesized on an AKTAoligopilot synthesizer or on an ABI 394 DNA/RNA synthesizer. Commercially available controlled pore glass solid support (dT-CPG, 500Å, Prime Synthesis) or the in-house synthesized solid support cholesterol-CPG, **<u>AI</u>** were used for the synthesis. Other ligand conjugated solid supports amenable to the invention are described in US patent application number 10/946,873 filed September 21, 2004, which is hereby incorporated by reference for all purposes. RNA phosphoramidites and 2'-*O*-methyl modified RNA phosphoramidites with standard protecting groups (5'-*O*-dimethoxytrityl-N6-benzoyl-2'-*t*-butyldimethylsilyl-adenosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-*t*-butyldimethylsilyl-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N2-isobutryl-2'-*t*-butyldimethylsilyl-guanosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-2'-*t*-butyldimethylsilyl-uridine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N6-benzoyl-2'-*O*-methyl-adenosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-*O*-methyl-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N2-isobutryl-2'-*O*-methyl-guanosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-2'-*O*-methyl-uridine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite and 5'-*O*-dimethoxytrityl-2'-deoxy-thymidine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite) were obtained commercially (e.g. from Pierce Nucleic Acids Technologies and ChemGenes Research).

For the syntheses on AKTAoligopilot synthesizer, all phosphoramidites were used at a concentration of 0.2 M in CH_3CN except for guanosine and 2'-O-methyl-uridine, which were used at 0.2 M concentration in 10% THF/CH_3CN (v/v). Coupling/recycling time of 16 minutes was used for all phosphoramidite couplings. The activator was 5-ethyl-thio-tetrazole (0.75 M, American International Chemicals). For the PO-oxidation, 50 mM iodine in water/pyridine (10:90 v/v) was used and for the PS-oxidation 2% PADS (GL Synthesis) in 2,6-lutidine/CH_3CN (1:1 v/v) was used. For the syntheses on ABI 394 DNA/RNA synthesizer, all phosphoramidites were used at a concentration of 0.15 M in CH_3CN except for 2'-O-methyl-uridine, which was used at 0.15 M concentration in 10% THF/CH_3CN (v/v). Coupling time of 10 minutes was used for all phosphoramidite couplings. The activator was 5-ethyl-thio-tetrazole (0.25 M, Glen Research). For the PO-oxidation, 20 mM iodine in water/pyridine (Glen Research) was used and for the PS-oxidation 0.1M DDTT (AM Chemicals) in pyridine was used.

Step 2. Deprotection of oligonucleotides

After completion of synthesis, the support was transferred to a 100 mL glass bottle (VWR). The oligonucleotide was cleaved from the support with simultaneous deprotection of base and phosphate groups with 40 mL of a 40% aq. methyl amine (Aldrich) 90 mins at 45°C. The bottle was cooled briefly on ice and then the methylamine was filtered into a new 500 mL bottle. The CPG was washed three times with 40 mL portions of DMSO. The mixture was then cooled on dry ice.

In order to remove the *tert*-butyldimethylsilyl (TBDMS) groups at the 2' position, 60 mL triethylamine trihydrofluoride (Et3N-HF) was added to the above mixture. The mixture was heated at 40°C for 60 minutes. The reaction was then quenched with 220 mL of 50 mM sodium acetate (pH 5.5) and stored in the freezer until purification.

Sequences synthesized on the ABI DNA/RNA synthesizer

After completion of synthesis, the support was transferred to a 15 mL tube (VWR). The oligonucleotide was cleaved from the support with simultaneous deprotection of base and phosphate groups with 7 mL of a 40% aq. methyl amine (Aldrich) 15 mins at 65°C. The bottle was cooled briefly on ice and then the methylamine solution was filtered into a 100 mL bottle (VWR). The CPG was washed three times with 7 mL portions of DMSO. The mixture was then cooled on dry ice.

In order to remove the *tert*-butyldimethylsilyl (TBDMS) groups at the 2' position, 10.5 mL triethylamine trihydrofluoride (Et3N-HF) was added to the above mixture. The mixture was heated at 60°C for 15 minutes. The reaction was then quenched with 38.5 mL of 50 mM sodium acetate (pH 5.5) and stored in the freezer until purification.

Step 3. Quantitation of Crude Oligonucleotides

For all samples, a 10 μL aliquot was diluted with 990 μL of deionised nuclease free water (1.0 mL) and the absorbance reading at 260 nm obtained.

Step 4. Purification of Oligonucleotides

Unconjugated oligonucleotides

The unconjugated samples were purified by HPLC on a TSK-Gel SuperQ-5PW (20) column packed in house (17.3 x 5 cm) or on a commercially available TSK-Gel SuperQ-5PW column (15 x 0.215cm) available from TOSOH Bioscience. The buffers were 20 mM phosphate in 10% CH_3CN, pH 8.5 (buffer A) and 20 mM phosphate, 1.0 M NaBr in 10% CH_3CN, pH 8.5 (buffer B). The flow rate was 50.0 mL/min for the in house packed column and 10.0ml/min for the commercially obtained column. Wavelengths of 260 and 294 nm were monitored. The fractions containing the full-length oligonucleotides were pooled together, evaporated, and reconstituted to ~100 mL with deionised water.

Cholesterol-conjugated oligonucleotides

The cholesterol conjugated sequences were HPLC purified on RPC-Source15 reverse-phase columns packed in house (17.3 x 5 cm or 15 x 2 cm). The buffers were 20 mM NaOAc in 10 % CH_3CN (buffer A) and 20 mM NaOAc in 70% CH_3CN (buffer B). The flow rate was 50.0 mL/min for the 17.3x 5cm column and 12.0ml/min for the 15 x 2 cm column. Wavelengths of 260 and 284 nm were monitored. The fractions containing the full-length oligonucleotides were pooled, evaporated, and reconstituted to 100 mL with deionised water.

Step 5. Desalting of Purified Oligonucleotides

The purified oligonucleotides were desalted on either an AKTA Explorer or an AKTA Prime system (Amersham Biosciences) using a Sephadex G-25 column packed in house. First, the column was washed with water at a flow rate of 40 mL/min for 20-30 min. The sample was then applied in 40-60 mL fractions. The eluted salt-free fractions were combined, dried, and reconstituted in ~50 mL of RNase free water.

Step 6. Purity Analysis

Approximately 0.3 OD of each of the desalted oligonucleotides was diluted in water to 300 μL and were analyzed by CGE, ion exchange HPLC, and LC/MS.

Step 7. Duplex Formation

For the preparation of duplexes, equimolar amounts of sense and antisense strand were heated in the required buffer (e.g. 1xPBS) at 95°C for 5 min and slowly cooled to room temperature. Integrity of the duplex was confirmed by HPLC analysis.

Tables of dsRNA sequences

Table 2 provides sequences used for design of dsRNA targeting human GNAQ that will cross react with monkey GNAQ. Table 3 provides sequences used for design of dsRNA targeting human GNAQ that will cross react with both monkey and rat GNAQ. Table 4 provides sequences used for design of dsRNA targeting rat GNAQ that will cross react with mouse GNAQ.

Tables 2a, 3a, and 4a following tables provide the sense and antisense strand of GNAQ target sequences. Tables 2b, 3b, and 4b provide exemplary sense and antisense dsRNA strands with a NN 2 base overhang. Tables 2c, 3c, and 4c provide exemplary sense and antisense dsRNA strands with dTdT 2 base overhang. Tables 2d, 3d, and 4d provide sequences of dsRNA that were synthesized, including the dTdT 2 base overhang and modified nucleotides.

Table 2a: GNAQ (human X monkey): target sequences

Numbering for target sequences is based on Human GNAQ NM_002072.

Start of target sequence	SEQ ID NO.	Target sequence, sense strand (5'-3')	SEQ ID NO.	Target sequence, antisense strand (5'-3')
1217	1	CUAAUUUAUUGCCGUCCUG	74	CAGGACGGCAAUAAAUUAG
1213	2	AAUACUAAUUUAUUGCCGU	75	ACGGCAAUAAAUUAGUAUU
1810	3	CAGCCAUAGCUUGAUUGCU	76	AGCAAUCAAGCUAUGGCUG
1590	4	GUCAGGACACAUCGUUCGA	77	UCGAACGAUGUGUCCUGAC
1149	5	CUUCCCUGGUGGGCUAUUG	78	CAAUAGCCCACCAGGGAAG
1971	6	GACACUACAUUACCCUAAU	79	AUUAGGGUAAUGUAGUGUC
1237	7	ACUCUGUGUGAGCGUGUCC	80	GGACACGCUCACACAGAGU
1152	8	CCCUGGUGGGCUAUUGAAG	81	CUUCAAUAGCCCACCAGGG
1216	9	ACUAAUUUAUUGCCGUCCU	82	AGGACGGCAAUAAAUUAGU
1575	10	CUCUCAAAUGAUACAGUCA	83	UGACUGUAUCAUUUGAGAG
1105	11	AGUACAAUCUGGUCUAAUU	84	AAUUAGACCAGAUUGUACU
1407	12	CACAAAGAUAAGACUUGUU	85	AACAAGUCUUAUCUUUGUG
1108	13	ACAAUCUGGUCUAAUUGUG	86	CACAAUUAGACCAGAUUGU
1395	14	CAGUCAUGCACUCACAAAG	87	CUUUGUGAGUGCAUGACUG
1595	15	GACACAUCGUUCGAUUUAA	88	UUAAAUCGAACGAUGUGUC
1992	16	CUGCUACCCAGAACCUUUU	89	AAAAGGUUCUGGGUAGCAG
1809	17	UCAGCCAUAGCUUGAUUGC	90	GCAAUCAAGCUAUGGCUGA
1220	18	AUUUAUUGCCGUCCUGGAC	91	GUCCAGGACGGCAAUAAAU
1203	19	CAAUUUGCAUAAUACUAAU	92	AUUAGUAUUAUGCAAAUUG
1322	20	GUACAGUCCCAGCACAUUU	93	AAAUGUGCUGGGACUGUAC
1804	21	UACCUUCAGCCAUAGCUUG	94	CAAGCUAUGGCUGAAGGUA
1968	22	ACAGACACUACAUUACCCU	95	AGGGUAAUGUAGUGUCUGU
1214	23	AUACUAAUUUAUUGCCGUC	96	GACGGCAAUAAAUUAGUAU
1159	24	GGGCUAUUGAAGAUACACA	97	UGUGUAUCUUCAAUAGCCC
1603	25	GUUCGAUUUAAGCCAUCAU	98	AUGAUGGCUUAAAUCGAAC
1123	26	UGUGCCUCCUAGACACCCG	99	CGGGUGUCUAGGAGGCACA
1233	27	CUGGACUCUGUGUGAGCGU	100	ACGCUCACACAGAGUCCAG
1930	28	ACCCUCUCUUUCAAUUGCA	101	UGCAAUUGAAAGAGAGGGU
1969	29	CAGACACUACAUUACCCUA	102	UAGGGUAAUGUAGUGUCUG
1219	30	AAUUUAUUGCCGUCCUGGA	103	UCCAGGACGGCAAUAAAUU
1241	31	UGUGUGAGCGUGUCCACAG	104	CUGUGGACACGCUCACACA
1153	32	CCUGGUGGGCUAUUGAAGA	105	UCUUCAAUAGCCCACCAGG

Start of target sequence	SEQ ID NO.	Target sequence, sense strand (5'-3')	SEQ ID NO.	Target sequence, antisense strand (5'-3')
1805	33	ACCUUCAGCCAUAGCUUGA	106	UCAAGCUAUGGCUGAAGGU
1312	34	GGAUGCUGAAGUACAGUCC	107	GGACUGUACUUCAGCAUCC
1546	35	AUCCUAGUUCCAUUCUUGG	108	CCAAGAAUGGAACUAGGAU
1547	36	UCCUAGUUCCAUUCUUGGU	109	ACCAAGAAUGGAACUAGGA
1103	37	GGAGUACAAUCUGGUCUAA	110	UUAGACCAGAUUGUACUCC
1334	38	CACAUUUCCUCUCUAUCUU	111	AAGAUAGAGAGGAAAUGUG
1255	39	CACAGAGUUUGUAGUAAAU	112	AUUUACUACAAACUCUGUG
1967	40	AACAGACACUACAUUACCC	113	GGGUAAUGUAGUGUCUGUU
1391	41	UUCUCAGUCAUGCACUCAC	114	GUGAGUGCAUGACUGAGAA
1124	42	GUGCCUCCUAGACACCCGC	115	GCGGGUGUCUAGGAGGCAC
1612	43	AAGCCAUCAUCAGCUUAAU	116	AUUAAGCUGAUGAUGGCUU
1933	44	CUCUCUUUCAAUUGCAGAU	117	AUCUGCAAUUGAAAGAGAG
1078	45	ACACCAUCCUCCAGUUGAA	118	UUCAACUGGAGGAUGGUGU
1545	46	UAUCCUAGUUCCAUUCUUG	119	CAAGAAUGGAACUAGGAUA
1109	47	CAAUCUGGUCUAAUUGUGC	120	GCACAAUUAGACCAGAUUG
1398	48	UCAUGCACUCACAAAGAUA	121	UAUCUUUGUGAGUGCAUGA
1970	49	AGACACUACAUUACCCUAA	122	UUAGGGUAAUGUAGUGUCU
1173	50	ACACAAGAGGGACUGUAUU	123	AAUACAGUCCCUCUUGUGU
1313	51	GAUGCUGAAGUACAGUCCC	124	GGGACUGUACUUCAGCAUC
1811	52	AGCCAUAGCUUGAUUGCUC	125	GAGCAAUCAAGCUAUGGCU
1862	53	CACAGGAGUCCUUUCUUUU	126	AAAAGAAAGGACUCCUGUG
1600	54	AUCGUUCGAUUUAAGCCAU	127	AUGGCUUAAAUCGAACGAU
1618	55	UCAUCAGCUUAAUUUAAGU	128	ACUUAAAUUAAGCUGAUGA
1332	56	AGCACAUUUCCUCUCUAUC	129	GAUAGAGAGGAAAUGUGCU
1157	57	GUGGGCUAUUGAAGAUACA	130	UGUAUCUUCAAUAGCCCAC
888	58	AUCAUGUAUUCCCAUCUAG	131	CUAGAUGGGAAUACAUGAU
1855	59	AAAGACACACAGGAGUCCU	132	AGGACUCCUGUGUGUCUUU
1579	60	CAAAUGAUACAGUCAGGAC	133	GUCCUGACUGUAUCAUUUG
805	61	UUAGAACAAUUAUCACAUA	134	UAUGUGAUAAUUGUUCUAA
1554	62	UCCAUUCUUGGUCAAGUUU	135	AAACUUGACCAAGAAUGGA
1113	63	CUGGUCUAAUUGUGCCUCC	136	GGAGGCACAAUUAGACCAG
1174	64	CACAAGAGGGACUGUAUUU	137	AAAUACAGUCCCUCUUGUG
1735	65	UCUUGUCUCACUUUGGACU	138	AGUCCAAAGUGAGACAAGA
1450	66	UUUUCUAUGGAGCAAAACA	139	UGUUUUGCUCCAUAGAAAA
1285	67	AUUUAAACUAUUCAGAGGA	140	UCCUCUGAAUAGUUUAAAU
804	68	UUUAGAACAAUUAUCACAU	141	AUGUGAUAAUUGUUCUAAA
1866	69	GGAGUCCUUUCUUUUGAAA	142	UUUCAAAAGAAAGGACUCC
1610	70	UUAAGCCAUCAUCAGCUUA	143	UAAGCUGAUGAUGGCUUAA
1117	71	UCUAAUUGUGCCUCCUAGA	144	UCUAGGAGGCACAAUUAGA
1320	72	AAGUACAGUCCCAGCACAU	145	AUGUGCUGGGACUGUACUU
1317	73	CUGAAGUACAGUCCCAGCA	146	UGCUGGGACUGUACUUCAG

Table 2b: GNAQ (human and monkey): sense and antisense sequences with 2 base overhangs;

Numbering for target sequences is based on Human GNAQ NM_002072.

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
147	CUAAUUUAUUGCCGUCCUGNN	sense	1217
148	CAGGACGGCAAUAAAUUAGNN	antis	1217
149	AAUACUAAUUUAUUGCCGUNN	sense	1213
150	ACGGCAAUAAAUUAGUAUUNN	antis	1213
151	CAGCCAUAGCUUGAUUGCUNN	sense	1810
152	AGCAAUCAAGCUAUGGCUGNN	antis	1810
153	GUCAGGACACAUCGUUCGANN	sense	1590
154	UCGAACGAUGUGUCCUGACNN	antis	1590
155	CUUCCCUGGUGGGCUAUUGNN	sense	1149
156	CAAUAGCCCACCAGGGAAGNN	antis	1149
157	GACACUACAUUACCCUAAUNN	sense	1971
158	AUUAGGGUAAUGUAGUGUCNN	antis	1971
159	ACUCUGUGUGAGCGUGUCCNN	sense	1237
160	GGACACGCUCACACAGAGUNN	antis	1237
161	CCCUGGUGGGCUAUUGAAGNN	sense	1152
162	CUUCAAUAGCCCACCAGGGNN	antis	1152
163	ACUAAUUUAUUGCCGUCCUNN	sense	1216
164	AGGACGGCAAUAAAUUAGUNN	antis	1216
165	CUCUCAAAUGAUACAGUCANN	sense	1575
166	UGACUGUAUCAUUUGAGAGNN	antis	1575
167	AGUACAAUCUGGUCUAAUUNN	sense	1105
168	AAUUAGACCAGAUUGUACUNN	antis	1105
169	CACAAAGAUAAGACUUGUUNN	sense	1407
170	AACAAGUCUUAUCUUUGUGNN	antis	1407
171	ACAAUCUGGUCUAAUUGUGNN	sense	1108
172	CACAAUUAGACCAGAUUGUNN	antis	1108
173	CAGUCAUGCACUCACAAAGNN	sense	1395
174	CUUUGUGAGUGCAUGACUGNN	antis	1395
175	GACACAUCGUUCGAUUUAANN	sense	1595
176	UUAAAUCGAACGAUGUGUCNN	antis	1595
177	CUGCUACCCAGAACCUUUUNN	sense	1992
178	AAAAGGUUCUGGGUAGCAGNN	antis	1992
179	UCAGCCAUAGCUUGAUUGCNN	sense	1809
180	GCAAUCAAGCUAUGGCUGANN	antis	1809
181	AUUUAUUGCCGUCCUGGACNN	sense	1220
182	GUCCAGGACGGCAAUAAAUNN	antis	1220
183	CAAUUUGCAUAAUACUAAUNN	sense	1203
184	AUUAGUAUUAUGCAAAUUGNN	antis	1203
185	GUACAGUCCCAGCACAUUUNN	sense	1322
186	AAAUGUGCUGGGACUGUACNN	antis	1322
187	UACCUUCAGCCAUAGCUUGNN	sense	1804
188	CAAGCUAUGGCUGAAGGUANN	antis	1804

SEQ ID NO	SEQUENCE (5′-3′)	Strand	Start of target sequence
189	ACAGACACUACAUUACCCUNN	sense	1968
190	AGGGUAAUGUAGUGUCUGUNN	antis	1968
191	AUACUAAUUUAUUGCCGUCNN	sense	1214
192	GACGGCAAUAAAUUAGUAUNN	antis	1214
193	GGGCUAUUGAAGAUACACANN	sense	1159
194	UGUGUAUCUUCAAUAGCCCNN	antis	1159
195	GUUCGAUUUAAGCCAUCAUNN	sense	1603
196	AUGAUGGCUUAAAUCGAACNN	antis	1603
197	UGUGCCUCCUAGACACCCGNN	sense	1123
198	CGGGUGUCUAGGAGGCACANN	antis	1123
199	CUGGACUCUGUGUGAGCGUNN	sense	1233
200	ACGCUCACACAGAGUCCAGNN	antis	1233
201	ACCCUCUCUUUCAAUUGCANN	sense	1930
202	UGCAAUUGAAAGAGAGGGUNN	antis	1930
203	CAGACACUACAUUACCCUANN	sense	1969
204	UAGGGUAAUGUAGUGUCUGNN	antis	1969
205	AAUUUAUUGCCGUCCUGGANN	sense	1219
206	UCCAGGACGGCAAUAAAUUNN	antis	1219
207	UGUGUGAGCGUGUCCACAGNN	sense	1241
208	CUGUGGACACGCUCACACANN	antis	1241
209	CCUGGUGGGCUAUUGAAGANN	sense	1153
210	UCUUCAAUAGCCCACCAGGNN	antis	1153
211	ACCUUCAGCCAUAGCUUGANN	sense	1805
212	UCAAGCUAUGGCUGAAGGUNN	antis	1805
213	GGAUGCUGAAGUACAGUCCNN	sense	1312
214	GGACUGUACUUCAGCAUCCNN	antis	1312
215	AUCCUAGUUCCAUUCUUGGNN	sense	1546
216	CCAAGAAUGGAACUAGGAUNN	antis	1546
217	UCCUAGUUCCAUUCUUGGUNN	sense	1547
218	ACCAAGAAUGGAACUAGGANN	antis	1547
219	GGAGUACAAUCUGGUCUAANN	sense	1103
220	UUAGACCAGAUUGUACUCCNN	antis	1103
221	CACAUUUCCUCUCUAUCUUNN	sense	1334
222	AAGAUAGAGAGGAAAUGUGNN	antis	1334
223	CACAGAGUUUGUAGUAAAUNN	sense	1255
224	AUUUACUACAAACUCUGUGNN	antis	1255
225	AACAGACACUACAUUACCCNN	sense	1967
226	GGGUAAUGUAGUGUCUGUUNN	antis	1967
227	UUCUCAGUCAUGCACUCACNN	sense	1391
228	GUGAGUGCAUGACUGAGAANN	antis	1391
229	GUGCCUCCUAGACACCCGCNN	sense	1124
230	GCGGGUGUCUAGGAGGCACNN	antis	1124
231	AAGCCAUCAUCAGCUUAAUNN	sense	1612
232	AUUAAGCUGAUGAUGGCUUNN	antis	1612
233	CUCUCUUUCAAUUGCAGAUNN	sense	1933
234	AUCUGCAAUUGAAAGAGAGNN	antis	1933
235	ACACCAUCCUCCAGUUGAANN	sense	1078

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
236	UUCAACUGGAGGAUGGUGUNN	antis	1078
237	UAUCCUAGUUCCAUUCUUGNN	sense	1545
238	CAAGAAUGGAACUAGGAUANN	antis	1545
239	CAAUCUGGUCUAAUUGUGCNN	sense	1109
240	GCACAAUUAGACCAGAUUGNN	antis	1109
241	UCAUGCACUCACAAAGAUANN	sense	1398
242	UAUCUUUGUGAGUGCAUGANN	antis	1398
243	AGACACUACAUUACCCUAANN	sense	1970
244	UUAGGGUAAUGUAGUGUCUNN	antis	1970
245	ACACAAGAGGGACUGUAUUNN	sense	1173
246	AAUACAGUCCCUCUUGUGUNN	antis	1173
247	GAUGCUGAAGUACAGUCCCNN	sense	1313
248	GGGACUGUACUUCAGCAUCNN	antis	1313
249	AGCCAUAGCUUGAUUGCUCNN	sense	1811
250	GAGCAAUCAAGCUAUGGCUNN	antis	1811
251	CACAGGAGUCCUUUCUUUUNN	sense	1862
252	AAAAGAAAGGACUCCUGUGNN	antis	1862
253	AUCGUUCGAUUUAAGCCAUNN	sense	1600
254	AUGGCUUAAAUCGAACGAUNN	antis	1600
255	UCAUCAGCUUAAUUUAAGUNN	sense	1618
256	ACUUAAAUUAAGCUGAUGANN	antis	1618
257	AGCACAUUUCCUCUCUAUCNN	sense	1332
258	GAUAGAGAGGAAAUGUGCUNN	antis	1332
259	GUGGGCUAUUGAAGAUACANN	sense	1157
260	UGUAUCUUCAAUAGCCCACNN	antis	1157
261	AUCAUGUAUUCCCAUCUAGNN	sense	888
262	CUAGAUGGGAAUACAUGAUNN	antis	888
263	AAAGACACACAGGAGUCCUNN	sense	1855
264	AGGACUCCUGUGUGUCUUUNN	antis	1855
265	CAAAUGAUACAGUCAGGACNN	sense	1579
266	GUCCUGACUGUAUCAUUUGNN	antis	1579
267	UUAGAACAAUUAUCACAUANN	sense	805
268	UAUGUGAUAAUUGUUCUAANN	antis	805
269	UCCAUUCUUGGUCAAGUUUNN	sense	1554
270	AAACUUGACCAAGAAUGGANN	antis	1554
271	CUGGUCUAAUUGUGCCUCCNN	sense	1113
272	GGAGGCACAAUUAGACCAGNN	antis	1113
273	CACAAGAGGGACUGUAUUUNN	sense	1174
274	AAAUACAGUCCCUCUUGUGNN	antis	1174
275	UCUUGUCUCACUUUGGACUNN	sense	1735
276	AGUCCAAAGUGAGACAAGANN	antis	1735
277	UUUUCUAUGGAGCAAAACANN	sense	1450
278	UGUUUUGCUCCAUAGAAAANN	antis	1450
279	AUUUAAACUAUUCAGAGGANN	sense	1285
280	UCCUCUGAAUAGUUUAAAUNN	antis	1285
281	UUUAGAACAAUUAUCACAUNN	sense	804
282	AUGUGAUAAUUGUUCUAAANN	antis	804

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
283	GGAGUCCUUUCUUUUGAAANN	sense	1866
284	UUUCAAAAGAAAGGACUCCNN	antis	1866
285	UUAAGCCAUCAUCAGCUUANN	sense	1610
286	UAAGCUGAUGAUGGCUUAANN	antis	1610
287	UCUAAUUGUGCCUCCUAGANN	sense	1117
288	UCUAGGAGGCACAAUUAGANN	antis	1117
289	AAGUACAGUCCCAGCACAUNN	sense	1320
290	AUGUGCUGGGACUGUACUUNN	antis	1320
291	CUGAAGUACAGUCCCAGCANN	sense	1317
292	UGCUGGGACUGUACUUCAGNN	antis	1317

Table 2c: GNAQ (human and monkey): sense and antisense sequences with dTdT overhangs

Numbering for target sequences is based on Human GNAQ NM_002072

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
293	CUAAUUUAUUGCCGUCCUGdTdT	sense	1217
294	CAGGACGGCAAUAAAUUAGdTdT	antis	1217
295	AAUACUAAUUUAUUGCCGUdTdT	sense	1213
296	ACGGCAAUAAAUUAGUAUUdTdT	antis	1213
297	CAGCCAUAGCUUGAUUGCUdTdT	sense	1810
298	AGCAAUCAAGCUAUGGCUGdTdT	antis	1810
299	GUCAGGACACAUCGUUCGAdTdT	sense	1590
300	UCGAACGAUGUGUCCUGACdTdT	antis	1590
301	CUUCCCUGGUGGGCUAUUGdTdT	sense	1149
302	CAAUAGCCCACCAGGGAAGdTdT	antis	1149
303	GACACUACAUUACCCUAAUdTdT	sense	1971
304	AUUAGGGUAAUGUAGUGUCdTdT	antis	1971
305	ACUCUGUGUGAGCGUGUCCdTdT	sense	1237
306	GGACACGCUCACACAGAGUdTdT	antis	1237
307	CCCUGGUGGGCUAUUGAAGdTdT	sense	1152
308	CUUCAAUAGCCCACCAGGGdTdT	antis	1152
309	ACUAAUUUAUUGCCGUCCUdTdT	sense	1216
310	AGGACGGCAAUAAAUUAGUdTdT	antis	1216
311	CUCUCAAAUGAUACAGUCAdTdT	sense	1575
312	UGACUGUAUCAUUUGAGAGdTdT	antis	1575
313	AGUACAAUCUGGUCUAAUUdTdT	sense	1105
314	AAUUAGACCAGAUUGUACUdTdT	antis	1105
315	CACAAAGAUAAGACUUGUUdTdT	sense	1407
316	AACAAGUCUUAUCUUUGUGdTdT	antis	1407
317	ACAAUCUGGUCUAAUUGUGdTdT	sense	1108
318	CACAAUUAGACCAGAUUGUdTdT	antis	1108
319	CAGUCAUGCACUCACAAAGdTdT	sense	1395
320	CUUUGUGAGUGCAUGACUGdTdT	antis	1395
321	GACACAUCGUUCGAUUUAAdTdT	sense	1595
322	UUAAAUCGAACGAUGUGUCdTdT	antis	1595
323	CUGCUACCCAGAACCUUUUdTdT	sense	1992
324	AAAAGGUUCUGGGUAGCAGdTdT	antis	1992
325	UCAGCCAUAGCUUGAUUGCdTdT	sense	1809
326	GCAAUCAAGCUAUGGCUGAdTdT	antis	1809
327	AUUUAUUGCCGUCCUGGACdTdT	sense	1220
328	GUCCAGGACGGCAAUAAAUdTdT	antis	1220
329	CAAUUUGCAUAAUACUAAUdTdT	sense	1203
330	AUUAGUAUUAUGCAAAUUGdTdT	antis	1203
331	GUACAGUCCCAGCACAUUUdTdT	sense	1322
332	AAAUGUGCUGGGACUGUACdTdT	antis	1322
333	UACCUUCAGCCAUAGCUUGdTdT	sense	1804
334	CAAGCUAUGGCUGAAGGUAdTdT	antis	1804

SEQ ID NO	SEQUENCE (5′-3′)	Strand	Start of target sequence
335	ACAGACACUACAUUACCCUdTdT	sense	1968
336	AGGGUAAUGUAGUGUCUGUdTdT	antis	1968
337	AUACUAAUUUAUUGCCGUCdTdT	sense	1214
338	GACGGCAAUAAAUUAGUAUdTdT	antis	1214
339	GGGCUAUUGAAGAUACACAdTdT	sense	1159
340	UGUGUAUCUUCAAUAGCCCdTdT	antis	1159
341	GUUCGAUUUAAGCCAUCAUdTdT	sense	1603
342	AUGAUGGCUUAAAUCGAACdTdT	antis	1603
343	UGUGCCUCCUAGACACCCGdTdT	sense	1123
344	CGGGUGUCUAGGAGGCACAdTdT	antis	1123
345	CUGGACUCUGUGUGAGCGUdTdT	sense	1233
346	ACGCUCACACAGAGUCCAGdTdT	antis	1233
347	ACCCUCUCUUUCAAUUGCAdTdT	sense	1930
348	UGCAAUUGAAAGAGAGGGUdTdT	antis	1930
349	CAGACACUACAUUACCCUAdTdT	sense	1969
350	UAGGGUAAUGUAGUGUCUGdTdT	antis	1969
351	AAUUUAUUGCCGUCCUGGAdTdT	sense	1219
352	UCCAGGACGGCAAUAAAUUdTdT	antis	1219
353	UGUGUGAGCGUGUCCACAGdTdT	sense	1241
354	CUGUGGACACGCUCACACAdTdT	antis	1241
355	CCUGGUGGGCUAUUGAAGAdTdT	sense	1153
356	UCUUCAAUAGCCCACCAGGdTdT	antis	1153
357	ACCUUCAGCCAUAGCUUGAdTdT	sense	1805
358	UCAAGCUAUGGCUGAAGGUdTdT	antis	1805
359	GGAUGCUGAAGUACAGUCCdTdT	sense	1312
360	GGACUGUACUUCAGCAUCCdTdT	antis	1312
361	AUCCUAGUUCCAUUCUUGGdTdT	sense	1546
362	CCAAGAAUGGAACUAGGAUdTdT	antis	1546
363	UCCUAGUUCCAUUCUUGGUdTdT	sense	1547
364	ACCAAGAAUGGAACUAGGAdTdT	antis	1547
365	GGAGUACAAUCUGGUCUAAdTdT	sense	1103
366	UUAGACCAGAUUGUACUCCdTdT	antis	1103
367	CACAUUUCCUCUCUAUCUUdTdT	sense	1334
368	AAGAUAGAGAGGAAAUGUGdTdT	antis	1334
369	CACAGAGUUUGUAGUAAAUdTdT	sense	1255
370	AUUUACUACAAACUCUGUGdTdT	antis	1255
371	AACAGACACUACAUUACCCdTdT	sense	1967
372	GGGUAAUGUAGUGUCUGUUdTdT	antis	1967
373	UUCUCAGUCAUGCACUCACdTdT	sense	1391
374	GUGAGUGCAUGACUGAGAAdTdT	antis	1391
375	GUGCCUCCUAGACACCCGCdTdT	sense	1124
376	GCGGGUGUCUAGGAGGCACdTdT	antis	1124
377	AAGCCAUCAUCAGCUUAAUdTdT	sense	1612
378	AUUAAGCUGAUGAUGGCUUdTdT	antis	1612
379	CUCUCUUUCAAUUGCAGAUdTdT	sense	1933
380	AUCUGCAAUUGAAAGAGAGdTdT	antis	1933
381	ACACCAUCCUCCAGUUGAAdTdT	sense	1078

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
382	UUCAACUGGAGGAUGGUGUdTdT	antis	1078
383	UAUCCUAGUUCCAUUCUUGdTdT	sense	1545
384	CAAGAAUGGAACUAGGAUAdTdT	antis	1545
385	CAAUCUGGUCUAAUUGUGCdTdT	sense	1109
386	GCACAAUUAGACCAGAUUGdTdT	antis	1109
387	UCAUGCACUCACAAAGAUAdTdT	sense	1398
388	UAUCUUUGUGAGUGCAUGAdTdT	antis	1398
389	AGACACUACAUUACCCUAAdTdT	sense	1970
390	UUAGGGUAAUGUAGUGUCUdTdT	antis	1970
391	ACACAAGAGGGACUGUAUUdTdT	sense	1173
392	AAUACAGUCCCUCUUGUGUdTdT	antis	1173
393	GAUGCUGAAGUACAGUCCCdTdT	sense	1313
394	GGGACUGUACUUCAGCAUCdTdT	antis	1313
395	AGCCAUAGCUUGAUUGCUCdTdT	sense	1811
396	GAGCAAUCAAGCUAUGGCUdTdT	antis	1811
397	CACAGGAGUCCUUUCUUUUdTdT	sense	1862
398	AAAAGAAAGGACUCCUGUGdTdT	antis	1862
399	AUCGUUCGAUUUAAGCCAUdTdT	sense	1600
400	AUGGCUUAAAUCGAACGAUdTdT	antis	1600
401	UCAUCAGCUUAAUUUAAGUdTdT	sense	1618
402	ACUUAAAUUAAGCUGAUGAdTdT	antis	1618
403	AGCACAUUUCCUCUCUAUCdTdT	sense	1332
404	GAUAGAGAGGAAAUGUGCUdTdT	antis	1332
405	GUGGGCUAUUGAAGAUACAdTdT	sense	1157
406	UGUAUCUUCAAUAGCCCACdTdT	antis	1157
407	AUCAUGUAUUCCCAUCUAGdTdT	sense	888
408	CUAGAUGGGAAUACAUGAUdTdT	antis	888
409	AAAGACACACAGGAGUCCUdTdT	sense	1855
410	AGGACUCCUGUGUGUCUUUdTdT	antis	1855
411	CAAAUGAUACAGUCAGGACdTdT	sense	1579
412	GUCCUGACUGUAUCAUUUGdTdT	antis	1579
413	UUAGAACAAUUAUCACAUAdTdT	sense	805
414	UAUGUGAUAAUUGUUCUAAdTdT	antis	805
415	UCCAUUCUUGGUCAAGUUUdTdT	sense	1554
416	AAACUUGACCAAGAAUGGAdTdT	antis	1554
417	CUGGUCUAAUUGUGCCUCCdTdT	sense	1113
418	GGAGGCACAAUUAGACCAGdTdT	antis	1113
419	CACAAGAGGGACUGUAUUUdTdT	sense	1174
420	AAAUACAGUCCCUCUUGUGdTdT	antis	1174
421	UCUUGUCUCACUUUGGACUdTdT	sense	1735
422	AGUCCAAAGUGAGACAAGAdTdT	antis	1735
423	UUUUCUAUGGAGCAAAACAdTdT	sense	1450
424	UGUUUUGCUCCAUAGAAAAdTdT	antis	1450
425	AUUUAAACUAUUCAGAGGAdTdT	sense	1285
426	UCCUCUGAAUAGUUUAAAUdTdT	antis	1285
427	UUUAGAACAAUUAUCACAUdTdT	sense	804
428	AUGUGAUAAUUGUUCUAAAdTdT	antis	804

SEQ ID NO	SEQUENCE (5′-3′)	Strand	Start of target sequence
429	GGAGUCCUUUCUUUUGAAAdTdT	sense	1866
430	UUUCAAAAGAAAGGACUCCdTdT	antis	1866
431	UUAAGCCAUCAUCAGCUUAdTdT	sense	1610
432	UAAGCUGAUGAUGGCUUAAdTdT	antis	1610
433	UCUAAUUGUGCCUCCUAGAdTdT	sense	1117
434	UCUAGGAGGCACAAUUAGAdTdT	antis	1117
435	AAGUACAGUCCCAGCACAUdTdT	sense	1320
436	AUGUGCUGGGACUGUACUUdTdT	antis	1320
437	CUGAAGUACAGUCCCAGCAdTdT	sense	1317
438	UGCUGGGACUGUACUUCAGdTdT	antis	1317

Table 2d: GNAQ (human and monkey): modified sense and antisense strands

Numbering for target sequences is based on Human GNAQ NM_002072.

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
Modifications :Sense strand - all pyrimidines (U, C)are 2'OMe; antisense strand - pyrimidines adjacent to A (UA, CA) are 2'Ome; 3' end is dTdT			
cuAAuuuAuuGccGuccuGdTdT	sense	1217	439
cAGGACGGcAAuAAAUuAGdTdT	antis	1217	440
AAuAcuAAuuuAuuGccGudTdT	sense	1213	441
ACGGcAAuAAAUuAGuAUUdTdT	antis	1213	442
cAGccAuAGcuuGAuuGcudTdT	sense	1810	443
AGcAAUcAAGCuAUGGCUGdTdT	antis	1810	444
GucAGGAcAcAucGuucGAdTdT	sense	1590	445
UCGAACGAUGUGUCCUGACdTdT	antis	1590	446
cuucccuGGuGGGcuAuuGdTdT	sense	1149	447
cAAuAGCCcACcAGGGAAGdTdT	antis	1149	448
GAcAcuAcAuuAcccuAAudTdT	sense	1971	449
AUuAGGGuAAUGuAGUGUCdTdT	antis	1971	450
AcucuGuGuGAGcGuGuccdTdT	sense	1237	451
GGAcACGCUcAcAcAGAGUdTdT	antis	1237	452
cccuGGuGGGcuAuuGAAGdTdT	sense	1152	453
CUUcAAuAGCCcACcAGGGdTdT	antis	1152	454
AcuAAuuuAuuGccGuccudTdT	sense	1216	455
AGGACGGcAAuAAAUuAGUdTdT	antis	1216	456
cucucAAAuGAuAcAGucAdTdT	sense	1575	457
UGACUGuAUcAUUUGAGAGdTdT	antis	1575	458
AGuAcAAucuGGucuAAuudTdT	sense	1105	459
AAUuAGACcAGAUUGuACUdTdT	antis	1105	460
cAcAAAGAuAAGAcuuGuudTdT	sense	1407	461
AAcAAGUCUuAUCUUUGUGdTdT	antis	1407	462
AcAAucuGGucuAAuuGuGdTdT	sense	1108	463
cAcAAUuAGACcAGAUUGUdTdT	antis	1108	464
cAGucAuGcAcucAcAAAGdTdT	sense	1395	465
CUUUGUGAGUGcAUGACUGdTdT	antis	1395	466
GAcAcAucGuucGAuuuAAdTdT	sense	1595	467
UuAAAUCGAACGAUGUGUCdTdT	antis	1595	468
cuGcuAcccAGAAccuuuudTdT	sense	1992	469
AAAAGGUUCUGGGuAGcAGdTdT	antis	1992	470
ucAGccAuAGcuuGAuuGcdTdT	sense	1809	471
GcAAUcAAGCuAUGGCUGAdTdT	antis	1809	472
AuuuAuuGccGuccuGGAcdTdT	sense	1220	473
GUCcAGGACGGcAAuAAAUdTdT	antis	1220	474
cAAuuuGcAuAAuAcuAAudTdT	sense	1203	475
AUuAGuAUuAUGcAAAUUGdTdT	antis	1203	476
GuAcAGucccAGcAcAuuudTdT	sense	1322	477
AAAUGUGCUGGGACUGuACdTdT	antis	1322	478

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
uAccuucAGccAuAGcuuGdTdT	sense	1804	479
cAAGCuAUGGCUGAAGGuAdTdT	antis	1804	480
AcAGAcAcuAcAuuAcccudTdT	sense	1968	481
AGGGuAAUGuAGUGUCUGUdTdT	antis	1968	482
AuAcuAAuuuAuuGccGucdTdT	sense	1214	483
GACGGcAAuAAAUuAGuAUdTdT	antis	1214	484
GGGcuAuuGAAGAuAcAcAdTdT	sense	1159	485
UGUGuAUCUUcAAuAGCCCdTdT	antis	1159	486
GuucGAuuuAAGccAucAudTdT	sense	1603	487
AUGAUGGCUuAAAUCGAACdTdT	antis	1603	488
uGuGccuccuAGAcAcccGdTdT	sense	1123	489
CGGGUGUCuAGGAGGcAcAdTdT	antis	1123	490
cuGGAcucuGuGuGAGcGudTdT	sense	1233	491
ACGCUcAcAcAGAGUCcAGdTdT	antis	1233	492
AcccucucuuucAAuuGcAdTdT	sense	1930	493
UGcAAUUGAAAGAGAGGGUdTdT	antis	1930	494
cAGAcAcuAcAuuAcccuAdTdT	sense	1969	495
uAGGGuAAUGuAGUGUCUGdTdT	antis	1969	496
AAuuuAuuGccGuccuGGAdTdT	sense	1219	497
UCcAGGACGGcAAuAAAUUdTdT	antis	1219	498
uGuGuGAGcGuGuccAcAGdTdT	sense	1241	499
CUGUGGAcACGCUcAcAcAdTdT	antis	1241	500
ccuGGuGGGcuAuuGAAGAdTdT	sense	1153	501
UCUUcAAuAGCCcACcAGGdTdT	antis	1153	502
AccuucAGccAuAGcuuGAdTdT	sense	1805	503
UcAAGCuAUGGCUGAAGGUdTdT	antis	1805	504
GGAuGcuGAAGuAcAGuccdTdT	sense	1312	505
GGACUGuACUUcAGcAUCCdTdT	antis	1312	506
AuccuAGuuccAuucuuGGdTdT	sense	1546	507
CcAAGAAUGGAACuAGGAUdTdT	antis	1546	508
uccuAGuuccAuucuuGGudTdT	sense	1547	509
ACcAAGAAUGGAACuAGGAdTdT	antis	1547	510
GGAGuAcAAucuGGucuAAdTdT	sense	1103	511
UuAGACcAGAUUGuACUCCdTdT	antis	1103	512
cAcAuuuccucucuAucuudTdT	sense	1334	513
AAGAuAGAGAGGAAAUGUGdTdT	antis	1334	514
cAcAGAGuuuGuAGuAAAudTdT	sense	1255	515
AUUuACuAcAAACUCUGUGdTdT	antis	1255	516
AAcAGAcAcuAcAuuAcccdTdT	sense	1967	517
GGGuAAUGuAGUGUCUGUUdTdT	antis	1967	518
uucucAGucAuGcAcucAcdTdT	sense	1391	519
GUGAGUGcAUGACUGAGAAdTdT	antis	1391	520
GuGccuccuAGAcAcccGcdTdT	sense	1124	521
GCGGGUGUCuAGGAGGcACdTdT	antis	1124	522
AAGccAucAucAGcuuAAudTdT	sense	1612	523
AUuAAGCUGAUGAUGGCUUdTdT	antis	1612	524

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
cucucuuucAAuuGcAGAudTdT	sense	1933	525
AUCUGcAAUUGAAAGAGAGdTdT	antis	1933	526
AcAccAuccuccAGuuGAAdTdT	sense	1078	527
UUcAACUGGAGGAUGGUGUdTdT	antis	1078	528
uAuccuAGuuccAuucuuGdTdT	sense	1545	529
cAAGAAUGGAACuAGGAuAdTdT	antis	1545	530
cAAucuGGucuAAuuGuGcdTdT	sense	1109	531
GcAcAAUuAGACcAGAUUGdTdT	antis	1109	532
ucAuGcAcucAcAAAGAuAdTdT	sense	1398	533
uAUCUUUGUGAGUGcAUGAdTdT	antis	1398	534
AGAcAcuAcAuuAcccuAAdTdT	sense	1970	535
UuAGGGuAAUGuAGUGUCUdTdT	antis	1970	536
AcAcAAGAGGGAcuGuAuudTdT	sense	1173	537
AAuAcAGUCCCUCUUGUGUdTdT	antis	1173	538
GAuGcuGAAGuAcAGucccdTdT	sense	1313	539
GGGACUGuACUUcAGcAUCdTdT	antis	1313	540
AGccAuAGcuuGAuuGcucdTdT	sense	1811	541
GAGcAAUcAAGCuAUGGCUdTdT	antis	1811	542
cAcAGGAGuccuuucuuuudTdT	sense	1862	543
AAAAGAAAGGACUCCUGUGdTdT	antis	1862	544
AucGuucGAuuuAAGccAudTdT	sense	1600	545
AUGGCUuAAAUCGAACGAUdTdT	antis	1600	546
ucAucAGcuuAAuuuAAGudTdT	sense	1618	547
ACUuAAAUuAAGCUGAUGAdTdT	antis	1618	548
AGcAcAuuuccucucuAucdTdT	sense	1332	549
GAuAGAGAGGAAAUGUGCUdTdT	antis	1332	550
GuGGGcuAuuGAAGAuAcAdTdT	sense	1157	551
UGuAUCUUcAAuAGCCcACdTdT	antis	1157	552
AucAuGuAuucccAucuAGdTdT	sense	888	553
CuAGAUGGGAAuAcAUGAUdTdT	antis	888	554
AAAGAcAcAcAGGAGuccudTdT	sense	1855	555
AGGACUCCUGUGUGUCUUUdTdT	antis	1855	556
cAAAuGAuAcAGucAGGAcdTdT	sense	1579	557
GUCCUGACUGuAUcAUUUGdTdT	antis	1579	558
uuAGAAcAAuuAucAcAuAdTdT	sense	805	559
uAUGUGAuAAUUGUUCuAAdTdT	antis	805	560
uccAuucuuGGucAAGuuudTdT	sense	1554	561
AAACUUGACcAAGAAUGGAdTdT	antis	1554	562
cuGGucuAAuuGuGccuccdTdT	sense	1113	563
GGAGGcAcAAUuAGACcAGdTdT	antis	1113	564
cAcAAGAGGGAcuGuAuuudTdT	sense	1174	565
AAAuAcAGUCCCUCUUGUGdTdT	antis	1174	566
ucuuGucucAcuuuGGAcudTdT	sense	1735	567
AGUCcAAAGUGAGAcAAGAdTdT	antis	1735	568
uuuucuAuGGAGcAAAcAdTdT	sense	1450	569
UGUUUUGCUCcAuAGAAAAdTdT	antis	1450	570

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
AuuuAAAcuAuucAGAGGAdTdT	sense	1285	571
UCCUCUGAAuAGUUuAAAUdTdT	antis	1285	572
uuuAGAAcAAuuAucAcAudTdT	sense	804	573
AUGUGAuAAUUGUUCuAAAdTdT	antis	804	574
GGAGuccuuucuuuuGAAAdTdT	sense	1866	575
UUUcAAAAGAAAGGACUCCdTdT	antis	1866	576
uuAAGccAucAucAGcuuAdTdT	sense	1610	577
uAAGCUGAUGAUGGCUuAAdTdT	antis	1610	578
ucuAAuuGuGccuccuAGAdTdT	sense	1117	579
UCuAGGAGGcAcAAUuAGAdTdT	antis	1117	580
AAGuAcAGucccAGcAcAudTdT	sense	1320	581
AUGUGCUGGGACUGuACUUdTdT	antis	1320	582
cuGAAGuAcAGucccAGcAdTdT	sense	1317	583
UGCUGGGACUGuACUUcAGdTdT	antis	1317	584
Modifications :Sense strand - all pyrimidines (U, C)are 2'OMe; antisense strand - pyrimidines adjacent to A (UA, CA) are 2'Ome; 3' end is thio (dTsdT).			
cuAAuuuAuuGccGuccuGdTsdT	sense	1217	585
cAGGACGGcAAuAAAUuAGdTsdT	antis	1217	586
AAuAcuAAuuuAuuGccGudTsdT	sense	1213	587
ACGGcAAuAAAUuAGuAUUdTsdT	antis	1213	588
cAGccAuAGcuuGAuuGcudTsdT	sense	1810	589
AGcAAUcAAGCuAUGGCUGdTsdT	antis	1810	590
GucAGGAcAcAucGuucGAdTsdT	sense	1590	591
UCGAACGAUGUGUCCUGACdTsdT	antis	1590	592
cuucccuGGuGGGcuAuuGdTsdT	sense	1149	593
cAAuAGCCcACcAGGGAAGdTsdT	antis	1149	594
GAcAcuAcAuuAcccuAAudTsdT	sense	1971	595
AUuAGGGuAAUGuAGUGUCdTsdT	antis	1971	596
AcucuGuGuGAGcGuGuccdTsdT	sense	1237	597
GGAcACGCUcAcAcAGAGUdTsdT	antis	1237	598
cccuGGuGGGcuAuuGAAGdTsdT	sense	1152	599
CUUcAAuAGCCcACcAGGGdTsdT	antis	1152	600
AcuAAuuuAuuGccGuccudTsdT	sense	1216	601
AGGACGGcAAuAAAUuAGUdTsdT	antis	1216	602
cucucAAAuGAuAcAGucAdTsdT	sense	1575	603
UGACUGuAUcAUUUGAGAGdTsdT	antis	1575	604
AGuAcAAucuGGucuAAuudTsdT	sense	1105	605
AAUuAGACcAGAUUGuACUdTsdT	antis	1105	606
cAcAAAGAuAAGAcuuGuudTsdT	sense	1407	607
AAcAAGUCUuAUCUUUGUGdTsdT	antis	1407	608
AcAAucuGGucuAAuuGuGdTsdT	sense	1108	609
cAcAAUuAGACcAGAUUGUdTsdT	antis	1108	610
cAGucAuGcAcucAcAAAGdTsdT	sense	1395	611
CUUUGUGAGUGcAUGACUGdTsdT	antis	1395	612
GAcAcAucGuucGAuuuAAdTsdT	sense	1595	613

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
UuAAAUCGAACGAUGUGUCdTsdT	antis	1595	614
cuGcuAcccAGAAccuuuudTsdT	sense	1992	615
AAAAGGUUCUGGGuAGcAGdTsdT	antis	1992	616
ucAGccAuAGcuuGAuuGcdTsdT	sense	1809	617
GcAAUcAAGCuAUGGCUGAdTsdT	antis	1809	618
AuuuAuuGccGuccuGGAcdTsdT	sense	1220	619
GUCcAGGACGGcAAuAAAUdTsdT	antis	1220	620
cAAuuuGcAuAAuAcuAAudTsdT	sense	1203	621
AUuAGuAUuAUGcAAAUUGdTsdT	antis	1203	622
GuAcAGucccAGcAcAuuudTsdT	sense	1322	623
AAAUGUGCUGGGACUGuACdTsdT	antis	1322	624
uAccuucAGccAuAGcuuGdTsdT	sense	1804	625
cAAGCuAUGGCUGAAGGuAdTsdT	antis	1804	626
AcAGAcAcuAcAuuAcccudTsdT	sense	1968	627
AGGGuAAUGuAGUGUCUGUdTsdT	antis	1968	628
AuAcuAAuuuAuuGccGucdTsdT	sense	1214	629
GACGGcAAuAAAUuAGuAUdTsdT	antis	1214	630
GGGcuAuuGAAGAuAcAcAdTsdT	sense	1159	631
UGUGuAUCUUcAAuAGCCCdTsdT	antis	1159	632
GuucGAuuuAAGccAucAudTsdT	sense	1603	633
AUGAUGGCUuAAAUCGAACdTsdT	antis	1603	634
uGuGccuccuAGAcAcccGdTsdT	sense	1123	635
CGGGUGUCuAGGAGGcAcAdTsdT	antis	1123	636
cuGGAcucuGuGuGAGcGudTsdT	sense	1233	637
ACGCUcAcAcAGAGUCcAGdTsdT	antis	1233	638
AcccucucuuucAAuuGcAdTsdT	sense	1930	639
UGcAAUUGAAAGAGAGGGUdTsdT	antis	1930	640
cAGAcAcuAcAuuAcccuAdTsdT	sense	1969	641
uAGGGuAAUGuAGUGUCUGdTsdT	antis	1969	642
AAuuuAuuGccGuccuGGAdTsdT	sense	1219	643
UCcAGGACGGcAAuAAAUUdTsdT	antis	1219	644
uGuGuGAGcGuGuccAcAGdTsdT	sense	1241	645
CUGUGGAcACGCUcAcAcAdTsdT	antis	1241	646
ccuGGuGGGcuAuuGAAGAdTsdT	sense	1153	647
UCUUcAAuAGCCcACcAGGdTsdT	antis	1153	648
AccuucAGccAuAGcuuGAdTsdT	sense	1805	649
UcAAGCuAUGGCUGAAGGUdTsdT	antis	1805	650
GGAuGcuGAAGuAcAGuccdTsdT	sense	1312	651
GGACUGuACUUcAGcAUCCdTsdT	antis	1312	652
AuccuAGuuccAuucuuGGdTsdT	sense	1546	653
CcAAGAAUGGAACuAGGAUdTsdT	antis	1546	654
uccuAGuuccAuucuuGGudTsdT	sense	1547	655
ACcAAGAAUGGAACuAGGAdTsdT	antis	1547	656
GGAGuAcAAucuGGucuAAdTsdT	sense	1103	657
UuAGACcAGAUUGuACUCCdTsdT	antis	1103	658
cAcAuuuccucucuAucuudTsdT	sense	1334	659

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
AAGAuAGAGAGGAAAUGUGdTsdT	antis	1334	660
cAcAGAGuuuGuAGuAAAudTsdT	sense	1255	661
AUUuACuAcAAACUCUGUGdTsdT	antis	1255	662
AAcAGAcAcuAcAuuAcccdTsdT	sense	1967	663
GGGuAAUGuAGUGUCUGUUdTsdT	antis	1967	664
uucucAGucAuGcAcucAcdTsdT	sense	1391	665
GUGAGUGcAUGACUGAGAAdTsdT	antis	1391	666
GuGccuccuAGAcAcccGcdTsdT	sense	1124	667
GCGGGUGUCuAGGAGGcACdTsdT	antis	1124	668
AAGccAucAucAGcuuAAudTsdT	sense	1612	669
AUuAAGCUGAUGAUGGCUUdTsdT	antis	1612	670
cucucuuucAAuuGcAGAudTsdT	sense	1933	671
AUCUGcAAUUGAAAGAGAGdTsdT	antis	1933	672
AcAccAuccuccAGuuGAAdTsdT	sense	1078	673
UUcAACUGGAGGAUGGUGUdTsdT	antis	1078	674
uAuccuAGuuccAuucuuGdTsdT	sense	1545	675
cAAGAAUGGAACuAGGAuAdTsdT	antis	1545	676
cAAucuGGucuAAuuGuGcdTsdT	sense	1109	677
GcAcAAUuAGACcAGAUUGdTsdT	antis	1109	678
ucAuGcAcucAcAAAGAuAdTsdT	sense	1398	679
uAUCUUUGUGAGUGcAUGAdTsdT	antis	1398	680
AGAcAcuAcAuuAcccuAAdTsdT	sense	1970	681
UuAGGGuAAUGuAGUGUCUdTsdT	antis	1970	682
AcAcAAGAGGGAcuGuAuudTsdT	sense	1173	683
AAuAcAGUCCCUCUUGUGUdTsdT	antis	1173	684
GAuGcuGAAGuAcAGucccdTsdT	sense	1313	685
GGGACUGuACUUcAGcAUCdTsdT	antis	1313	686
AGccAuAGcuuGAuuGcucdTsdT	sense	1811	687
GAGcAAUcAAGCuAUGGCUdTsdT	antis	1811	688
cAcAGGAGuccuuucuuuudTsdT	sense	1862	689
AAAAGAAAGGACUCCUGUGdTsdT	antis	1862	690
AucGuucGAuuuAAGccAudTsdT	sense	1600	691
AUGGCUuAAAUCGAACGAUdTsdT	antis	1600	692
ucAucAGcuuAAuuuAAGudTsdT	sense	1618	693
ACUuAAAUuAAGCUGAUGAdTsdT	antis	1618	694
AGcAcAuuuccucucuAucdTsdT	sense	1332	695
GAuAGAGAGGAAAUGUGCUdTsdT	antis	1332	696
GuGGGcuAuuGAAGAuAcAdTsdT	sense	1157	697
UGuAUCUUcAAuAGCCcACdTsdT	antis	1157	698
AucAuGuAuucccAucuAGdTsdT	sense	888	699
CuAGAUGGGAAuAcAUGAUdTsdT	antis	888	700
AAAGAcAcAcAGGAGuccudTsdT	sense	1855	701
AGGACUCCUGUGUGUCUUUdTsdT	antis	1855	702
cAAAuGAuAcAGucAGGAcdTsdT	sense	1579	703
GUCCUGACUGuAUcAUUUGdTsdT	antis	1579	704
uuAGAAcAAuuAucAcAuAdTsdT	sense	805	705

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
uAUGUGAuAAUUGUUCuAAdTsdT	antis	805	706
uccAuucuuGGucAAGuuudTsdT	sense	1554	707
AAACUUGACcAAGAAUGGAdTsdT	antis	1554	708
cuGGucuAAuuGuGccuccdTsdT	sense	1113	709
GGAGGcAcAAUuAGACcAGdTsdT	antis	1113	710
cAcAAGAGGGAcuGuAuuudTsdT	sense	1174	711
AAAuAcAGUCCCUCUUGUGdTsdT	antis	1174	712
ucuuGucucAcuuuGGAcudTsdT	sense	1735	713
AGUCcAAAGUGAGAcAAGAdTsdT	antis	1735	714
uuuucuAuGGAGcAAAAcAdTsdT	sense	1450	715
UGUUUUGCUCcAuAGAAAAdTsdT	antis	1450	716
AuuuAAAcuAuucAGAGGAdTsdT	sense	1285	717
UCCUCUGAAuAGUUuAAAUdTsdT	antis	1285	718
uuuAGAAcAAuuAucAcAudTsdT	sense	804	719
AUGUGAuAAUUGUUCuAAAdTsdT	antis	804	720
GGAGuccuuucuuuuGAAAdTsdT	sense	1866	721
UUUcAAAAGAAAGGACUCCdTsdT	antis	1866	722
uuAAGccAucAucAGcuuAdTsdT	sense	1610	723
uAAGCUGAUGAUGGCUuAAdTsdT	antis	1610	724
ucuAAuuGuGccuccuAGAdTsdT	sense	1117	725
UCuAGGAGGcAcAAUuAGAdTsdT	antis	1117	726
AAGuAcAGucccAGcAcAudTsdT	sense	1320	727
AUGUGCUGGGACUGuACUUdTsdT	antis	1320	728
cuGAAGuAcAGucccAGcAdTsdT	sense	1317	729
UGCUGGGACUGuACUUcAGdTsdT	antis	1317	730
Modifications: Sense strand - all pyrimidines are 2'OMe; antisense strand - pyrimidines adjacent to A (UA, CA) + U adjacent to another U (UU) or G (UG) are 2'Ome; 3' end is thio (dTsdT).			
cuAAuuuAuuGccGuccuGdTsdT	sense	1217	731
cAGGACGGcAAuAAAUuAGdTsdT	antis	1217	732
AAuAcuAAuuuAuuGccGudTsdT	sense	1213	733
ACGGcAAuAAAUuAGuAuUdTsdT	antis	1213	734
cAGccAuAGcuuGAuuGcudTsdT	sense	1810	735
AGcAAUcAAGCuAuGGCuGdTsdT	antis	1810	736
GucAGGAcAcAucGuucGAdTsdT	sense	1590	737
UCGAACGAuGuGUCCuGACdTsdT	antis	1590	738
cuucccuGGuGGGcuAuuGdTsdT	sense	1149	739
cAAuAGCCcACcAGGGAAGdTsdT	antis	1149	740
GAcAcuAcAuuAcccuAAudTsdT	sense	1971	741
AUuAGGGuAAuGuAGuGUCdTsdT	antis	1971	742
AcucuGuGuGAGcGuGuccdTsdT	sense	1237	743
GGAcACGCUcAcAcAGAGUdTsdT	antis	1237	744
cccuGGuGGGcuAuuGAAGdTsdT	sense	1152	745
CuUcAAuAGCCcACcAGGGdTsdT	antis	1152	746
AcuAAuuuAuuGccGuccudTsdT	sense	1216	747
AGGACGGcAAuAAAUuAGUdTsdT	antis	1216	748

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
cucucAAAuGAuAcAGucAdTsdT	sense	1575	749
uGACuGuAUcAuUuGAGAGdTsdT	antis	1575	750
AGuAcAAucuGGucuAAuudTsdT	sense	1105	751
AAUuAGACcAGAuuGuACUdTsdT	antis	1105	752
cAcAAAGAuAAGAcuuGuudTsdT	sense	1407	753
AAcAAGUCUuAUCuUuGuGdTsdT	antis	1407	754
AcAAucuGGucuAAuuGuGdTsdT	sense	1108	755
cAcAAUuAGACcAGAuuGUdTsdT	antis	1108	756
cAGucAuGcAcucAcAAAGdTsdT	sense	1395	757
CuUuGuGAGuGcAuGACuGdTsdT	antis	1395	758
GAcAcAucGuucGAuuuAAdTsdT	sense	1595	759
UuAAAUCGAACGAuGuGUCdTsdT	antis	1595	760
cuGcuAcccAGAAccuuuudTsdT	sense	1992	761
AAAAGGuUCuGGGuAGcAGdTsdT	antis	1992	762
ucAGccAuAGcuuGAuuGcdTsdT	sense	1809	763
GcAAUcAAGCuAuGGCuGAdTsdT	antis	1809	764
AuuuAuuGccGuccuGGAcdTsdT	sense	1220	765
GUCcAGGACGGcAAuAAAUdTsdT	antis	1220	766
cAAuuuGcAuAAuAcuAAudTsdT	sense	1203	767
AUuAGuAUuAuGcAAAuuGdTsdT	antis	1203	768
GuAcAGucccAGcAcAuuudTsdT	sense	1322	769
AAAuGuGCuGGGACuGuACdTsdT	antis	1322	770
uAccuucAGccAuAGcuuGdTsdT	sense	1804	771
cAAGCuAuGGCuGAAGGuAdTsdT	antis	1804	772
AcAGAcAcuAcAuuAcccudTsdT	sense	1968	773
AGGGuAAuGuAGuGUCuGUdTsdT	antis	1968	774
AuAcuAAuuuAuuGccGucdTsdT	sense	1214	775
GACGGcAAuAAAUuAGuAUdTsdT	antis	1214	776
GGGcuAuuGAAGAuAcAcAdTsdT	sense	1159	777
uGuGuAUCuUcAAuAGCCCdTsdT	antis	1159	778
GuucGAuuuAAGccAucAudTsdT	sense	1603	779
AuGAuGGCUuAAAUCGAACdTsdT	antis	1603	780
uGuGccuccuAGAcAcccGdTsdT	sense	1123	781
CGGGuGUCuAGGAGGcAcAdTsdT	antis	1123	782
cuGGAcucuGuGuGAGcGudTsdT	sense	1233	783
ACGCUcAcAcAGAGUCcAGdTsdT	antis	1233	784
AcccucucuuucAAuuGcAdTsdT	sense	1930	785
uGcAAuuGAAAGAGAGGGUdTsdT	antis	1930	786
cAGAcAcuAcAuuAcccuAdTsdT	sense	1969	787
uAGGGuAAuGuAGuGUCuGdTsdT	antis	1969	788
AAuuuAuuGccGuccuGGAdTsdT	sense	1219	789
UCcAGGACGGcAAuAAAuUdTsdT	antis	1219	790
uGuGuGAGcGuGuccAcAGdTsdT	sense	1241	791
CuGuGGAcACGCUcAcAcAdTsdT	antis	1241	792
ccuGGuGGGcuAuuGAAGAdTsdT	sense	1153	793
UCuUcAAuAGCCcACcAGGdTsdT	antis	1153	794

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
AccuucAGccAuAGcuuGAdTsdT	sense	1805	795
UcAAGCuAuGGCuGAAGGUdTsdT	antis	1805	796
GGAuGcuGAAGuAcAGuccdTsdT	sense	1312	797
GGACuGuACuUcAGcAUCCdTsdT	antis	1312	798
AuccuAGuuccAuucuuGGdTsdT	sense	1546	799
CcAAGAAuGGAACuAGGAUdTsdT	antis	1546	800
uccuAGuuccAuucuuGGudTsdT	sense	1547	801
ACcAAGAAuGGAACuAGGAdTsdT	antis	1547	802
GGAGuAcAAucuGGucuAAdTsdT	sense	1103	803
UuAGACcAGAuuGuACUCCdTsdT	antis	1103	804
cAcAuuuccucucuAucuudTsdT	sense	1334	805
AAGAuAGAGAGGAAAuGuGdTsdT	antis	1334	806
cAcAGAGuuuGuAGuAAAudTsdT	sense	1255	807
AuUuACuAcAAACUCuGuGdTsdT	antis	1255	808
AAcAGAcAcuAcAuuAcccdTsdT	sense	1967	809
GGGuAAuGuAGuGUCuGuUdTsdT	antis	1967	810
uucucAGucAuGcAcucAcdTsdT	sense	1391	811
GuGAGuGcAuGACuGAGAAdTsdT	antis	1391	812
GuGccuccuAGAcAcccGcdTsdT	sense	1124	813
GCGGGuGUCuAGGAGGcACdTsdT	antis	1124	814
AAGccAucAucAGcuuAAudTsdT	sense	1612	815
AUuAAGCuGAuGAuGGCuUdTsdT	antis	1612	816
cucucuuucAAuuGcAGAudTsdT	sense	1933	817
AUCuGcAAuuGAAAGAGAGdTsdT	antis	1933	818
AcAccAuccuccAGuuGAAdTsdT	sense	1078	819
uUcAACuGGAGGAuGGuGUdTsdT	antis	1078	820
uAuccuAGuuccAuucuuGdTsdT	sense	1545	821
cAAGAAuGGAACuAGGAuAdTsdT	antis	1545	822
cAAucuGGucuAAuuGuGcdTsdT	sense	1109	823
GcAcAAUuAGACcAGAuuGdTsdT	antis	1109	824
ucAuGcAcucAcAAAGAuAdTsdT	sense	1398	825
uAUCuUuGuGAGuGcAuGAdTsdT	antis	1398	826
AGAcAcuAcAuuAcccuAAdTsdT	sense	1970	827
UuAGGGuAAuGuAGuGUCUdTsdT	antis	1970	828
AcAcAAGAGGGAcuGuAuudTsdT	sense	1173	829
AAuAcAGUCCCUCuuGuGUdTsdT	antis	1173	830
GAuGcuGAAGuAcAGucccdTsdT	sense	1313	831
GGGACuGuACuUcAGcAUCdTsdT	antis	1313	832
AGccAuAGcuuGAuuGcucdTsdT	sense	1811	833
GAGcAAUcAAGCuAuGGCUdTsdT	antis	1811	834
cAcAGGAGuccuuucuuuudTsdT	sense	1862	835
AAAAGAAAGGACUCCuGuGdTsdT	antis	1862	836
AucGuucGAuuuAAGccAudTsdT	sense	1600	837
AuGGCUuAAAUCGAACGAUdTsdT	antis	1600	838
ucAucAGcuuAAuuuAAGudTsdT	sense	1618	839
ACUuAAAUuAAGCuGAuGAdTsdT	antis	1618	840

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
AGcAcAuuuccucucuAucdTsdT	sense	1332	841
GAuAGAGAGGAAAuGuGCUdTsdT	antis	1332	842
GuGGGcuAuuGAAGAuAcAdTsdT	sense	1157	843
uGuAUCuUcAAuAGCCcACdTsdT	antis	1157	844
AucAuGuAuucccAucuAGdTsdT	sense	888	845
CuAGAuGGGAAuAcAuGAUdTsdT	antis	888	846
AAAGAcAcAcAGGAGuccudTsdT	sense	1855	847
AGGACUCCuGuGuGUCuUUdTsdT	antis	1855	848
cAAAuGAuAcAGucAGGAcdTsdT	sense	1579	849
GUCCuGACuGuAUcAuUuGdTsdT	antis	1579	850
uuAGAAcAAuuAucAcAuAdTsdT	sense	805	851
uAuGuGAuAAuuGuUCuAAdTsdT	antis	805	852
uccAuucuuGGucAAGuuudTsdT	sense	1554	853
AAACuuGACcAAGAAuGGAdTsdT	antis	1554	854
cuGGucuAAuuGuGccuccdTsdT	sense	1113	855
GGAGGcAcAAUuAGACcAGdTsdT	antis	1113	856
cAcAAGAGGGAcuGuAuuudTsdT	sense	1174	857
AAAuAcAGUCCCUCuuGuGdTsdT	antis	1174	858
ucuuGucucAcuuuGGAcudTsdT	sense	1735	859
AGUCcAAAGuGAGAcAAGAdTsdT	antis	1735	860
uuuucuAuGGAGcAAAcAdTsdT	sense	1450	861
uGuUuuGCUCcAuAGAAAAdTsdT	antis	1450	862
AuuuAAAcuAuucAGAGGAdTsdT	sense	1285	863
UCCUCuGAAuAGuUuAAAUdTsdT	antis	1285	864
uuuAGAAcAAuuAucAcAudTsdT	sense	804	865
AuGuGAuAAuuGuUCuAAAdTsdT	antis	804	866
GGAGuccuuucuuuGAAAdTsdT	sense	1866	867
uUUcAAAAGAAAGGACUCCdTsdT	antis	1866	868
uuAAGccAucAucAGcuuAdTsdT	sense	1610	869
uAAGCuGAuGAuGGCUuAAdTsdT	antis	1610	870
ucuAAuuGuGccuccuAGAdTsdT	sense	1117	871
UCuAGGAGGcAcAAUuAGAdTsdT	antis	1117	872
AAGuAcAGucccAGcAcAudTsdT	sense	1320	873
AuGuGCuGGGACuGuACuUdTsdT	antis	1320	874
cuGAAGuAcAGucccAGcAdTsdT	sense	1317	875
uGCuGGGACuGuACuUcAGdTsdT	antis	1317	876

Table 3a: GNAQ (Human, monkey and mouse): target sequences

Numbering for target sequence is Human GNAQ NM_002072.

Start of target sequence	SEQ ID NO.	Target sequence, sense strand (5'-3')	SEQ ID NO.	Target sequence, antisense strand (5'-3')
1215	877	UACUAAUUUAUUGCCGUCC	888	GGACGGCAAUAAAUUAGUA
1217	878	CUAAUUUAUUGCCGUCCUG	889	CAGGACGGCAAUAAAUUAG
1216	879	ACUAAUUUAUUGCCGUCCU	890	AGGACGGCAAUAAAUUAGU
1322	880	GUACAGUCCCAGCACAUUU	891	AAAUGUGCUGGGACUGUAC
1220	881	AUUUAUUGCCGUCCUGGAC	892	GUCCAGGACGGCAAUAAAU
1265	882	GUAGUAAAUAUUAUGAUUU	893	AAAUCAUAAUAUUUACUAC
1218	883	UAAUUUAUUGCCGUCCUGG	894	CCAGGACGGCAAUAAAUUA
1175	884	ACAAGAGGGACUGUAUUUC	895	GAAAUACAGUCCCUCUUGU
1223	885	UAUUGCCGUCCUGGACUCU	896	AGAGUCCAGGACGGCAAUA
1319	886	GAAGUACAGUCCCAGCACA	897	UGUGCUGGGACUGUACUUC
1285	887	AUUUAAACUAUUCAGAGGA	898	UCCUCUGAAUAGUUUAAAU

Table 3b: GNAQ (Human, monkey and mouse): sense and antisense sequences with 2 base overhangs

Numbering for target sequence is Human GNAQ NM_002072.

SEQ ID NO	**SEQUENCE (5′-3′)**	**Strand**	**Start of target sequence**
899	UACUAAUUUAUUGCCGUCCNN	sense	1215
900	GGACGGCAAUAAAUUAGUANN	antis	1215
901	CUAAUUUAUUGCCGUCCUGNN	sense	1217
902	CAGGACGGCAAUAAAUUAGNN	antis	1217
903	ACUAAUUUAUUGCCGUCCUNN	sense	1216
904	AGGACGGCAAUAAAUUAGUNN	antis	1216
905	GUACAGUCCCAGCACAUUUNN	sense	1322
906	AAAUGUGCUGGGACUGUACNN	antis	1322
907	AUUUAUUGCCGUCCUGGACNN	sense	1220
908	GUCCAGGACGGCAAUAAAUNN	antis	1220
909	GUAGUAAAUAUUAUGAUUUNN	sense	1265
910	AAAUCAUAAUAUUUACUACNN	antis	1265
911	UAAUUUAUUGCCGUCCUGGNN	sense	1218
912	CCAGGACGGCAAUAAAUUANN	antis	1218
913	ACAAGAGGGACUGUAUUUCNN	sense	1175
914	GAAAUACAGUCCCUCUUGUNN	antis	1175
915	UAUUGCCGUCCUGGACUCUNN	sense	1223
916	AGAGUCCAGGACGGCAAUANN	antis	1223
917	GAAGUACAGUCCCAGCACANN	sense	1319
918	UGUGCUGGGACUGUACUUCNN	antis	1319
919	AUUUAAACUAUUCAGAGGANN	sense	1285
920	UCCUCUGAAUAGUUUAAAUNN	antis	1285

Table 3c: GNAQ (Human, monkey and mouse): sense and antisense sequences with dTdT overhangs

Numbering for target sequence is Human GNAQ NM_002072.

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
921	UACUAAUUUAUUGCCGUCCdTdT	sense	1215
922	GGACGGCAAUAAAUUAGUAdTdT	antis	1215
923	CUAAUUUAUUGCCGUCCUGdTdT	sense	1217
924	CAGGACGGCAAUAAAUUAGdTdT	antis	1217
925	ACUAAUUUAUUGCCGUCCUdTdT	sense	1216
926	AGGACGGCAAUAAAUUAGUdTdT	antis	1216
927	GUACAGUCCCAGCACAUUUdTdT	sense	1322
928	AAAUGUGCUGGGACUGUACdTdT	antis	1322
929	AUUUAUUGCCGUCCUGGACdTdT	sense	1220
930	GUCCAGGACGGCAAUAAAUdTdT	antis	1220
931	GUAGUAAAUAUUAUGAUUUdTdT	sense	1265
932	AAAUCAUAAUAUUUACUACdTdT	antis	1265
933	UAAUUUAUUGCCGUCCUGGdTdT	sense	1218
934	CCAGGACGGCAAUAAAUUAdTdT	antis	1218
935	ACAAGAGGGACUGUAUUUCdTdT	sense	1175
936	GAAAUACAGUCCCUCUUGUdTdT	antis	1175
937	UAUUGCCGUCCUGGACUCUdTdT	sense	1223
938	AGAGUCCAGGACGGCAAUAdTdT	antis	1223
939	GAAGUACAGUCCCAGCACAdTdT	sense	1319
940	UGUGCUGGGACUGUACUUCdTdT	antis	1319
941	AUUUAAACUAUUCAGAGGAdTdT	sense	1285
942	UCCUCUGAAUAGUUUAAAUdTdT	antis	1285

Table 3d: GNAQ (Human, monkey and mouse): modified sense and antisense strands

Numbering for target sequence is Human GNAQ NM_002072.

SEQUENCE (5′-3′)	Strand	Start of target sequence	SEQ ID NO
Modifications: Sense strand - all pyrimidines (U, C)are 2′OMe; antisense strand - pyrimidines adjacent to A (UA, CA) are 2′Ome; 3′ end is dTdT			
uAcuAAuuuAuuGccGuccdTdT	sense	1215	943
GGACGGcAAuAAAUuAGuAdTdT	antis	1215	944
cuAAuuuAuuGccGuccuGdTdT	sense	1217	945
cAGGACGGcAAuAAAUuAGdTdT	antis	1217	946
AcuAAuuuAuuGccGuccudTdT	sense	1216	947
AGGACGGcAAuAAAUuAGUdTdT	antis	1216	948
GuAcAGucccAGcAcAuuudTdT	sense	1322	949
AAAUGUGCUGGGACUGuACdTdT	antis	1322	950
AuuuAuuGccGuccuGGAcdTdT	sense	1220	951
GUCcAGGACGGcAAuAAAUdTdT	antis	1220	952
GuAGuAAAuAuuAuGAuuudTdT	sense	1265	953
AAAUcAuAAuAUUuACuACdTdT	antis	1265	954
uAAuuuAuuGccGuccuGGdTdT	sense	1218	955
CcAGGACGGcAAuAAAUuAdTdT	antis	1218	956
AcAAGAGGGAcuGuAuuucdTdT	sense	1175	957
GAAAuAcAGUCCCUCUUGUdTdT	antis	1175	958
uAuuGccGuccuGGAcucudTdT	sense	1223	959
AGAGUCcAGGACGGcAAuAdTdT	antis	1223	960
GAAGuAcAGucccAGcAcAdTdT	sense	1319	961
UGUGCUGGGACUGuACUUCdTdT	antis	1319	962
AuuuAAAcuAuucAGAGGAdTdT	sense	1285	963
UCCUCUGAAuAGUUuAAAUdTdT	antis	1285	964
Modifications: Sense strand - all pyrimidines (U, C) are 2′OMe; antisense strand - pyrimidines adjacent to A (UA, CA) are 2′Ome; 3′ end is thio (dTsdT)			
uAcuAAuuuAuuGccGuccdTsdT	sense	1215	965
GGACGGcAAuAAAUuAGuAdTsdT	antis	1215	966
cuAAuuuAuuGccGuccuGdTsdT	sense	1217	967
cAGGACGGcAAuAAAUuAGdTsdT	antis	1217	968
AcuAAuuuAuuGccGuccudTsdT	sense	1216	969
AGGACGGcAAuAAAUuAGUdTsdT	antis	1216	970
GuAcAGucccAGcAcAuuudTsdT	sense	1322	971
AAAUGUGCUGGGACUGuACdTsdT	antis	1322	972
AuuuAuuGccGuccuGGAcdTsdT	sense	1220	973
GUCcAGGACGGcAAuAAAUdTsdT	antis	1220	974
GuAGuAAAuAuuAuGAuuudTsdT	sense	1265	975
AAAUcAuAAuAUUuACuACdTsdT	antis	1265	976
uAAuuuAuuGccGuccuGGdTsdT	sense	1218	977
CcAGGACGGcAAuAAAUuAdTsdT	antis	1218	978

AcAAGAGGGAcuGuAuuucdTsdT	sense	1175	979
GAAAuAcAGUCCCUCUUGUdTsdT	antis	1175	980
uAuuGccGuccuGGAcucudTsdT	sense	1223	981
AGAGUCcAGGACGGcAAuAdTsdT	antis	1223	982
GAAGuAcAGucccAGcAcAdTsdT	sense	1319	983
UGUGCUGGGACUGuACUUCdTsdT	antis	1319	984
AuuuAAAcuAuucAGAGGAdTsdT	sense	1285	985
UCCUCUGAAuAGUUuAAAUdTsdT	antis	1285	986
Modifications: Sense strand - all pyrimidines are 2'OMe; antisense strand - pyrimidines adjacent to A (UA, CA) + U adjacent to another U (UU) or G (UG) are 2'Ome; 3' end is thio (dTsdT).			
uAcuAAuuuAuuGccGuccdTsdT	sense	1215	987
GGACGGcAAuAAAUuAGuAdTsdT	antis	1215	988
cuAAuuuAuuGccGuccuGdTsdT	sense	1217	989
cAGGACGGcAAuAAAUuAGdTsdT	antis	1217	990
AcuAAuuuAuuGccGuccudTsdT	sense	1216	991
AGGACGGcAAuAAAUuAGUdTsdT	antis	1216	992
GuAcAGucccAGcAcAuuudTsdT	sense	1322	993
AAAuGuGCuGGGACuGuACdTsdT	antis	1322	994
AuuuAuuGccGuccuGGAcdTsdT	sense	1220	995
GUCcAGGACGGcAAuAAAUdTsdT	antis	1220	996
GuAGuAAAuAuuAuGAuuudTsdT	sense	1265	997
AAAUcAuAAuAuUuACuACdTsdT	antis	1265	998
uAAuuuAuuGccGuccuGGdTsdT	sense	1218	999
CcAGGACGGcAAuAAAUuAdTsdT	antis	1218	1000
AcAAGAGGGAcuGuAuuucdTsdT	sense	1175	1001
GAAAuAcAGUCCCUCuuGUdTsdT	antis	1175	1002
uAuuGccGuccuGGAcucudTsdT	sense	1223	1003
AGAGUCcAGGACGGcAAuAdTsdT	antis	1223	1004
GAAGuAcAGucccAGcAcAdTsdT	sense	1319	1005
uGuGCuGGGACuGuACuUCdTsdT	antis	1319	1006
AuuuAAAcuAuucAGAGGAdTsdT	sense	1285	1007
UCCUCuGAAuAGuUuAAAUdTsdT	antis	1285	1008

Table 4a: GNAQ (rat and mouse): target sequences

Numbering for target sequences is Rat GNAQ NM_031036.

Start of target sequence	SEQ ID NO.	Target sequence, sense strand (5'-3')	SEQ ID NO.	Target sequence, antisense strand (5'-3')
853	1009	UAUUCCCACCUAGUCGACU	1039	AGUCGACUAGGUGGGAAUA
855	1010	UUCCCACCUAGUCGACUAC	1040	GUAGUCGACUAGGUGGGAA
367	1011	GCUUUUGAGAAUCCAUAUG	1041	CAUAUGGAUUCUCAAAAGC
55	1012	CGGAGGAUCAACGACGAGA	1042	UCUCGUCGUUGAUCCUCCG
459	1013	AUCUGACUCUACCAAAUAC	1043	GUAUUUGGUAGAGUCAGAU
312	1014	ACACAAUAAGGCUCAUGCA	1044	UGCAUGAGCCUUAUUGUGU
178	1015	AGGAUCAUCCACGGGUCGG	1045	CCGACCCGUGGAUGAUCCU
297	1016	CCCAUACAAGUAUGAACAC	1046	GUGUUCAUACUUGUAUGGG
315	1017	CAAUAAGGCUCAUGCACAA	1047	UUGUGCAUGAGCCUUAUUG
58	1018	AGGAUCAACGACGAGAUCG	1048	CGAUCUCGUCGUUGAUCCU
324	1019	UCAUGCACAAUUGGUUCGA	1049	UCGAACCAAUUGUGCAUGA
59	1020	GGAUCAACGACGAGAUCGA	1050	UCGAUCUCGUCGUUGAUCC
398	1021	AGAGCUUGUGGAAUGAUCC	1051	GGAUCAUUCCACAAGCUCU
57	1022	GAGGAUCAACGACGAGAUC	1052	GAUCUCGUCGUUGAUCCUC
56	1023	GGAGGAUCAACGACGAGAU	1053	AUCUCGUCGUUGAUCCUCC
369	1024	UUUUGAGAAUCCAUAUGUA	1054	UACAUAUGGAUUCUCAAAA
45	1025	CAAGGAAGCCCGGAGGAUC	1055	GAUCCUCCGGGCUUCCUUG
460	1026	UCUGACUCUACCAAAUACU	1056	AGUAUUUGGUAGAGUCAGA
97	1027	AAGCGCGACGCCCGCCGGG	1057	CCCGGCGGGCGUCGCGCUU
314	1028	ACAAUAAGGCUCAUGCACA	1058	UGUGCAUGAGCCUUAUUGU
318	1029	UAAGGCUCAUGCACAAUUG	1059	CAAUUGUGCAUGAGCCUUA
50	1030	AAGCCCGGAGGAUCAACGA	1060	UCGUUGAUCCUCCGGGCUU
323	1031	CUCAUGCACAAUUGGUUCG	1061	CGAACCAAUUGUGCAUGAG
327	1032	UGCACAAUUGGUUCGAGAG	1062	CUCUCGAACCAAUUGUGCA
329	1033	CACAAUUGGUUCGAGAGGU	1063	ACCUCUCGAACCAAUUGUG
862	1034	CUAGUCGACUACUUCCCAG	1064	CUGGGAAGUAGUCGACUAG
89	1035	GCAGGGACAAGCGCGACGC	1065	GCGUCGCGCUUGUCCCUGC
371	1036	UUGAGAAUCCAUAUGUAGA	1066	UCUACAUAUGGAUUCUCAA
868	1037	GACUACUUCCCAGAAUAUG	1067	CAUAUUCUGGGAAGUAGUC
62	1038	UCAACGACGAGAUCGAGCG	1068	CGCUCGAUCUCGUCGUUGA

Table 4b: GNAQ (rat and mouse): sense and antisense sequences with 2 base overhangs

Numbering for target sequences is Rat GNAQ NM_031036.

SEQ ID NO	**SEQUENCE (5'-3')**	**Type**	**Start of target sequence**
1069	UAUUCCCACCUAGUCGACUNN	sense	853
1070	AGUCGACUAGGUGGGAAUANN	antis	853
1071	UUCCCACCUAGUCGACUACNN	sense	855
1072	GUAGUCGACUAGGUGGGAANN	antis	855
1073	GCUUUUGAGAAUCCAUAUGNN	sense	367
1074	CAUAUGGAUUCUCAAAAGCNN	antis	367
1075	CGGAGGAUCAACGACGAGANN	sense	55
1076	UCUCGUCGUUGAUCCUCCGNN	antis	55
1077	AUCUGACUCUACCAAAUACNN	sense	459
1078	GUAUUUGGUAGAGUCAGAUNN	antis	459
1079	ACACAAUAAGGCUCAUGCANN	sense	312
1080	UGCAUGAGCCUUAUUGUGUNN	antis	312
1081	AGGAUCAUCCACGGGUCGGNN	sense	178
1082	CCGACCCGUGGAUGAUCCUNN	antis	178
1083	CCCAUACAAGUAUGAACACNN	sense	297
1084	GUGUUCAUACUUGUAUGGGNN	antis	297
1085	CAAUAAGGCUCAUGCACAANN	sense	315
1086	UUGUGCAUGAGCCUUAUUGNN	antis	315
1087	AGGAUCAACGACGAGAUCGNN	sense	58
1088	CGAUCUCGUCGUUGAUCCUNN	antis	58
1089	UCAUGCACAAUUGGUUCGANN	sense	324
1090	UCGAACCAAUUGUGCAUGANN	antis	324
1091	GGAUCAACGACGAGAUCGANN	sense	59
1092	UCGAUCUCGUCGUUGAUCCNN	antis	59
1093	AGAGCUUGUGGAAUGAUCCNN	sense	398
1094	GGAUCAUUCCACAAGCUCUNN	antis	398
1095	GAGGAUCAACGACGAGAUCNN	sense	57
1096	GAUCUCGUCGUUGAUCCUCNN	antis	57
1097	GGAGGAUCAACGACGAGAUNN	sense	56
1098	AUCUCGUCGUUGAUCCUCCNN	antis	56
1099	UUUUGAGAAUCCAUAUGUANN	sense	369
1100	UACAUAUGGAUUCUCAAAANN	antis	369
1101	CAAGGAAGCCCGGAGGAUCNN	sense	45
1102	GAUCCUCCGGGCUUCCUUGNN	antis	45
1103	UCUGACUCUACCAAAUACUNN	sense	460
1104	AGUAUUUGGUAGAGUCAGANN	antis	460
1105	AAGCGCGACGCCCGCCGGGNN	sense	97
1106	CCCGGCGGGCGUCGCGCUUNN	antis	97
1107	ACAAUAAGGCUCAUGCACANN	sense	314
1108	UGUGCAUGAGCCUUAUUGUNN	antis	314
1109	UAAGGCUCAUGCACAAUUGNN	sense	318
1110	CAAUUGUGCAUGAGCCUUANN	antis	318
1111	AAGCCCGGAGGAUCAACGANN	sense	50

SEQ ID NO	SEQUENCE (5′-3′)	Type	Start of target sequence
1112	UCGUUGAUCCUCCGGGCUUNN	antis	50
1113	CUCAUGCACAAUUGGUUCGNN	sense	323
1114	CGAACCAAUUGUGCAUGAGNN	antis	323
1115	UGCACAAUUGGUUCGAGAGNN	sense	327
1116	CUCUCGAACCAAUUGUGCANN	antis	327
1117	CACAAUUGGUUCGAGAGGUNN	sense	329
1118	ACCUCUCGAACCAAUUGUGNN	antis	329
1119	CUAGUCGACUACUUCCCAGNN	sense	862
1120	CUGGGAAGUAGUCGACUAGNN	antis	862
1121	GCAGGGACAAGCGCGACGCNN	sense	89
1122	GCGUCGCGCUUGUCCCUGCNN	antis	89
1123	UUGAGAAUCCAUAUGUAGANN	sense	371
1124	UCUACAUAUGGAUUCUCAANN	antis	371
1125	GACUACUUCCCAGAAUAUGNN	sense	868
1126	CAUAUUCUGGGAAGUAGUCNN	antis	868
1127	UCAACGACGAGAUCGAGCGNN	sense	62
1128	CGCUCGAUCUCGUCGUUGANN	antis	62

Table 4c: GNAQ (rat and mouse): sense and antisense sequences with dTdT overhangs

Numbering for target sequences is Rat GNAQ NM_031036.

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
1129	UAUUCCCACCUAGUCGACUdTdT	sense	853
1130	AGUCGACUAGGUGGGAAUAdTdT	antis	853
1131	UUCCCACCUAGUCGACUACdTdT	sense	855
1132	GUAGUCGACUAGGUGGGAAdTdT	antis	855
1133	GCUUUUGAGAAUCCAUAUGdTdT	sense	367
1134	CAUAUGGAUUCUCAAAAGCdTdT	antis	367
1135	CGGAGGAUCAACGACGAGAdTdT	sense	55
1136	UCUCGUCGUUGAUCCUCCGdTdT	antis	55
1137	AUCUGACUCUACCAAAUACdTdT	sense	459
1138	GUAUUUGGUAGAGUCAGAUdTdT	antis	459
1139	ACACAAUAAGGCUCAUGCAdTdT	sense	312
1140	UGCAUGAGCCUUAUUGUGUdTdT	antis	312
1141	AGGAUCAUCCACGGGUCGGdTdT	sense	178
1142	CCGACCCGUGGAUGAUCCUdTdT	antis	178
1143	CCCAUACAAGUAUGAACACdTdT	sense	297
1144	GUGUUCAUACUUGUAUGGGdTdT	antis	297
1145	CAAUAAGGCUCAUGCACAAdTdT	sense	315
1146	UUGUGCAUGAGCCUUAUUGdTdT	antis	315
1147	AGGAUCAACGACGAGAUCGdTdT	sense	58
1148	CGAUCUCGUCGUUGAUCCUdTdT	antis	58
1149	UCAUGCACAAUUGGUUCGAdTdT	sense	324
1150	UCGAACCAAUUGUGCAUGAdTdT	antis	324
1151	GGAUCAACGACGAGAUCGAdTdT	sense	59
1152	UCGAUCUCGUCGUUGAUCCdTdT	antis	59
1153	AGAGCUUGUGGAAUGAUCCdTdT	sense	398
1154	GGAUCAUUCCACAAGCUCUdTdT	antis	398
1155	GAGGAUCAACGACGAGAUCdTdT	sense	57
1156	GAUCUCGUCGUUGAUCCUCdTdT	antis	57
1157	GGAGGAUCAACGACGAGAUdTdT	sense	56
1158	AUCUCGUCGUUGAUCCUCCdTdT	antis	56
1159	UUUUGAGAAUCCAUAUGUAdTdT	sense	369
1160	UACAUAUGGAUUCUCAAAAdTdT	antis	369
1161	CAAGGAAGCCCGGAGGAUCdTdT	sense	45
1162	GAUCCUCCGGGCUUCCUUGdTdT	antis	45
1163	UCUGACUCUACCAAAUACUdTdT	sense	460
1164	AGUAUUUGGUAGAGUCAGAdTdT	antis	460
1165	AAGCGCGACGCCCGCCGGGdTdT	sense	97
1166	CCCGGCGGGCGUCGCGCUUdTdT	antis	97
1167	ACAAUAAGGCUCAUGCACAdTdT	sense	314
1168	UGUGCAUGAGCCUUAUUGUdTdT	antis	314

SEQ ID NO	SEQUENCE (5'-3')	Strand	Start of target sequence
1169	UAAGGCUCAUGCACAAUUGdTdT	sense	318
1170	CAAUUGUGCAUGAGCCUUAdTdT	antis	318
1171	AAGCCCGGAGGAUCAACGAdTdT	sense	50
1172	UCGUUGAUCCUCCGGGCUUdTdT	antis	50
1173	CUCAUGCACAAUUGGUUCGdTdT	sense	323
1174	CGAACCAAUUGUGCAUGAGdTdT	antis	323
1175	UGCACAAUUGGUUCGAGAGdTdT	sense	327
1176	CUCUCGAACCAAUUGUGCAdTdT	antis	327
1177	CACAAUUGGUUCGAGAGGUdTdT	sense	329
1178	ACCUCUCGAACCAAUUGUGdTdT	antis	329
1179	CUAGUCGACUACUUCCCAGdTdT	sense	862
1180	CUGGGAAGUAGUCGACUAGdTdT	antis	862
1181	GCAGGGACAAGCGCGACGCdTdT	sense	89
1182	GCGUCGCGCUUGUCCCUGCdTdT	antis	89
1183	UUGAGAAUCCAUAUGUAGAdTdT	sense	371
1184	UCUACAUAUGGAUUCUCAAdTdT	antis	371
1185	GACUACUUCCCAGAAUAUGdTdT	sense	868
1186	CAUAUUCUGGGAAGUAGUCdTdT	antis	868
1187	UCAACGACGAGAUCGAGCGdTdT	sense	62
1188	CGCUCGAUCUCGUCGUUGAdTdT	antis	62

Table 4d: GNAQ dsRNA (rat and mouse): modified sense and antisense strands

Numbering for target sequences is Rat GNAQ NM_031036.

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
Modifications: Sense strand - all pyrimidines (U, C)are 2'OMe; antisense strand – pyrimidines adjacent to A (UA, CA) are 2'Ome; 3' end is dTdT			
uAuucccAccuAGucGAcudTdT	sense	853	1189
AGUCGACuAGGUGGGAAuAdTdT	antis	853	1190
uucccAccuAGucGAcuAcdTdT	sense	855	1191
GuAGUCGACuAGGUGGGAAdTdT	antis	855	1192
GcuuuuGAGAAuccAuAuGdTdT	sense	367	1193
cAuAUGGAUUCUcAAAAGCdTdT	antis	367	1194
cGGAGGAucAAcGAcGAGAdTdT	sense	55	1195
UCUCGUCGUUGAUCCUCCGdTdT	antis	55	1196
AucuGAcucuAccAAAuAcdTdT	sense	459	1197
GuAUUUGGuAGAGUcAGAUdTdT	antis	459	1198
AcAcAAuAAGGcucAuGcAdTdT	sense	312	1199
UGcAUGAGCCUuAUUGUGUdTdT	antis	312	1200
AGGAucAuccAcGGGucGGdTdT	sense	178	1201
CCGACCCGUGGAUGAUCCUdTdT	antis	178	1202
cccAuAcAAGuAuGAAcAcdTdT	sense	297	1203
GUGUUcAuACUUGuAUGGGdTdT	antis	297	1204
cAAuAAGGcucAuGcAcAAdTdT	sense	315	1205
UUGUGcAUGAGCCUuAUUGdTdT	antis	315	1206
AGGAucAAcGAcGAGAucGdTdT	sense	58	1207
CGAUCUCGUCGUUGAUCCUdTdT	antis	58	1208
ucAuGcAcAAuuGGuucGAdTdT	sense	324	1209
UCGAACcAAUUGUGcAUGAdTdT	antis	324	1210
GGAucAAcGAcGAGAucGAdTdT	sense	59	1211
UCGAUCUCGUCGUUGAUCCdTdT	antis	59	1212
AGAGcuuGuGGAAuGAuccdTdT	sense	398	1213
GGAUcAUUCcAcAAGCUCUdTdT	antis	398	1214
GAGGAucAAcGAcGAGAucdTdT	sense	57	1215
GAUCUCGUCGUUGAUCCUCdTdT	antis	57	1216
GGAGGAucAAcGAcGAGAudTdT	sense	56	1217
AUCUCGUCGUUGAUCCUCCdTdT	antis	56	1218
uuuuGAGAAuccAuAuGuAdTdT	sense	369	1219
uAcAuAUGGAUUCUcAAAAdTdT	antis	369	1220
cAAGGAAGcccGGAGGAucdTdT	sense	45	1221
GAUCCUCCGGGCUUCCUUGdTdT	antis	45	1222
ucuGAcucuAccAAAuAcudTdT	sense	460	1223
AGuAUUUGGuAGAGUcAGAdTdT	antis	460	1224
AAGcGcGAcGccсGccGGGdTdT	sense	97	1225
CCCGGCGGGCGUCGCGCUUdTdT	antis	97	1226
AcAAuAAGGcucAuGcAcAdTdT	sense	314	1227

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
UGUGcAUGAGCCUuAUUGUdTdT	antis	314	1228
uAAGGcucAuGcAcAAuuGdTdT	sense	318	1229
cAAUUGUGcAUGAGCCUuAdTdT	antis	318	1230
AAGcccGGAGGAucAAcGAdTdT	sense	50	1231
UCGUUGAUCCUCCGGGCUUdTdT	antis	50	1232
cucAuGcAcAAuuGGuucGdTdT	sense	323	1233
CGAACcAAUUGUGcAUGAGdTdT	antis	323	1234
uGcAcAAuuGGuucGAGAGdTdT	sense	327	1235
CUCUCGAACcAAUUGUGcAdTdT	antis	327	1236
cAcAAuuGGuucGAGAGGudTdT	sense	329	1237
ACCUCUCGAACcAAUUGUGdTdT	antis	329	1238
cuAGucGAcuAcuucccAGdTdT	sense	862	1239
CUGGGAAGuAGUCGACuAGdTdT	antis	862	1240
GcAGGGAcAAGcGcGAcGcdTdT	sense	89	1241
GCGUCGCGCUUGUCCCUGCdTdT	antis	89	1242
uuGAGAAuccAuAuGuAGAdTdT	sense	371	1243
UCuAcAuAUGGAUUCUcAAdTdT	antis	371	1244
GAcuAcuucccAGAAuAuGdTdT	sense	868	1245
cAuAUUCUGGGAAGuAGUCdTdT	antis	868	1246
ucAAcGAcGAGAucGAGcGdTdT	sense	62	1247
CGCUCGAUCUCGUCGUUGAdTdT	antis	62	1248
Modifications: Sense strand - all pyrimidines (U, C) are 2'OMe; antisense strand – pyrimidines adjacent to A (UA, CA) are 2'Ome; 3' end is thio (dTsdT)			
uAuucccAccuAGucGAcudTsdT	sense	853	1249
AGUCGACuAGGUGGGAAuAdTsdT	antis	853	1250
uucccAccuAGucGAcuAcdTsdT	sense	855	1251
GuAGUCGACuAGGUGGGAAdTsdT	antis	855	1252
GcuuuuGAGAAuccAuAuGdTsdT	sense	367	1253
cAuAUGGAUUCUcAAAAGCdTsdT	antis	367	1254
cGGAGGAucAAcGAcGAGAdTsdT	sense	55	1255
UCUCGUCGUUGAUCCUCCGdTsdT	antis	55	1256
AucuGAcucuAccAAAuAcdTsdT	sense	459	1257
GuAUUUGGuAGAGUcAGAUdTsdT	antis	459	1258
AcAcAAuAAGGcucAuGcAdTsdT	sense	312	1259
UGcAUGAGCCUuAUUGUGUdTsdT	antis	312	1260
AGGAucAuccAcGGGucGGdTsdT	sense	178	1261
CCGACCCGUGGAUGAUCCUdTsdT	antis	178	1262
cccAuAcAAGuAuGAAcAcdTsdT	sense	297	1263
GUGUUcAuACUUGuAUGGGdTsdT	antis	297	1264
cAAuAAGGcucAuGcAcAAdTsdT	sense	315	1265
UUGUGcAUGAGCCUuAUUGdTsdT	antis	315	1266
AGGAucAAcGAcGAGAucGdTsdT	sense	58	1267
CGAUCUCGUCGUUGAUCCUdTsdT	antis	58	1268
ucAuGcAcAAuuGGuucGAdTsdT	sense	324	1269
UCGAACcAAUUGUGcAUGAdTsdT	antis	324	1270

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
GGAucAAcGAcGAGAucGAdTsdT	sense	59	1271
UCGAUCUCGUCGUUGAUCCdTsdT	antis	59	1272
AGAGcuuGuGGAAuGAuccdTsdT	sense	398	1273
GGAUcAUUCcAcAAGCUCUdTsdT	antis	398	1274
GAGGAucAAcGAcGAGAucdTsdT	sense	57	1275
GAUCUCGUCGUUGAUCCUCdTsdT	antis	57	1276
GGAGGAucAAcGAcGAGAudTsdT	sense	56	1277
AUCUCGUCGUUGAUCCUCCdTsdT	antis	56	1278
uuuuGAGAAuccAuAuGuAdTsdT	sense	369	1279
uAcAuAUGGAUUCUcAAAAdTsdT	antis	369	1280
cAAGGAAGcccGGAGGAucdTsdT	sense	45	1281
GAUCCUCCGGGCUUCCUUGdTsdT	antis	45	1282
ucuGAcucuAccAAAuAcudTsdT	sense	460	1283
AGuAUUUGGuAGAGUcAGAdTsdT	antis	460	1284
AAGcGcGAcGcccGccGGGdTsdT	sense	97	1285
CCCGGCGGGCGUCGCGCUUdTsdT	antis	97	1286
AcAAuAAGGcucAuGcAcAdTsdT	sense	314	1287
UGUGcAUGAGCCUuAUUGUdTsdT	antis	314	1288
uAAGGcucAuGcAcAAuuGdTsdT	sense	318	1289
cAAUUGUGcAUGAGCCUuAdTsdT	antis	318	1290
AAGcccGGAGGAucAAcGAdTsdT	sense	50	1291
UCGUUGAUCCUCCGGGCUUdTsdT	antis	50	1292
cucAuGcAcAAuuGGuucGdTsdT	sense	323	1293
CGAACcAAUUGUGcAUGAGdTsdT	antis	323	1294
uGcAcAAuuGGuucGAGAGdTsdT	sense	327	1295
CUCUCGAACcAAUUGUGcAdTsdT	antis	327	1296
cAcAAuuGGuucGAGAGGudTsdT	sense	329	1297
ACCUCUCGAACcAAUUGUGdTsdT	antis	329	1298
cuAGucGAcuAcuucccAGdTsdT	sense	862	1299
CUGGGAAGuAGUCGACuAGdTsdT	antis	862	1300
GcAGGGAcAAGcGcGAcGcdTsdT	sense	89	1301
GCGUCGCGCUUGUCCCUGCdTsdT	antis	89	1302
uuGAGAAuccAuAuGuAGAdTsdT	sense	371	1303
UCuAcAuAUGGAUUCUcAAdTsdT	antis	371	1304
GAcuAcuucccAGAAuAuGdTsdT	sense	868	1305
cAuAUUCUGGGAAGuAGUCdTsdT	antis	868	1306
ucAAcGAcGAGAucGAGcGdTsdT	sense	62	1307
CGCUCGAUCUCGUCGUUGAdTsdT	antis	62	1308

Modifications: Sense strand – all pyrimidines are 2'OMe; antisense strand – pyrimidines adjacent to A (UA, CA) + U adjacent to another U (UU) or G (UG) are 2'Ome; 3' end is thio (dTsdT).

uAuucccAccuAGucGAcudTsdT	sense	853	1309
AGUCGACuAGGuGGGAAuAdTsdT	antis	853	1310
uucccAccuAGucGAcuAcdTsdT	sense	855	1311
GuAGUCGACuAGGuGGGAAdTsdT	antis	855	1312
GcuuuuGAGAAuccAuAuGdTsdT	sense	367	1313

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
cAuAuGGAuUCUcAAAAGCdTsdT	antis	367	1314
cGGAGGAucAAcGAcGAGAdTsdT	sense	55	1315
UCUCGUCGuuGAUCCUCCGdTsdT	antis	55	1316
AucuGAcucuAccAAAuAcdTsdT	sense	459	1317
GuAuUuGGuAGAGUcAGAUdTsdT	antis	459	1318
AcAcAAuAAGGcucAuGcAdTsdT	sense	312	1319
uGcAuGAGCCUuAuuGuGUdTsdT	antis	312	1320
AGGAucAuccAcGGGucGGdTsdT	sense	178	1321
CCGACCCGuGGAuGAUCCUdTsdT	antis	178	1322
cccAuAcAAGuAuGAAcAcdTsdT	sense	297	1323
GuGuUcAuACuuGuAuGGGdTsdT	antis	297	1324
cAAuAAGGcucAuGcAcAAdTsdT	sense	315	1325
uuGuGcAuGAGCCUuAuuGdTsdT	antis	315	1326
AGGAucAAcGAcGAGAucGdTsdT	sense	58	1327
CGAUCUCGUCGuuGAUCCUdTsdT	antis	58	1328
ucAuGcAcAAuuGGuucGAdTsdT	sense	324	1329
UCGAACcAAuuGuGcAuGAdTsdT	antis	324	1330
GGAucAAcGAcGAGAucGAdTsdT	sense	59	1331
UCGAUCUCGUCGuuGAUCCdTsdT	antis	59	1332
AGAGcuuGuGGAAuGAuccdTsdT	sense	398	1333
GGAUcAuUCcAcAAGCUCUdTsdT	antis	398	1334
GAGGAucAAcGAcGAGAucdTsdT	sense	57	1335
GAUCUCGUCGuuGAUCCUCdTsdT	antis	57	1336
GGAGGAucAAcGAcGAGAudTsdT	sense	56	1337
AUCUCGUCGuuGAUCCUCCdTsdT	antis	56	1338
uuuuGAGAAuccAuAuGuAdTsdT	sense	369	1339
uAcAuAuGGAuUCUcAAAAdTsdT	antis	369	1340
cAAGGAAGcccGGAGGAucdTsdT	sense	45	1341
GAUCCUCCGGGCuUCCuuGdTsdT	antis	45	1342
ucuGAcucuAccAAAuAcudTsdT	sense	460	1343
AGuAuUuGGuAGAGUcAGAdTsdT	antis	460	1344
AAGcGcGAcGcccGccGGGdTsdT	sense	97	1345
CCCGGCGGGCGUCGCGCuUdTsdT	antis	97	1346
AcAAuAAGGcucAuGcAcAdTsdT	sense	314	1347
uGuGcAuGAGCCUuAuuGUdTsdT	antis	314	1348
uAAGGcucAuGcAcAAuuGdTsdT	sense	318	1349
cAAuuGuGcAuGAGCCUuAdTsdT	antis	318	1350
AAGcccGGAGGAucAAcGAdTsdT	sense	50	1351
UCGuuGAUCCUCCGGGCuUdTsdT	antis	50	1352
cucAuGcAcAAuuGGuucGdTsdT	sense	323	1353
CGAACcAAuuGuGcAuGAGdTsdT	antis	323	1354
uGcAcAAuuGGuucGAGAGdTsdT	sense	327	1355
CUCUCGAACcAAuuGuGcAdTsdT	antis	327	1356
cAcAAuuGGuucGAGAGGudTsdT	sense	329	1357
ACCUCUCGAACcAAuuGuGdTsdT	antis	329	1358

SEQUENCE (5'-3')	Strand	Start of target sequence	SEQ ID NO:
cuAGucGAcuAcuucccAGdTsdT	sense	862	1359
CuGGGAAGuAGUCGACuAGdTsdT	antis	862	1360
GcAGGGAcAAGcGcGAcGcdTsdT	sense	89	1361
GCGUCGCGCuuGUCCCuGCdTsdT	antis	89	1362
uuGAGAAuccAuAuGuAGAdTsdT	sense	371	1363
UCuAcAuAuGGAuUCUcAAdTsdT	antis	371	1364
GAcuAcuucccAGAAuAuGdTsdT	sense	868	1365
cAuAuUCuGGGAAGuAGUCdTsdT	antis	868	1366
ucAAcGAcGAGAucGAGcGdTsdT	sense	62	1367
CGCUCGAUCUCGUCGuuGAdTsdT	antis	62	1368

Example 3: *In vitro* screening

For *in vitro* screening, cells expressing GNAQ were utilized. Some exemplary cell lines expressing GNAQ include, but are not limited to, human melanoma cell lines OMM1.3 and MEL 285, and Mel 202. OMM1.3 are liver metastisis cells that include a mutant GNAQ gene. MEL285 are primary uveal melanoma cells that include a WT GNAQ gene. MEL202 are also primary uveal melanoma but include a mutant GNAQ gene. A549 (lung carcinoma) and A375 (malignant melanoma) are cancer cell lines expressing WT GNAQ.

Cells expressing human GNAQ with the activating GNAQ mutation were obtained following the method outlined in PCT publication number WO2008/098208, which is incorporated herein in its entirety for all purposes.

The dsRNAs were screened for *in vitro* inhibition of the target gene. Tissue culture cells were transfected with the dsRNA. Target gene mRNA levels were assayed using qPCR (real time PCR).

Cell culture and transfections:

A549, A375, OMM1.3 and UMEL202 cells were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in specific medium (ATCC) supplemented with 10% FBS, streptomycin, and glutamine (ATCC) before being released from the plate by trypsinization. Reverse transfection was carried out by adding 5μl of Opti-MEM to 5μl of siRNA duplexes (Tables 5-7) per well into a 96-well plate along with 10μl of Opti-MEM plus 0.2μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) and incubated at room temperature for 15 minutes. 80μl of complete growth media without antibiotics containing $2x10^4$ cells were then added. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at either 0.1 nM, 1.0 nM, or and 10.0 nM final duplex concentration and dose response experiments were done with 10, 1.66, 0.27, 0.046, 0.0077, 0.0012, 0.00021, 0.000035 nM of selected duplexes.

Table 5: Duplex (dsRNA) names and corresponding sample names

Sample name	Duplex Name	ssRNA name
1	AD-20032	36864
		36865
2	AD-20033	36866
		36867
3	AD-20034	36868
		36869
4	AD-20035	36870
		36871
5	AD-20036	36872
		36873
6	AD-20037	36874
		36875

Sample name	Duplex Name	ssRNA name
7	AD-20038	36876
		36877
8	AD-20039	36878
		36879
9	AD-20040	36880
		36881
10	AD-20041	36882
		36883
11	AD-20042	36884
		36885
12	AD-20043	36886
		36887
13	AD-20044	36888
		36889
14	AD-20045	36890
		36891
15	AD-20046	36892
		36893
16	AD-20047	36894
		36895
17	AD-20048	36896
		36897
18	AD-20049	36898
		36899
19	AD-20050	36900
		36901
20	AD-20051	36902
		36903
21	AD-20052	36904
		36905
22	AD-20053	36906
		36907
23	AD-20054	36910
		36911
24	AD-20055	36912
		36913
25	AD-20056	36914
		36915
26	AD-20057	36916
		36917
27	AD-20058	36918
		36919
28	AD-20059	36920
		36921
29	AD-20060	36922
		36923
30	AD-20061	36924
		36925
31	AD-20062	36926
		36927
32	AD-20063	36928
		36929
33	AD-20064	36930
		36931
34	AD-20065	36932
		36933
35	AD-20066	36934
		36935
36	AD-20067	36936

Sample name	Duplex Name	ssRNA name
		36937
37	AD-20068	36938
		36939
38	AD-20069	36940
		36941
39	AD-20070	36942
		36943
40	AD-20071	36946
		36947
41	AD-20072	36948
		36949
42	AD-20073	36950
		36951
43	AD-20074	36954
		36955
87	AD-20075	36956
		36957
44	AD-20076	36958
		36959
45	AD-20077	36960
		36961
46	AD-20078	36962
		36963
47	AD-20079	36964
		36965
48	AD-20080	36966
		36967
49	AD-20081	36968
		36969
50	AD-20082	36970
		36971
51	AD-20083	36972
		36973
52	AD-20084	36974
		36975
53	AD-20085	36976
		36977
54	AD-20086	36978
		36979
55	AD-20087	36980
		36981
56	AD-20088	36982
		36983
57	AD-20089	36984
		36985
58	AD-20090	36986
		36987
59	AD-20091	36988
		36989
60	AD-20092	36990
		36991
61	AD-20093	36992
		36993
62	AD-20094	36994
		36995
63	AD-20095	36996
		36997
64	AD-20096	36998
		36999

Sample name	Duplex Name	ssRNA name
65	AD-20097	37000
		37001
88	AD-20098	37002
		37003
66	AD-20099	37004
		37005
67	AD-20100	37006
		37007
68	AD-20101	37008
		37009
69	AD-20102	37010
		37011
89	AD-20103	37012
		37013
70	AD-20104	37014
		37015
95	AD-20105	37016
		37017
71	AD-20106	37018
		37019
72	AD-20107	37022
		37023
73	AD-20108	37024
		37025
74	AD-20109	37026
		37027
75	AD-20110	37032
		37033
76	AD-20111	37034
		37035
77	AD-20112	37036
		37037
78	AD-20113	37038
		37039
79	AD-20114	37040
		37041
80	AD-20115	37042
		37043
81	AD-20116	37044
		37045
82	AD-20117	37046
		37047
83	AD-20118	37048
		37049
84	AD-20119	37050
		37051
85	AD-20120	37052
		37053
86	AD-20121	37054
		37055
91	AD-20193	36908
		36909
92	AD-20194	36945
		36944
93	AD-20195	37020
		37021
94	AD-20196	37028
		37029
95	AD-20197	37030

Sample name	Duplex Name	ssRNA name
		37031

Table 6: Sequences of dsRNA targeting Human GNAQ (NM_002072.2)

(target is position of 5' base on transcript of NM_002072.2

Duplex name	Strand	Target	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
AD-20032	S	1215	1369	UACUAAUUUAUUGCCGUCC	1527	uAcuAAuuuAuuGccGuccdTdT
	A	1215	1370	GGACGGCAAUAAAUUAGUA	1528	GGACGGcAAuAAAUuAGuAdTdT
AD-20033	S	1217	1371	CUAAUUUAUUGCCGUCCUG	1529	cuAAuuuAuuGccGuccuGdTdT
	A	1217	1372	CAGGACGGCAAUAAAUUAG	1530	cAGGACGGcAAuAAAUuAGdTdT
AD-20034	S	1216	1373	ACUAAUUUAUUGCCGUCCU	1531	AcuAAuuuAuuGccGuccudTdT
	A	1216	1374	AGGACGGCAAUAAAUUAGU	1532	AGGACGGcAAuAAAUuAGUdTdT
AD-20035	S	1322	1375	GUACAGUCCCAGCACAUUU	1533	GuAcAGucccAGcAcAuuudTdT
	A	1322	1376	AAAUGUGCUGGGACUGUAC	1534	AAAUGUGCUGGGACUGuACdTdT
AD-20036	S	1220	1377	AUUUAUUGCCGUCCUGGAC	1535	AuuuAuuGccGuccuGGAcdTdT
	A	1220	1378	GUCCAGGACGGCAAUAAAU	1536	GUCcAGGACGGcAAuAAAUdTdT
AD-20037	S	1265	1379	GUAGUAAAUAUUAUGAUUU	1537	GuAGuAAAuAuuAuGAuuudTdT
	A	1265	1380	AAAUCAUAAUAUUUACUAC	1538	AAAUcAuAAuAUUuACuACdTdT
AD-20038	S	1218	1381	UAAUUUAUUGCCGUCCUGG	1539	uAAuuuAuuGccGuccuGGdTdT
	A	1218	1382	CCAGGACGGCAAUAAAUUA	1540	CcAGGACGGcAAuAAAUuAdTdT
AD-20039	S	1175	1383	ACAAGAGGGACUGUAUUUC	1541	AcAAGAGGGAcuGuAuuucdTdT
	A	1175	1384	GAAAUACAGUCCCUCUUGU	1542	GAAAuAcAGUCCCUCUUGUdTdT
AD-20040	S	1223	1385	UAUUGCCGUCCUGGACUCU	1543	uAuuGccGuccuGGAcucudTdT
	A	1223	1386	AGAGUCCAGGACGGCAAUA	1544	AGAGUCcAGGACGGcAAuAdTdT
AD-20041	S	1319	1387	GAAGUACAGUCCCAGCACA	1545	GAAGuAcAGucccAGcAcAdTdT
	A	1319	1388	UGUGCUGGGACUGUACUUC	1546	UGUGCUGGGACUGuACUUCdTdT
AD-20042	S	1285	1389	AUUUAAACUAUUCAGAGGA	1547	AuuuAAAcuAuucAGAGGAdTdT
	A	1285	1390	UCCUCUGAAUAGUUUAAAU	1548	UCCUCUGAAuAGUUuAAAUdTdT
AD-20043	S	1213	1391	AAUACUAAUUUAUUGCCGU	1549	AAuAcuAAuuuAuuGccGudTdT
	A	1213	1392	ACGGCAAUAAAUUAGUAUU	1550	ACGGcAAuAAAUuAGuAUUdTdT
AD-20044	S	1810	1393	CAGCCAUAGCUUGAUUGCU	1551	cAGccAuAGcuuGAuuGcudTdT
	A	1810	1394	AGCAAUCAAGCUAUGGCUG	1552	AGcAAUcAAGCuAUGGCUGdTdT
AD-20045	S	1590	1395	GUCAGGACACAUCGUUCGA	1553	GucAGGAcAcAucGuucGAdTdT
	A	1590	1396	UCGAACGAUGUGUCCUGAC	1554	UCGAACGAUGUGUCCUGACdTdT
AD-20046	S	1149	1397	CUUCCCUGGUGGGCUAUUG	1555	cuucccuGGuGGGcuAuuGdTdT
	A	1149	1398	CAAUAGCCCACCAGGGAAG	1556	cAAuAGCCcACcAGGGAAGdTdT
AD-20047	S	1971	1399	GACACUACAUUACCCUAAU	1557	GAcAcuAcAuuAcccuAAudTdT
	A	1971	1400	AUUAGGGUAAUGUAGUGUC	1558	AUuAGGGuAAUGuAGUGUCdTdT
AD-20048	S	1237	1401	ACUCUGUGUGAGCGUGUCC	1559	AcucuGuGuGAGcGuGuccdTdT
	A	1237	1402	GGACACGCUCACACAGAGU	1560	GGAcACGCUcAcAcAGAGUdTdT
AD-20049	S	1152	1403	CCCUGGUGGGCUAUUGAAG	1561	cccuGGuGGGcuAuuGAAGdTdT
	A	1152	1404	CUUCAAUAGCCCACCAGGG	1562	CUUcAAuAGCCcACcAGGGdTdT
AD-20050	S	1575	1405	CUCUCAAAUGAUACAGUCA	1563	cucucAAAuGAuAcAGucAdTdT

Duplex name	Strand	Target	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
	A	1575	1406	UGACUGUAUCAUUUGAGAG	1564	UGACUGuAUcAUUUGAGAGdTdT
AD-20051	S	1105	1407	AGUACAAUCUGGUCUAAUU	1565	AGuAcAAucuGGucuAAuudTdT
	A	1105	1408	AAUUAGACCAGAUUGUACU	1566	AAUuAGACcAGAUUGuACUdTdT
AD-20052	S	1407	1409	CACAAAGAUAAGACUUGUU	1567	cAcAAAGAuAAGAcuuGuudTdT
	A	1407	1410	AACAAGUCUUAUCUUUGUG	1568	AAcAAGUCUuAUCUUUGUGdTdT
AD-20053	S	1108	1411	ACAAUCUGGUCUAAUUGUG	1569	AcAAucuGGucuAAuuGuGdTdT
	A	1108	1412	CACAAUUAGACCAGAUUGU	1570	cAcAAUuAGACcAGAUUGUdTdT
AD-20193	S	1395	1413	CAGUCAUGCACUCACAAAG	1571	cAGucAuGcAcucAcAAAGdTdT
	A	1395	1414	CUUUGUGAGUGCAUGACUG	1572	CUUUGUGAGUGcAUGACUGdTdT
AD-20054	S	1595	1415	GACACAUCGUUCGAUUUAA	1573	GAcAcAucGuucGAuuuAAdTdT
	A	1595	1416	UUAAAUCGAACGAUGUGUC	1574	UuAAAUCGAACGAUGUGUCdTdT
AD-20055	S	1992	1417	CUGCUACCCAGAACCUUUU	1575	cuGcuAcccAGAAccuuuudTdT
	A	1992	1418	AAAAGGUUCUGGGUAGCAG	1576	AAAAGGUUCUGGGuAGcAGdTdT
AD-20056	S	1809	1419	UCAGCCAUAGCUUGAUUGC	1577	ucAGccAuAGcuuGAuuGcdTdT
	A	1809	1420	GCAAUCAAGCUAUGGCUGA	1578	GcAAUcAAGCuAUGGCUGAdTdT
AD-20057	S	1203	1421	CAAUUUGCAUAAUACUAAU	1579	cAAuuuGcAuAAuAcuAAudTdT
	A	1203	1422	AUUAGUAUUAUGCAAAUUG	1580	AUuAGuAUuAUGcAAAUUGdTdT
AD-20058	S	1804	1423	UACCUUCAGCCAUAGCUUG	1581	uAccuucAGccAuAGcuuGdTdT
	A	1804	1424	CAAGCUAUGGCUGAAGGUA	1582	cAAGCuAUGGCUGAAGGuAdTdT
AD-20059	S	1968	1425	ACAGACACUACAUUACCCU	1583	AcAGAcAcuAcAuuAcccudTdT
	A	1968	1426	AGGGUAAUGUAGUGUCUGU	1584	AGGGuAAUGuAGUGUCUGUdTdT
AD-20060	S	1214	1427	AUACUAAUUUAUUGCCGUC	1585	AuAcuAAuuuAuuGccGucdTdT
	A	1214	1428	GACGGCAAUAAAUUAGUAU	1586	GACGGcAAuAAAUuAGuAUdTdT
AD-20061	S	1159	1429	GGGCUAUUGAAGAUACACA	1587	GGGcuAuuGAAGAuAcAcAdTdT
	A	1159	1430	UGUGUAUCUUCAAUAGCCC	1588	UGUGuAUCUUcAAuAGCCCdTdT
AD-20062	S	1603	1431	GUUCGAUUUAAGCCAUCAU	1589	GuucGAuuuAAGccAucAudTdT
	A	1603	1432	AUGAUGGCUUAAAUCGAAC	1590	AUGAUGGCUuAAAUCGAACdTdT
AD-20063	S	1123	1433	UGUGCCUCCUAGACACCCG	1591	uGuGccuccuAGAcAcccGdTdT
	A	1123	1434	CGGGUGUCUAGGAGGCACA	1592	CGGGUGUCuAGGAGGcAcAdTdT
AD-20064	S	1233	1435	CUGGACUCUGUGUGAGCGU	1593	cuGGAcucuGuGuGAGcGudTdT
	A	1233	1436	ACGCUCACACAGAGUCCAG	1594	ACGCUcAcAcAGAGUCcAGdTdT
AD-20065	S	1930	1437	ACCCUCUCUUUCAAUUGCA	1595	AcccucucuuucAAuuGcAdTdT
	A	1930	1438	UGCAAUUGAAAGAGAGGGU	1596	UGcAAUUGAAAGAGAGGGUdTdT
AD-20066	S	1969	1439	CAGACACUACAUUACCCUA	1597	cAGAcAcuAcAuuAcccuAdTdT
	A	1969	1440	UAGGGUAAUGUAGUGUCUG	1598	uAGGGuAAUGuAGUGUCUGdTdT
AD-20067	S	1219	1441	AAUUUAUUGCCGUCCUGGA	1599	AAuuuAuuGccGuccuGGAdTdT
	A	1219	1442	UCCAGGACGGCAAUAAAUU	1600	UCcAGGACGGcAAuAAAUUdTdT
AD-20068	S	1241	1443	UGUGUGAGCGUGUCCACAG	1601	uGuGuGAGcGuGuccAcAGdTdT
	A	1241	1444	CUGUGGACACGCUCACACA	1602	CUGUGGAcACGCUcAcAcAdTdT
AD-20069	S	1153	1445	CCUGGUGGGCUAUUGAAGA	1603	ccuGGuGGGcuAuuGAAGAdTdT
	A	1153	1446	UCUUCAAUAGCCCACCAGG	1604	UCUUcAAuAGCCcACcAGGdTdT
AD-20070	S	1805	1447	ACCUUCAGCCAUAGCUUGA	1605	AccuucAGccAuAGcuuGAdTdT
	A	1805	1448	UCAAGCUAUGGCUGAAGGU	1606	UcAAGCuAUGGCUGAAGGUdTdT

Duplex name	Strand	Target	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
AD-20194	S	1312	1449	GGAUGCUGAAGUACAGUCC	1607	GGAuGcuGAAGuAcAGuccdTdT
	A	1312	1450	GGACUGUACUUCAGCAUCC	1608	GGACUGuACUUcAGcAUCCdTdT
AD-20071	S	1546	1451	AUCCUAGUUCCAUUCUUGG	1609	AuccuAGuuccAuucuuGGdTdT
	A	1546	1452	CCAAGAAUGGAACUAGGAU	1610	CcAAGAAUGGAACuAGGAUdTdT
AD-20072	S	1547	1453	UCCUAGUUCCAUUCUUGGU	1611	uccuAGuuccAuucuuGGudTdT
	A	1547	1454	ACCAAGAAUGGAACUAGGA	1612	ACcAAGAAUGGAACuAGGAdTdT
AD-20073	S	1103	1455	GGAGUACAAUCUGGUCUAA	1613	GGAGuAcAAucuGGucuAAdTdT
	A	1103	1456	UUAGACCAGAUUGUACUCC	1614	UuAGACcAGAUUGuACUCCdTdT
	A	1334	1457	CACAUUUCCUCUCUAUCUU	1615	cAcAuuuccucucuAucuudTdT
	A	1334	1458	AAGAUAGAGAGGAAAUGUG	1616	AAGAuAGAGAGGAAAUGUGdTdT
AD-20074	S	1255	1459	CACAGAGUUUGUAGUAAAU	1617	cAcAGAGuuuGuAGuAAAudTdT
	A	1255	1460	AUUUACUACAAACUCUGUG	1618	AUUuACuAcAAACUCUGUGdTdT
AD-20075	S	1967	1461	AACAGACACUACAUUACCC	1619	AAcAGAcAcuAcAuuAcccdTdT
	A	1967	1462	GGGUAAUGUAGUGUCUGUU	1620	GGGuAAUGuAGUGUCUGUUdTdT
AD-20076	S	1391	1463	UUCUCAGUCAUGCACUCAC	1621	uucucAGucAuGcAcucAcdTdT
	A	1391	1464	GUGAGUGCAUGACUGAGAA	1622	GUGAGUGcAUGACUGAGAAdTdT
AD-20077	S	1124	1465	GUGCCUCCUAGACACCCGC	1623	GuGccuccuAGAcAcccGcdTdT
	A	1124	1466	GCGGGUGUCUAGGAGGCAC	1624	GCGGGUGUCuAGGAGGcACdTdT
AD-20078	S	1612	1467	AAGCCAUCAUCAGCUUAAU	1625	AAGccAucAucAGcuuAAudTdT
	A	1612	1468	AUUAAGCUGAUGAUGGCUU	1626	AUuAAGCUGAUGAUGGCUUdTdT
AD-20079	S	1933	1469	CUCUCUUUCAAUUGCAGAU	1627	cucucuuucAAuuGcAGAudTdT
	A	1933	1470	AUCUGCAAUUGAAAGAGAG	1628	AUCUGcAAUUGAAAGAGAGdTdT
AD-20080	S	1078	1471	ACACCAUCCUCCAGUUGAA	1629	AcAccAuccuccAGuuGAAdTdT
	A	1078	1472	UUCAACUGGAGGAUGGUGU	1630	UUcAACUGGAGGAUGGUGUdTdT
AD-20081	S	1545	1473	UAUCCUAGUUCCAUUCUUG	1631	uAuccuAGuuccAuucuuGdTdT
	A	1545	1474	CAAGAAUGGAACUAGGAUA	1632	cAAGAAUGGAACuAGGAuAdTdT
AD-20082	S	1109	1475	CAAUCUGGUCUAAUUGUGC	1633	cAAucuGGucuAAuuGuGcdTdT
	A	1109	1476	GCACAAUUAGACCAGAUUG	1634	GcAcAAUuAGACcAGAUUGdTdT
AD-20083	S	1398	1477	UCAUGCACUCACAAAGAUA	1635	ucAuGcAcucAcAAAGAuAdTdT
	A	1398	1478	UAUCUUUGUGAGUGCAUGA	1636	uAUCUUUGUGAGUGcAUGAdTdT
AD-20084	S	1970	1479	AGACACUACAUUACCCUAA	1637	AGAcAcuAcAuuAcccuAAdTdT
	A	1970	1480	UUAGGGUAAUGUAGUGUCU	1638	UuAGGGuAAUGuAGUGUCUdTdT
AD-20085	S	1173	1481	ACACAAGAGGGACUGUAUU	1639	AcAcAAGAGGGAcuGuAuudTdT
	A	1173	1482	AAUACAGUCCCUCUUGUGU	1640	AAuAcAGUCCCUCUUGUGUdTdT
AD-20086	S	1313	1483	GAUGCUGAAGUACAGUCCC	1641	GAuGcuGAAGuAcAGucccdTdT
	A	1313	1484	GGGACUGUACUUCAGCAUC	1642	GGGACUGuACUUcAGcAUCdTdT
AD-20087	S	1811	1485	AGCCAUAGCUUGAUUGCUC	1643	AGccAuAGcuuGAuuGcucdTdT
	A	1811	1486	GAGCAAUCAAGCUAUGGCU	1644	GAGcAAUcAAGCuAUGGCUdTdT
AD-20088	S	1862	1487	CACAGGAGUCCUUUCUUUU	1645	cAcAGGAGuccuuucuuuudTdT
	A	1862	1488	AAAAGAAAGGACUCCUGUG	1646	AAAAGAAAGGACUCCUGUGdTdT
AD-20089	S	1600	1489	AUCGUUCGAUUUAAGCCAU	1647	AucGuucGAuuuAAGccAudTdT
	A	1600	1490	AUGGCUUAAAUCGAACGAU	1648	AUGGCUuAAAUCGAACGAUdTdT
AD-20090	S	1618	1491	UCAUCAGCUUAAUUUAAGU	1649	ucAucAGcuuAAuuuAAGudTdT

Duplex name	Strand	Target	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
	A	1618	1492	ACUUAAAUUAAGCUGAUGA	1650	ACUuAAAUuAAGCUGAUGAdTdT
AD-20091	S	1332	1493	AGCACAUUUCCUCUCUAUC	1651	AGcAcAuuuccucucuAucdTdT
	A	1332	1494	GAUAGAGAGGAAAUGUGCU	1652	GAuAGAGAGGAAAUGUGCUdTdT
AD-20092	S	1157	1495	GUGGGCUAUUGAAGAUACA	1653	GuGGGcuAuuGAAGAuAcAdTdT
	A	1157	1496	UGUAUCUUCAAUAGCCCAC	1654	UGuAUCUUcAAuAGCCcACdTdT
AD-20093	S	888	1497	AUCAUGUAUUCCCAUCUAG	1655	AucAuGuAuucccAucuAGdTdT
	A	888	1498	CUAGAUGGGAAUACAUGAU	1656	CuAGAUGGGAAuAcAUGAUdTdT
AD-20094	S	1855	1499	AAAGACACACAGGAGUCCU	1657	AAAGAcAcAcAGGAGuccudTdT
	A	1855	1500	AGGACUCCUGUGUGUCUUU	1658	AGGACUCCUGUGUGUCUUUdTdT
AD-20095	S	1579	1501	CAAAUGAUACAGUCAGGAC	1659	cAAAuGAuAcAGucAGGAcdTdT
	A	1579	1502	GUCCUGACUGUAUCAUUUG	1660	GUCCUGACUGuAUcAUUUGdTdT
AD-20096	S	805	1503	UUAGAACAAUUAUCACAUA	1661	uuAGAAcAAuuAucAcAuAdTdT
	A	805	1504	UAUGUGAUAAUUGUUCUAA	1662	uAUGUGAuAAUUGUUCuAAdTdT
AD-20097	S	1554	1505	UCCAUUCUUGGUCAAGUUU	1663	uccAuucuuGGucAAGuuudTdT
	A	1554	1506	AAACUUGACCAAGAAUGGA	1664	AAACUUGACcAAGAAUGGAdTdT
AD-20098	S	1113	1507	CUGGUCUAAUUGUGCCUCC	1665	cuGGucuAAuuGuGccuccdTdT
	A	1113	1508	GGAGGCACAAUUAGACCAG	1666	GGAGGcAcAAUuAGACcAGdTdT
AD-20099	S	1174	1509	CACAAGAGGGACUGUAUUU	1667	cAcAAGAGGGAcuGuAuuudTdT
	A	1174	1510	AAAUACAGUCCCUCUUGUG	1668	AAAuAcAGUCCCUCUUGUGdTdT
AD-20100	S	1735	1511	UCUUGUCUCACUUUGGACU	1669	ucuuGucucAcuuuGGAcudTdT
	A	1735	1512	AGUCCAAAGUGAGACAAGA	1670	AGUCcAAAGUGAGAcAAGAdTdT
AD-20101	S	1450	1513	UUUUCUAUGGAGCAAAACA	1671	uuuucuAuGGAGcAAAAcAdTdT
	A	1450	1514	UGUUUUGCUCCAUAGAAAA	1672	UGUUUUGCUCcAuAGAAAAdTdT
AD-20102	S	804	1515	UUUAGAACAAUUAUCACAU	1673	uuuAGAAcAAuuAucAcAudTdT
	A	804	1516	AUGUGAUAAUUGUUCUAAA	1674	AUGUGAuAAUUGUUCuAAAdTdT
AD-20103	S	1866	1517	GGAGUCCUUUCUUUUGAAA	1675	GGAGuccuuucuuuuGAAAdTdT
	A	1866	1518	UUUCAAAAGAAAGGACUCC	1676	UUUcAAAAGAAAGGACUCCdTdT
AD-20104	S	1610	1519	UUAAGCCAUCAUCAGCUUA	1677	uuAAGccAucAucAGcuuAdTdT
	A	1610	1520	UAAGCUGAUGAUGGCUUAA	1678	uAAGCUGAUGAUGGCUuAAdTdT
AD-20105	S	1117	1521	UCUAAUUGUGCCUCCUAGA	1679	ucuAAuuGuGccuccuAGAdTdT
	A	1117	1522	UCUAGGAGGCACAAUUAGA	1680	UCuAGGAGGcAcAAUuAGAdTdT
AD-20106	S	1320	1523	AAGUACAGUCCCAGCACAU	1681	AAGuAcAGucccAGcAcAudTdT
	A	1320	1524	AUGUGCUGGGACUGUACUU	1682	AUGUGCUGGGACUGuACUUdTdT
AD-20195	S	1317	1525	CUGAAGUACAGUCCCAGCA	1683	cuGAAGuAcAGucccAGcAdTdT
	A	1317	1526	UGCUGGGACUGUACUUCAG	1684	UGCUGGGACUGuACUUcAGdTdT

Table 7a – – Sequences of dsRNA targeting Mouse GNAQ (NM_031036)

(target is position of 5' base on transcript of NM_031036

Duplex Name	Strand	Target	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
AD-20107	S	853	1685	UAUUCCCACCUAGUCGACU	1719	uAuucccAccuAGucGAcudTdT
	A	853	1686	AGUCGACUAGGUGGGAAUA	1720	AGUCGACuAGGUGGGAAuAdTdT
AD-20108	S	855	1687	UUCCCACCUAGUCGACUAC	1721	uucccAccuAGucGAcuAcdTdT
	A	855	1688	GUAGUCGACUAGGUGGGAA	1722	GuAGUCGACuAGGUGGGAAdTdT
AD-20109	S	367	1689	GCUUUUGAGAAUCCAUAUG	1723	GcuuuuGAGAAuccAuAuGdTdT
	A	367	1690	CAUAUGGAUUCUCAAAAGC	1724	cAuAUGGAUUCUcAAAAGCdTdT
AD-20196	S	55	1691	CGGAGGAUCAACGACGAGA	1725	cGGAGGAucAAcGAcGAGAdTdT
	A	55	1692	UCUCGUCGUUGAUCCUCCG	1726	UCUCGUCGUUGAUCCUCCGdTdT
AD-20197	S	459	1693	AUCUGACUCUACCAAAUAC	1727	AucuGAcucuAccAAAuAcdTdT
	A	459	1694	GUAUUUGGUAGAGUCAGAU	1728	GuAUUUGGuAGAGUcAGAUdTdT
AD-20110	S	312	1695	ACACAAUAAGGCUCAUGCA	1729	AcAcAAuAAGGcucAuGcAdTdT
	A	312	1696	UGCAUGAGCCUUAUUGUGU	1730	UGcAUGAGCCUuAUUGUGUdTdT
AD-20111	S	178	1697	AGGAUCAUCCACGGGUCGG	1731	AGGAucAuccAcGGGucGGdTdT
	A	178	1698	CCGACCCGUGGAUGAUCCU	1732	CCGACCCGUGGAUGAUCCUdTdT
AD-20112	S	297	1699	CCCAUACAAGUAUGAACAC	1733	cccAuAcAAGuAuGAAcAcdTdT
	A	297	1700	GUGUUCAUACUUGUAUGGG	1734	GUGUUcAuACUUGuAUGGGdTdT
AD-20113	S	315	1701	CAAUAAGGCUCAUGCACAA	1735	cAAuAAGGcucAuGcAcAAdTdT
	A	315	1702	UUGUGCAUGAGCCUUAUUG	1736	UUGUGcAUGAGCCUuAUUGdTdT
AD-20114	S	58	1703	AGGAUCAACGACGAGAUCG	1737	AGGAucAAcGAcGAGAucGdTdT
	A	58	1704	CGAUCUCGUCGUUGAUCCU	1738	CGAUCUCGUCGUUGAUCCUdTdT
AD-20115	S	324	1705	UCAUGCACAAUUGGUUCGA	1739	ucAuGcAcAAuuGGuucGAdTdT
	A	324	1706	UCGAACCAAUUGUGCAUGA	1740	UCGAACcAAUUGUGcAUGAdTdT
AD-20116	S	59	1707	GGAUCAACGACGAGAUCGA	1741	GGAucAAcGAcGAGAucGAdTdT
	A	59	1708	UCGAUCUCGUCGUUGAUCC	1742	UCGAUCUCGUCGUUGAUCCdTdT
AD-20117	S	398	1709	AGAGCUUGUGGAAUGAUCC	1743	AGAGcuuGuGGAAuGAuccdTdT
	A	398	1710	GGAUCAUUCCACAAGCUCU	1744	GGAUcAUUCcAcAAGCUCUdTdT
AD-20118	S	57	1711	GAGGAUCAACGACGAGAUC	1745	GAGGAucAAcGAcGAGAucdTdT
	A	57	1712	GAUCUCGUCGUUGAUCCUC	1746	GAUCUCGUCGUUGAUCCUCdTdT
AD-20119	S	56	1713	GGAGGAUCAACGACGAGAU	1747	GGAGGAucAAcGAcGAGAudTdT
	A	56	1714	AUCUCGUCGUUGAUCCUCC	1748	AUCUCGUCGUUGAUCCUCCdTdT
AD-20120	S	369	1715	UUUUGAGAAUCCAUAUGUA	1749	uuuuGAGAAuccAuAuGuAdTdT
	A	369	1716	UACAUAUGGAUUCUCAAAA	1750	uAcAuAUGGAUUCUcAAAAdTdT
AD-20121	S	45	1717	CAAGGAAGCCCGGAGGAUC	1751	cAAGGAAGcccGGAGGAucdTdT
	A	45	1718	GAUCCUCCGGGCUUCCUUG	1752	GAUCCUCCGGGCUUCCUUGdTdT

Table 7b – – Sequences of dsRNA targeting GNAQ (AD-20196 and AD-20197 only)

Duplex Name	Strand	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
AD-20196	S	1753	CGGAGGAUCAACGACGAGA	1757	cGGAGGAucAAcGAcGAGAdTdT

Duplex Name	Strand	SEQ ID NO:	Unmodified sequence 5' to 3'	SEQ ID NO:	Modified sequence 5' to 3'
	A	1754	UCUCGUCGUUGAUCCUCCG	1758	UCUCGUCGUUGAUCCUCCGdTdT
AD-20197	S	1755	AUCUGACUCUACCAAAUAC	1759	AucuGAcucuAccAAAuAcdTdT
	A	1756	GUAUUUGGUAGAGUCAGAU	1760	GuAUUUGGuAGAGUcAGAUdTdT

Total RNA isolation using MagMAX-96 Total RNA Isolation Kit (Applied Biosystem, Foster City CA, part #: AM1830):

Cells were harvested and lysed in 140μl of Lysis/Binding Solution then mixed for 1 minute at 850rpm using and Eppendorf Thermomixer (the mixing speed was the same throughout the process). Twenty micro liters of magnetic beads were added into cell-lysate and mixed for 5 minutes. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, magnetic beads were washed with Wash Solution 1 (isopropanol added) and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150μl Wash Solution 2 (Ethanol added), captured and supernatant was removed. 50ul of DNase mixture (MagMax turbo DNase Buffer and Turbo DNase) was then added to the beads and they were mixed for 10 to 15 minutes. After mixing, 100μl of RNA Rebinding Solution was added and mixed for 3 minutes. Supernatant was removed and magnetic beads were washed again with 150μl Wash Solution 2 and mixed for 1 minute and supernatant was removed completely. The magnetic beads were mixed for 2 minutes to dry before RNA it was eluted with 50μl of water.

Total RNA isolation using RNAqueous®-96 well plate procedure (Applied Biosystem, Foster City CA, part #: 1812):

Cells were lysed for 5 minutes in 200μl of Lysis/Binding Solution. 100μl of 100% ethanol was added into each cell lysate and the total 300μl lysates were transferred into one wells of "filter plate". Filter plate was centrifuged at RCF of 10,000-15,000g for 2 minutes. 300μl Wash Solution was then added into each well and the plate was centrifuged at RCF of 10,000-15,000g for 2 minutes. For DNase treatment, 20ul of DNase mixture was added on top of each filter and the plate was incubated for 15 minutes at room temperature. RNA rebinding was performed by washing filters with 200 μL of Rebinding Mix and 1 minute later samples were centrifuged at RCF of 10,000-15,000g for 2 minutes. Filter was washed then twice with 200μl of Wash Solution and centrifuged at RCF of 10,000-15,000g for 2 minutes. A third centrifugation of 2 minutes was then applied after the reservoir unit was emptied and elution of the RNA was done into a clean culture plate by adding into the filters 50 μL of preheated (80^0C) Nuclease-free Water.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

A master mix of 2μl 10X Buffer, 0.8μl 25X dNTPs, 2μl Random primers, 1μl Reverse Transcriptase, 1μl RNase inhibitor and 3.2μl of H2O per reaction were added into 10μl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR:

2μl of cDNA was added to a master mix of 1μl GAPDH TaqMan Probe (Human GAPD Endogenous Control VIC / MGB Probe, Primer Limited Applied Biosystems Cat # 4326317E), 1μl GNAQ TaqMan probe (Applied Biosystems cat # HS00387073_M1) and 10μl TaqMan Universal PCR Master Mix (Applied Biosystems Cat #4324018) per well in a MicroAmp Optical 96 well plate (Applied Biosystems cat # 4326659). Real time PCR was done in an ABI 7900HT Real Time PCR system (Applied Biosystems) using the ΔΔCt(RQ) assay. All reactions were done in triplicate.

Real time data were analyzed using the ΔΔCt method and normalized to assays performed from cells transfected with 10nM BlockIT fluorescent Oligo (Invitrogen Cat # 2013) or 10nM AD-1955 a duplex that targets luciferase to calculate fold change.

Results

A total of 94 chemically modified siRNAs were screened. Single dose screens were performed in A549 (lung carcinoma), A375 (malignant melanoma) and uveal melanoma cell lines $GNAQ^{mut}$, OMM1.3, and MEL202. Tables 8-14 show the results of the single-dose *in vitro* siRNA screen.

Table 8: A375 cells (0.1 nM) GNAQ dsRNA single dose in vitro screen

A375 cells (0.1nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
26	AD-20057	44.52	4.74
21	AD-20052	49.83	4.55
20	AD-20051	51.94	6.86
38	AD-20069	53.68	5.80
60	AD-20092	54.34	5.94
66	AD-20099	56.06	5.86
14	AD-20045	56.35	5.74
91	AD-20193	57.53	3.82
68	AD-20101	58.44	4.72

A375 cells (0.1nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
23	AD-20054	60.08	6.13
89	AD-20103	60.82	5.02
16	AD-20047	61.66	5.59
53	AD-20085	61.99	8.52
56	AD-20088	62.09	7.48
81	AD-20116	63.84	7.76
19	AD-20050	64.39	8.36
78	AD-20113	64.84	7.95
8	AD-20039	65.29	9.23
51	AD-20083	70.34	8.09
65	AD-20097	71.57	5.92
11	AD-20042	74.74	8.67
43	AD-20074	74.87	6.70
47	AD-20079	75.39	6.12
24	AD-20055	77.24	36.15
58	AD-20090	77.65	9.17
57	AD-20089	78.32	9.94
44	AD-20076	78.59	5.98
46	AD-20078	79.00	5.54
64	AD-20096	80.39	5.96
48	AD-20080	80.66	10.31
84	AD-20119	80.94	5.22
3	AD-20034	81.37	7.63
10	AD-20041	81.65	6.39
12	AD-20043	81.65	11.97
6	AD-20037	81.79	11.99
59	AD-20091	81.79	9.24
13	AD-20044	81.79	6.42
30	AD-20061	85.41	8.80
63	AD-20095	85.71	8.69
18	AD-20049	85.71	10.26
75	AD-20110	86.60	13.52
52	AD-20084	87.81	11.94
69	AD-20102	87.81	6.48
94	AD-20196	88.58	6.22
71	AD-20106	88.73	9.38
70	AD-20104	89.35	14.88
35	AD-20066	89.81	7.73
54	AD-20086	89.97	12.64
45	AD-20077	90.28	10.05
72	AD-20107	90.59	5.99
83	AD-20118	90.75	8.80
34	AD-20065	91.54	12.41
62	AD-20094	92.02	10.71

A375 cells (0.1nM conc.)			
Sample Name	Duplex Name	% Target Remaining	St.Dev error
74	AD-20109	92.82	11.79
79	AD-20114	92.82	11.48
73	AD-20108	93.14	8.59
80	AD-20115	93.47	9.77
93	AD-20195	93.63	7.82
55	AD-20087	93.95	15.29
76	AD-20111	93.95	14.29
92	AD-20194	94.44	6.29
82	AD-20117	94.61	12.80
15	AD-20046	94.61	10.42
22	AD-20053	94.93	16.04
77	AD-20112	95.10	12.23
29	AD-20060	95.10	11.08
67	AD-20100	95.26	11.16
28	AD-20059	95.43	11.09
32	AD-20063	95.93	14.32
25	AD-20056	96.09	12.23
90		96.26	9.50
95	AD-20105	96.76	10.01
9	AD-20040	97.10	7.88
17	AD-20048	97.10	11.44
88	AD-20098	97.27	6.97
61	AD-20093	97.27	12.46
39	AD-20070	97.43	9.70
7	AD-20038	97.60	11.22
87		97.94	8.37
49	AD-20081	98.45	9.22
31	AD-20062	98.62	13.40
86	AD-20121	98.62	10.71
50	AD-20082	98.79	12.83
41	AD-20072	98.97	9.54
42	AD-20073	99.48	9.92
85	AD-20120	99.65	7.42
27	AD-20058	99.83	16.38
33	AD-20064	100.35	10.82
1	AD-20032	101.40	9.56
37	AD-20068	101.57	9.44
4	AD-20035	102.99	15.49
2	AD-20033	103.71	13.63
40	AD-20071	104.25	10.82
5	AD-20036	106.25	23.63

Table 9: A375 cells (1.0 nM) single dose GNAQ in vitro screen

A375 cells (1nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
26	AD-20057	39.55	7.92
21	AD-20052	41.23	9.20
38	AD-20069	44.42	6.19
68	AD-20101	45.04	6.93
20	AD-20051	45.11	8.89
14	AD-20045	45.98	7.80
19	AD-20050	47.11	11.07
53	AD-20085	47.52	9.93
56	AD-20088	47.60	9.91
16	AD-20047	48.35	8.35
66	AD-20099	48.44	8.52
78	AD-20113	48.69	8.81
81	AD-20116	49.20	9.77
23	AD-20054	49.71	8.20
89	AD-20103	49.80	7.27
91	AD-20193	51.29	8.73
65	AD-20097	52.27	9.60
60	AD-20092	52.46	6.04
51	AD-20083	55.64	9.34
58	AD-20090	57.30	9.63
8	AD-20039	57.70	15.80
11	AD-20042	58.51	9.24
43	AD-20074	59.43	9.18
24	AD-20055	59.53	13.24
47	AD-20079	59.74	8.98
57	AD-20089	59.94	11.78
46	AD-20078	61.10	12.31
18	AD-20049	61.31	8.08
30	AD-20061	63.14	11.19
6	AD-20037	63.91	10.65
10	AD-20041	64.25	12.25
59	AD-20091	64.36	10.87
3	AD-20034	65.26	12.38
13	AD-20044	65.26	10.78
64	AD-20096	66.51	10.16
44	AD-20076	66.86	8.87
93	AD-20195	67.44	8.49
12	AD-20043	68.74	12.02
94	AD-20196	68.98	12.81
35	AD-20066	69.70	10.38
54	AD-20086	70.79	13.09
45	AD-20077	71.04	10.55

A375 cells (1nM conc.)			
Sample Name	Duplex Name	% Target Remaining	St.Dev error
84	AD-20119	71.28	8.50
52	AD-20084	71.53	14.24
34	AD-20065	72.15	12.77
29	AD-20060	74.44	11.33
48	AD-20080	74.83	9.32
63	AD-20095	75.09	12.36
75	AD-20110	75.35	15.56
92	AD-20194	76.40	12.95
70	AD-20104	76.67	10.72
28	AD-20059	78.41	12.71
74	AD-20109	78.55	15.25
15	AD-20046	78.69	12.82
55	AD-20087	79.37	12.92
69	AD-20102	80.90	11.23
31	AD-20062	80.90	15.87
4	AD-20035	81.18	19.43
83	AD-20118	82.45	19.97
49	AD-20081	82.60	15.95
67	AD-20100	82.88	13.22
42	AD-20073	83.32	14.05
25	AD-20056	84.19	16.11
62	AD-20094	84.48	13.94
41	AD-20072	84.92	12.80
9	AD-20040	85.21	15.48
71	AD-20106	85.51	16.45
90		85.81	15.45
7	AD-20038	86.85	16.09
79	AD-20114	87.76	16.56
33	AD-20064	88.07	20.64
80	AD-20115	88.07	17.42
2	AD-20033	88.68	16.03
61	AD-20093	89.76	13.56
32	AD-20063	90.07	14.83
36	AD-20067	90.23	9.73
77	AD-20112	90.54	15.45
86	AD-20121	91.49	20.81
95	AD-20105	91.65	17.40
22	AD-20053	91.97	20.15
5	AD-20036	92.13	23.89
37	AD-20068	92.77	14.46
39	AD-20070	93.09	16.90
27	AD-20058	93.09	17.29
17	AD-20048	93.25	14.32
88	AD-20098	93.25	14.60

A375 cells (1nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
82	AD-20117	93.41	17.84
40	AD-20071	94.39	15.66
50	AD-20082	94.88	17.58
87		95.71	15.99
1	AD-20032	96.71	14.37
73	AD-20108	96.71	17.36
85	AD-20120	97.04	11.67
72	AD-20107	108.05	12.36

Table 10: A549 cells (1.0 nM) single dose GNAQ in vitro screen

A549 cells (1nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
78	AD-20113	13.33	2.98
53	AD-20085	15.79	3.53
81	AD-20116	16.44	3.68
21	AD-20052	16.90	3.78
20	AD-20051	17.31	3.87
38	AD-20069	17.71	3.96
66	AD-20099	17.77	3.98
19	AD-20050	18.11	4.05
64	AD-20096	18.17	4.07
26	AD-20057	18.75	4.20
89	AD-20103	19.11	4.28
43	AD-20074	19.28	4.31
51	AD-20083	19.41	4.34
68	AD-20101	19.61	4.39
14	AD-20045	20.06	4.49
8	AD-20039	20.20	4.52
11	AD-20042	20.41	4.57
65	AD-20097	20.99	4.70
60	AD-20092	21.02	4.70
56	AD-20088	22.53	5.04
44	AD-20076	22.57	5.05
58	AD-20090	23.29	5.21
57	AD-20089	23.29	5.21
47	AD-20079	23.69	5.30
74	AD-20109	23.86	5.34
16	AD-20047	24.02	5.38
63	AD-20095	24.36	5.45
59	AD-20091	25.04	5.60
23	AD-20054	25.17	5.63
45	AD-20077	25.61	5.73

A549 cells (1nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
48	AD-20080	25.84	5.78
91	AD-20193	28.92	6.47
13	AD-20044	29.83	6.68
6	AD-20037	30.89	6.91
46	AD-20078	31.10	6.96
24	AD-20055	31.64	7.08
85	AD-20120	31.70	7.09
18	AD-20049	33.74	7.55
84	AD-20119	34.75	7.77
3	AD-20034	35.85	8.02
35	AD-20066	36.73	8.22
70	AD-20104	36.92	8.26
12	AD-20043	38.62	8.64
54	AD-20086	38.96	8.72
15	AD-20046	39.98	8.95
34	AD-20065	40.19	8.99
93	AD-20195	41.18	9.21
75	AD-20110	41.18	9.21
69	AD-20102	41.68	9.33
52	AD-20084	42.19	9.44
30	AD-20061	44.29	9.91
94	AD-20196	48.13	10.77
40	AD-20071	48.21	10.79
49	AD-20081	48.72	10.90
10	AD-20041	48.80	10.92
36	AD-20067	48.97	10.96
29	AD-20060	50.79	11.36
31	AD-20062	51.05	11.42
90		52.12	11.66
55	AD-20087	52.30	11.70
61	AD-20093	52.85	11.83
2	AD-20033	53.50	11.97
25	AD-20056	55.77	12.48
4	AD-20035	56.25	12.59
1	AD-20032	57.43	12.85
92	AD-20194	60.19	13.47
42	AD-20073	61.03	13.65
5	AD-20036	61.45	13.75
28	AD-20059	61.99	13.87
50	AD-20082	62.09	13.89
67	AD-20100	63.29	14.16
83	AD-20118	64.06	14.33
62	AD-20094	64.17	14.36
27	AD-20058	64.95	14.53

A549 cells (1nM conc.)			
Sample Name	Duplex Name	% Target Remaining	St.Dev error
7	AD-20038	69.26	15.50
79	AD-20114	71.45	15.99
39	AD-20070	72.07	16.13
41	AD-20072	74.61	16.69
86	AD-20121	74.61	16.69
33	AD-20064	75.39	16.87
9	AD-20040	80.11	17.92
72	AD-20107	82.22	18.40
95	AD-20105	86.90	19.45
73	AD-20108	87.96	19.68
17	AD-20048	89.04	19.92
88	AD-20098	90.13	20.17
77	AD-20112	90.44	20.24
80	AD-20115	91.07	20.38
22	AD-20053	91.86	20.55
37	AD-20068	92.50	20.70
32	AD-20063	92.66	20.73
76	AD-20111	92.82	20.77
71	AD-20106	92.98	20.80
82	AD-20117	109.81	24.57
87		110.19	24.65

Table 11: OMM1.3 cells (10 nM) single dose GNAQ in vitro screen

OMM1.3 cells (10nM conc.)			
Sample Name	Duplex Name	% Target Remaining	St.Dev error
85	AD-20120	51.12	7.27
58	AD-20090	51.83	11.83
89	AD-20103	53.57	6.93
68	AD-20101	54.50	10.88
64	AD-20096	54.60	9.30
57	AD-20089	54.98	10.87
53	AD-20085	55.07	11.92
38	AD-20069	55.55	10.05
59	AD-20091	55.94	13.82
51	AD-20083	56.42	13.08
60	AD-20092	56.72	11.64
65	AD-20097	57.61	8.03
45	AD-20077	57.81	11.18
63	AD-20095	57.81	10.19
43	AD-20074	58.01	11.58
91	AD-20193	58.11	10.38
26	AD-20057	58.21	10.36

OMM1.3 cells (10nM conc.)			
Sample Name	Duplex Name	% Target Remaining	St.Dev error
20	AD-20051	58.41	7.60
24	AD-20055	58.92	9.65
66	AD-20099	59.74	10.83
44	AD-20076	59.74	12.63
23	AD-20054	59.95	8.25
47	AD-20079	60.06	11.09
56	AD-20088	60.06	12.78
61	AD-20093	60.06	13.48
41	AD-20072	60.37	12.49
13	AD-20044	61.11	12.23
35	AD-20066	61.32	11.53
90		61.53	10.72
19	AD-20050	61.64	10.53
14	AD-20045	61.85	7.21
15	AD-20046	61.96	10.96
21	AD-20052	62.07	7.36
34	AD-20065	62.61	8.87
29	AD-20060	62.71	11.52
16	AD-20047	62.93	8.91
93	AD-20195	63.26	10.94
69	AD-20102	63.59	7.49
54	AD-20086	64.25	16.58
50	AD-20082	64.59	16.58
94	AD-20196	64.70	9.71
48	AD-20080	64.70	12.16
30	AD-20061	64.81	9.02
A2		65.26	13.18
70	AD-20104	65.83	8.26
A3		66.41	11.43
18	AD-20049	68.27	11.57
49	AD-20081	68.75	15.03
55	AD-20087	69.35	14.25
31	AD-20062	69.71	10.58
52	AD-20084	71.42	17.10
A4		72.29	8.52
67	AD-20100	73.68	15.34
27	AD-20058	74.19	12.01
36	AD-20067	74.32	17.93
33	AD-20064	75.23	14.71
72	AD-20107	75.88	10.61
28	AD-20059	76.94	13.68
A1		76.94	14.61
71	AD-20106	77.08	12.79
25	AD-20056	79.11	12.52

OMM1.3 cells (10nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
8	AD-20039	80.21	10.01
39	AD-20070	80.49	14.56
88	AD-20098	80.63	11.15
40	AD-20071	80.77	16.38
62	AD-20094	81.75	16.23
86	AD-20121	84.49	9.13
17	AD-20048	84.64	16.94
12	AD-20043	86.87	14.40
22	AD-20053	87.93	14.30
11	AD-20042	88.23	13.27
37	AD-20068	91.66	17.18
32	AD-20063	91.98	14.78
87		94.56	10.00
9	AD-20040	96.89	12.28
6	AD-20037	97.90	16.58
2	AD-20033	100.48	17.62
3	AD-20034	100.83	12.65
1	AD-20032	105.84	19.01
7	AD-20038	114.62	16.88
5	AD-20036	115.42	14.21
4	AD-20035	123.49	11.58
10	AD-20041	135.05	65.85

Table 12: OMM1.3 cells (10 nM) single dose GNAQ in vitro screen

OMM1.3 (10nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
38	AD-20069	50.04	6.45
68	AD-20101	50.30	7.35
53	AD-20085	51.09	11.53
66	AD-20099	51.45	8.97
64	AD-20096	51.72	8.35
43	AD-20074	53.17	6.93
21	AD-20052	53.54	8.56
51	AD-20083	53.54	10.85
58	AD-20090	53.82	9.62
45	AD-20077	54.29	8.36
26	AD-20057	54.76	12.81
56	AD-20088	54.86	12.18
65	AD-20097	54.86	7.64
89	AD-20103	55.24	9.10
63	AD-20095	55.33	9.42
23	AD-20054	55.53	7.94

OMM1.3 (10nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
19	AD-20050	55.53	8.95
57	AD-20089	55.82	10.84
91	AD-20193	56.01	10.65
16	AD-20047	56.20	8.85
20	AD-20051	56.50	9.35
47	AD-20079	56.50	7.15
15	AD-20046	56.69	7.92
44	AD-20076	57.09	8.01
59	AD-20091	57.09	9.56
8	AD-20039	57.29	7.18
61	AD-20093	57.58	10.14
14	AD-20045	57.78	10.19
85	AD-20120	57.78	9.98
54	AD-20086	57.88	9.77
11	AD-20042	58.90	11.84
13	AD-20044	59.41	11.72
48	AD-20080	60.55	9.45
41	AD-20072	60.87	6.66
A2		61.08	9.29
12	AD-20043	61.72	13.93
35	AD-20066	61.72	11.27
6	AD-20037	61.72	9.69
69	AD-20102	61.93	10.90
34	AD-20065	62.15	12.75
60	AD-20092	62.25	10.76
50	AD-20082	62.80	11.11
3	AD-20034	63.12	7.93
10	AD-20041	63.89	9.55
18	AD-20049	64.00	9.43
30	AD-20061	64.12	10.78
29	AD-20060	64.23	12.25
70	AD-20104	65.35	10.65
52	AD-20084	67.54	14.15
55	AD-20087	67.77	12.89
90		67.77	10.53
24	AD-20055	68.36	10.68
9	AD-20040	68.60	11.16
5	AD-20036	69.08	10.37
93	AD-20195	69.44	10.54
2	AD-20033	70.04	12.33
31	AD-20062	71.02	11.73
49	AD-20081	71.02	11.45
39	AD-20070	71.51	9.18
27	AD-20058	71.88	11.58

OMM1.3 (10nM conc.)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
67	AD-20100	72.01	9.95
94	AD-20196	72.26	15.50
A1		72.89	8.79
33	AD-20064	73.39	13.32
A4		73.65	12.42
A3		74.55	14.37
25	AD-20056	74.81	12.70
28	AD-20059	74.94	14.91
40	AD-20071	75.20	10.84
36	AD-20067	76.64	12.54
71	AD-20106	76.64	10.67
4	AD-20035	76.91	10.25
22	AD-20053	78.80	15.37
86	AD-20121	79.48	10.26
7	AD-20038	79.62	10.32
17	AD-20048	80.59	13.99
88	AD-20098	81.01	12.34
72	AD-20107	82.00	16.19
62	AD-20094	82.43	14.56
32	AD-20063	84.60	12.39
37	AD-20068	93.22	16.05
87		94.52	14.29
1	AD-20032	115.87	15.00

Table 13: UMEL 202 cells (10 nM) single dose GNAQ in vitro screen

UMEL 202 cells (10nm Conc.			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
51	AD-20083	17.87	3.17
85	AD-20120	18.37	4.48
45	AD-20077	18.76	5.42
68	AD-20101	18.82	3.16
26	AD-20057	19.42	3.43
64	AD-20096	19.66	3.25
15	AD-20046	19.83	4.71
58	AD-20090	19.90	4.30
57	AD-20089	20.74	4.31
53	AD-20085	21.55	4.68
89	AD-20103	22.15	4.48
63	AD-20095	22.31	2.70
21	AD-20052	22.46	4.02
11	AD-20042	22.66	2.36
59	AD-20091	22.78	4.06
20	AD-20051	22.86	3.46

UMEL 202 cells (10nm Conc.			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
38	AD-20069	23.34	5.47
16	AD-20047	23.58	3.90
43	AD-20074	23.62	5.71
19	AD-20050	23.87	4.41
8	AD-20039	23.91	2.96
14	AD-20045	24.33	4.08
47	AD-20079	25.10	5.85
50	AD-20082	25.27	4.51
3	AD-20034	25.49	4.73
61	AD-20093	25.54	4.75
60	AD-20092	25.76	4.91
56	AD-20088	25.94	3.59
66	AD-20099	26.03	4.28
65	AD-20097	26.30	4.82
41	AD-20072	27.09	6.80
13	AD-20044	27.61	5.71
2	AD-20033	27.70	3.68
91	AD-20193	27.90	4.64
29	AD-20060	27.99	5.33
44	AD-20076	28.04	7.95
A2		28.29	6.73
54	AD-20086	28.78	5.43
69	AD-20102	29.18	4.79
48	AD-20080	29.28	8.11
5	AD-20036	30.90	4.82
A3		30.95	6.03
18	AD-20049	31.06	4.57
6	AD-20037	31.17	3.46
30	AD-20061	31.49	6.96
35	AD-20066	31.71	39.01
34	AD-20065	34.05	7.29
90		34.11	5.07
94	AD-20196	34.17	6.48
23	AD-20054	34.46	5.09
12	AD-20043	34.70	2.93
10	AD-20041	34.76	6.00
55	AD-20087	36.55	8.21
31	AD-20062	36.81	7.00
49	AD-20081	37.06	7.58
25	AD-20056	39.04	9.55
70	AD-20104	39.59	6.12
52	AD-20084	39.93	6.61
A4		40.42	7.46
93	AD-20195	41.99	7.10

UMEL 202 cells (10nm Conc.			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
40	AD-20071	42.28	8.86
27	AD-20058	43.40	9.27
4	AD-20035	47.00	6.36
24	AD-20055	47.08	6.37
A1		48.65	10.76
9	AD-20040	50.46	8.04
28	AD-20059	50.63	12.97
39	AD-20070	51.43	9.23
36	AD-20067	52.42	10.11
33	AD-20064	52.78	10.02
17	AD-20048	54.36	8.74
88	AD-20098	55.50	9.72
86	AD-20121	57.16	8.59
67	AD-20100	58.87	8.34
22	AD-20053	65.32	10.91
62	AD-20094	68.10	10.87
7	AD-20038	72.48	9.86
37	AD-20068	74.00	17.25
71	AD-20106	82.39	11.32
32	AD-20063	83.11	17.34
72	AD-20107	89.39	11.20
87		99.18	18.11
1	AD-20032	119.33	18.54

Table 14: ~~UMEL~~ MEL 202 cells (10 nM) single dose GNAQ in vitro screen

UMEL202 cells (10 nM)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
85	AD-20120	16.28	1.84
26	AD-20057	18.41	3.50
68	AD-20101	18.73	3.64
45	AD-20077	19.09	4.41
64	AD-20096	19.33	4.19
21	AD-20052	21.08	3.11
51	AD-20083	21.22	4.27
58	AD-20090	22.36	4.62
63	AD-20095	22.55	3.04
20	AD-20051	23.22	2.94
53	AD-20085	23.43	4.97
57	AD-20089	23.43	4.55
8	AD-20039	24.00	3.73
89	AD-20103	24.25	5.69
15	AD-20046	24.30	3.82
38	AD-20069	25.02	5.88

UMEL202 cells (10 nM)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
19	AD-20050	25.11	3.28
11	AD-20042	25.20	3.93
16	AD-20047	25.20	3.98
59	AD-20091	25.41	5.04
43	AD-20074	25.50	6.00
61	AD-20093	25.50	4.07
66	AD-20099	25.68	3.88
65	AD-20097	25.90	2.90
56	AD-20088	25.95	4.67
47	AD-20079	26.31	5.00
41	AD-20072	26.96	5.21
69	AD-20102	27.19	3.81
14	AD-20045	27.72	5.20
13	AD-20044	28.10	5.06
50	AD-20082	28.25	5.67
54	AD-20086	28.35	4.75
60	AD-20092	28.84	4.72
29	AD-20060	29.04	5.29
2	AD-20033	29.24	4.55
91	AD-20193	29.30	7.31
35	AD-20066	29.40	6.42
3	AD-20034	29.45	5.51
A2		30.70	5.81
48	AD-20080	30.86	7.14
44	AD-20076	31.07	7.63
12	AD-20043	31.29	7.00
30	AD-20061	31.40	5.57
94	AD-20196	32.22	8.75
A3		32.73	7.68
18	AD-20049	33.36	6.21
5	AD-20036	34.12	4.63
34	AD-20065	34.60	4.89
6	AD-20037	34.66	5.71
70	AD-20104	35.32	5.17
23	AD-20054	35.39	5.08
90		36.19	8.71
10	AD-20041	36.82	5.22
A4		36.89	11.72
93	AD-20195	37.73	9.95
31	AD-20062	37.93	7.86
25	AD-20056	40.51	8.37
55	AD-20087	40.65	10.00
52	AD-20084	41.72	7.24
24	AD-20055	43.26	6.08

UMEL202 cells (10 nM)			
Sample Name	**Duplex Name**	**% Target Remaining**	**St.Dev error**
49	AD-20081	43.34	13.40
27	AD-20058	45.57	7.25
A1		45.89	8.52
4	AD-20035	46.13	8.13
28	AD-20059	48.25	8.52
36	AD-20067	48.84	13.01
40	AD-20071	48.93	9.64
88	AD-20098	50.30	12.25
33	AD-20064	50.48	7.61
9	AD-20040	50.74	6.96
67	AD-20100	50.92	9.41
39	AD-20070	53.36	14.44
17	AD-20048	53.45	6.78
22	AD-20053	66.61	12.90
86	AD-20121	66.84	16.28
62	AD-20094	67.89	11.19
7	AD-20038	70.53	8.81
71	AD-20106	81.44	14.31
32	AD-20063	83.29	12.02
72	AD-20107	85.04	14.05
87		100.26	29.22
37	AD-20068	108.58	54.53
1	AD-20032	124.62	15.51

Duplexes with desirable levels of GNAQ inhibition were selected for further analysis of IC50 in A549 (lung carcinoma) MEL202 ($GNAQ_{mut}$ uveal melanoma), and OMM1.3 cells ($GNAQ_{mut}$ liver metastisis). Tables 15-17 show the results of the IC50 experiments in A549, MEL202, and OMM1.3 cells. Dose response screen identified pM IC50s in lung carcinoma cell line and GNAQmut uveal melanoma MEL202 and OMM1.3, including duplexes AD-20057 and AD-20051.

Table 15: IC50 in A549 cells

Rank	Duplex Name	IC50 in [nM]	IC50 in [pM]
1	AD-20057	0.0002	0.2
2	AD-20069	0.0026	2.6
3	AD-20051	0.0031	3.1
4	AD-20052	0.0032	3.2
5	AD-20099	0.0033	3.3
6	AD-20045	0.0052	5.2
7	AD-20193	0.0064	6.4
8	AD-20092	0.0094	9.4
9	AD-20116	0.0098	9.8

10	AD-20039	0.0137	13.7
11	AD-20042	0.0172	17.2

Table 16: IC50 in MEL 202 cells

Rank	Duplex Name / (Sample Name)	IC50 in [nM]
1	AD-20057 (26)	0.001
2	AD-20069 (38)	0.002
3	AD-20051 (20)	0.002
4	AD-20052 (21)	0.003
5	AD-20045 (14)	0.003
6	AD-20193 (91)	0.003
7	AD-20092 (60)	0.003
8	AD-20099 (66)	0.004
9	AD-20101 (68)	0.005
10	AD-20116 (81)	0.006
11	AD-20039 (8)	0.006
12	AD-20103 (89)	0.007
13	AD-20085 (53)	0.008
14	AD-20113 (78)	0.010
15	AD-20083 (51)	0.010
16	AD-20096 (64)	0.010
17	AD-20042 (11)	0.011
18	AD-20090 (58)	0.023
19	AD-20119 (84)	0.024
20	AD-20120 (85)	0.037
21	AD-20109 (74)	0.047
22	AD-20077 (45)	0.084

Table 17: IC50 in OMM1.3 cells

Rank	Duplex Name (Sample)	IC50 in [nM]
1	AD-20057 (26)	0.0043
2	AD-20069 (38)	0.0115
3	AD-20052 (21)	0.0183
4	AD-20051 (20)	0.0197
5	AD-20099 (66)	0.0270
6	AD-20092 (60)	0.0280
7	AD-20193 (91)	0.0335
8	AD-20101 (68)	0.0531
9	AD-20045 (14)	0.0538
10	AD-20113 (78)	0.0625
11	AD-20039 (8)	0.0693
12	AD-20103 (89)	0.0820
13	AD-20085 (53)	0.0842
14	AD-20116 (81)	0.1280
15	AD-20083 (51)	0.1653

16	AD-20042 (11)	0.2470
17	AD-20090 (58)	0.2593
18	AD-20096 (64)	0.3006
19	AD-20120 (85)	0.6189
20	AD-20119 (84)	1.2276
21	AD-20109 (74)	1.2558
22	AD-20077 (45)	2.0044

Example 4: *In vitro* dose response

For *in vitro* dose response experiments, cells expressing GNAQ were utilized. Some exemplary cell lines expressing GNAQ include, but are not limited to, human melanoma cell lines OMM1.3 and Mel 202 and MEL-285.

The dsRNAs were screened for *in vitro* inhibition of the target gene at 1nM, 0.1nM, 0.01nM, and 0.001nM. Tissue culture cells were transfected with the dsRNA. Target gene mRNA levels were assayed using qPCR (real time PCR).

Cell culture and transfection

For knockdown, OMM-1.3, MEL-202 and MEL-285 were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in RPMI (Invitrogen) supplemented with 10% FBS, streptomycin, and glutamine (ATCC) before being released from the plate by trypsinization. Reverse transfection was carried out by adding 5μl of Opti-MEM to 5μl of siRNA duplexes per well into a 96-well plate along with 10μl of Opti-MEM plus 0.2μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) and incubated at room temperature for 15 minutes. 80μl of complete growth media without antibiotic containing 2.0 x10^4 OMM-1.3, MEL-202 or MEL-285 cells were then added. Cells were incubated for 24 hours prior to RNA purification. Experiments were performed at 1, 0.1, 0.01 and 0.001nM final duplex concentration.

Total RNA isolation using MagMAX-96 Total RNA Isolation Kit (Applied Biosystem, Foster City CA, part #: AM1830):

Cells were harvested and lysed in 140μl of Lysis/Binding Solution then mixed for 1 minute at 850rpm using and Eppendorf Thermomixer (the mixing speed was the same throughout the process). Twenty micro liters of magnetic beads and Lysis/Binding Enhancer mixture were added into cell-lysate and mixed for 5 minutes. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, magnetic beads were washed with Wash Solution 1 (isopropanol added) and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150µl Wash Solution 2 (Ethanol added), captured and supernatant was removed. 50µl of DNase mixture (MagMax turbo DNase Buffer and Turbo DNase) was then added to the beads and they were mixed for 10 to 15 minutes. After mixing, 100µl of RNA Rebinding Solution was added and mixed for 3 minutes. Supernatant was removed and magnetic beads were washed again with 150µl Wash Solution 2 and mixed for 1 minute and supernatant was removed completely. The magnetic beads were mixed for 2 minutes to dry before RNA was eluted with 50µl of water.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

A master mix of 2µl 10X Buffer, 0.8µl 25X dNTPs, 2µl Random primers, 1µl Reverse Transcriptase, 1µl RNase inhibitor and 3.2µl of H2O per reaction were added into 10µl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR:

2µl of cDNA were added to a master mix containing 0.5µl GAPDH TaqMan Probe (Applied Biosystems Cat # 4326317E), 0.5µl GNAQ TaqMan probe (Applied Biosystems cat # Hs00387073_m1) and 5µl Roche Probes Master Mix (Roche Cat # 04887301001) per well in a LightCycler 480 384 well plate (Roche cat # 0472974001). Real time PCR was done in a LightCycler 480 Real Time PCR machine (Roche). Each duplex was tested in two independent transfections and each transfections was assayed in duplicate.

Real time data were analyzed using the ΔΔCt method. Each sample was normalized to GAPDH expression –and knockdown was assessed relative to cells transfected with the non-targeting duplex AD-1955.

The data are presented in Table 18a. Data are expressed as the fraction of message remaining relative to cells targeted with AD-1955. The calculated IC_{50}s are presented in Table 18b.

Table 18a: In vitro dose response in 3 cell lines

OMM-1.3				
Duplex name	1 nM	0.1 nM	0.01nM	0.001 nM
AD-20039	0.38	0.46	0.74	0.73
AD-20045	0.42	0.52	0.60	0.79
AD-20051	0.34	0.46	0.63	1.18
AD-20052	0.36	0.37	0.53	0.61
AD-20057	0.32	0.36	0.43	0.59

AD-20063	0.63	0.69	0.99	0.74
AD-20069	0.37	0.35	0.43	0.69
AD-20092	0.42	0.51	0.71	0.75
AD-20099	0.35	0.46	0.52	0.63
AD-20101	0.39	0.57	0.60	0.69
AD-20111	0.64	0.68	0.65	0.70
AD-20113	0.37	0.51	0.71	0.92
AD-20116	0.56	0.58	0.66	0.75
AD-20193	0.45	0.50	0.64	0.75
AD-1955	1.12	1.17	0.83	0.92

MEL-202				
Duplex name	1 nM	0.1 nM	0.01nM	0.001 nM
AD-20039	0.35	0.44	0.63	0.83
AD-20045	0.30	0.36	0.53	0.55
AD-20051	0.22	0.37	0.67	0.88
AD-20052	0.33	0.39	0.66	0.85
AD-20057	0.28	0.29	0.39	0.77
AD-20063	0.93	0.87	0.95	0.97
AD-20069	0.35	0.39	0.39	0.75
AD-20092	0.37	0.49	0.93	0.98
AD-20099	0.28	0.33	0.61	0.96
AD-20101	0.38	0.46	0.83	0.92
AD-20111	0.67	0.81	0.91	0.98
AD-20113	0.31	0.48	0.82	0.99
AD-20116	0.33	0.34	0.72	0.92
AD-20193	0.32	0.44	0.65	0.87
AD-1955	1.11	0.85	1.11	0.95

MEL-285				
Duplex name	1 nM	0.1 nM	0.01nM	0.001 nM
AD-20039	0.29	0.47	0.95	1.09
AD-20045	0.39	0.42	0.69	0.86
AD-20051	0.34	0.34	0.73	0.90
AD-20052	0.30	0.53	1.17	1.22
AD-20057	0.37	0.34	0.54	0.86
AD-20063	0.99	1.05	1.52	1.37
AD-20069	0.27	0.33	0.55	0.80
AD-20092	0.39	0.58	0.78	0.82
AD-20099	0.28	0.40	0.92	1.10
AD-20101	0.35	0.57	0.82	1.05
AD-20111	0.75	0.79	0.78	0.73
AD-20113	0.32	0.53	0.92	1.18

AD-20116	0.55	0.51	1.17	0.91
AD-20193	0.42	0.47	0.79	0.95
AD-1955	0.93	1.01	0.93	1.15

Table 18b: IC_{50} (pM) in 3 cell lines

duplex number	MEL202	OMM1.3	A549
AD-20057	0.7	4.3	0.2
AD-20069	1.8	11.5	2.6
AD-20051	2.5	19.7	3.1
AD-20052	2.6	18.3	3.2
AD-20045	2.8	53.8	5.2
AD-20193	3.2	33.5	6.4
AD-20092	3.5	28	9.4
AD-20099	3.6	27	3.3
AD-20101	4.9	53.1	
AD-20116	5.5	128	9.8
AD-20113	9.5	62.5	
AD-20039	6.1	69.3	13.7

Example 5: Immunostimulatory assays: Screening siRNA sequences for immunostimulatory ability

Twelve siRNA candidates were tested for induction of cytokines associated with immunostimulation (TNF-alpha and IFN-alpha).

Human PBMC were isolated from whole blood from healthy donors (Research Blood Components, Inc., Boston, MA) by a standard Ficoll-Hypaque density gradient centrifugation technique. PBMC ($1x10^5$/well/100μL) were seeded in 96-well flat bottom plates and cultured in RPMI 1640 GlutaMax-1 medium (Invitrogen) supplemented with 10% heat -inactivated fetal bovine serum (Omega Scientific) and 1% antibiotic/antimycotic (Invitrogen).

GNAC siRNAs was transfected into PBMC using N-[1-(2, 3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP; Roche). The DOTAP was first diluted in Opti-MEM Reduced Serum medium (Invitrogen) for 5 minutes before mixing with an equal volume of Opti-MEM containing the siRNA. siRNA/DOTAP complexes were incubated for 10-15 minutes at room temperature and subsequently added to PBMC (50μL/well) which were then cultured at 37°C 5% CO_2. siRNAs were used at a final concentration of 133nM. The ratio of RNA to transfection reagent was 16.5 pmoles per μL of DOTAP. Transfections were conducted in quadruplicate in all experiments and were performed within two hours of cell plating. Culture supernatants were collected after 20-24 h and assayed for IFN-α and TNF-α by ELISA.

Cytokines were detected and quantified in culture supernatants with a commercially available ELISA kit for IFN-α (BMS216INST) and TNF-α (BMS223INST) from Bender MedSystems (Vienna, Austria).

Results

The data in Table 19 are presented as a percentage to a AD-5048 stimulated cytokine response. AD-5048 (positive control) corresponds to a sequence that targets human Apolipoprotein B (Soutschek et al., 2004) and elicits both an IFN-α and TNF-α. FIG. 1 and FIG. 2 shows the cytokine induction following transfection with siRNAs.

None of the siRNAs tested demonstrated significant expression of IFN-α and TNF-α in Human PBMCs compared to AD-5048. In particular, AD-20051 and AD-20057 were found to be non immunostimulatory in HuPBMC assay.

Table 19: Immunostimulatory activity

Duplex name	% IFN-α/AD-5048	% TNF-α/AD-5048
AD-20039	0	0
AD-20045	0	0
AD-20051	0	0
AD-20052	0	0
AD-20057	0	0
AD-20069	0	0
AD-20092	0	0
AD-20099	0	0
AD-20101	0	0
AD-20113	0	0
AD-20116	0	0
AD-20193	0	0

Example 6: *In vitro* cell viability

A set of dsRNAs were screened for effects on *in vitro* cell viability. Tissue culture cells were transfected with the dsRNA and viability was assayed by staining with CellTiterBLue and microscopic evaluation..

Cell culture and transfection

For viability, OMM-1.3, MEL-202 and MEL-285 cells were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in RPMI, (Invitrogen) supplemented with 10% FBS, Penn/streptomycin, and glutamine (ATCC) before being released from the plate by trypsinization. Reverse transfection was carried out by adding 5μl of Opti-MEM to 5μl of siRNA duplexes per well into a 96-well plate along with 10μl of Opti-MEM plus 0.2μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) and incubated at room temperature for 15 minutes. 80μl of complete growth media without antibiotic containing 1.0 $x10^3$ OMM-1.3, MEL-202 or MEL-285 cells were then added. Cells were incubated for 3, 5 or 7 days prior to viability assays. Experiments were performed at 1, 0.1, 0.01 and 0.001nM final duplex concentration. All transfections were done in triplicate. The siRNAs PLK, and AD-19200 were included as positive controls (result in loss of viability) and AD-1955 was included as a negative control and was used for data normalization.

Cell viability assay

For viability assays, 20 μl of CellTiterBlue (Promega, Cat# G8080) was added and mixed into each well of the culture plate 3, 5 or 7 days after transfection with an siRNAs at 1, 0.1, 0.01 or 0.001nM final concentration. The plates, containing transfected, cultured cells, media and CellTiterBlue were incubated for 1.5 hours and then read on a SpectraMax M5 plate reader (Molecular Devices) at 560 nm (excitation) and 590 nm (emission).

To measure viability, three replicate wells were averaged and subtracted from background (wells containing media and CellTiterBlue, but no cells). Viability is expressed as a normalized value in which cells transfected with GNAQ specific siRNAs or other controls are compared to cells transfected with AD-1955, a non-targeting duplex, cultured under the same conditions.

Results

The results are shown in Table 20. Graphical summaries of the results comparing viability at 3, 5, and 7 days in a single cell line after treatment with each of the duplexes at a single concentration are shown in FIG. 3, FIG. 4, FIG. 5, and FIG. 6.

The results show decreased cell viability in vitro following GNAQ knockdown that was specific for GNAQ mutant cell lines (e.g., OMM1.3, MEL202), but not GNAQ wild-type (e.g., MEL285) cell lines. In particular these results were shown for duplexes AD-20057, AD-20051, AD-20069, and AD-20093 as illustrated by the graphs in FIG. 7 and FIG. 8.

Table 20: Cell viability after treatment with siRNA

		Day 3				Day 5				Day 7			
	Conc. (in nM)	1nM	0.1nM	0.01nM	0.001nM	1nM	0.1nM	0.01nM	0.001nM	1nM	0.1nM	0.01nM	0.001nM
OMM-1.3	AD-20039	0.95	0.96	1.18	1.27	0.46	0.48	0.79	0.94	0.25	0.52	1.18	1.32

		Day 3				Day 5				Day 7			
	AD-20045	0.99	0.94	1.07	1.20	0.53	0.53	0.70	1.03	0.42	0.44	0.71	1.16
	AD-20051	0.78	0.90	1.01	0.63	0.35	0.42	0.65	0.94	0.23	0.35	0.74	1.15
	AD-20052	0.82	0.90	1.17	1.38	0.41	0.47	0.79	1.02	0.31	0.45	1.06	1.24
	AD-20057	0.86	0.88	0.90	1.31	0.36	0.39	0.49	0.83	0.22	0.31	0.55	1.03
	AD-20063	1.26	1.26	1.10	0.53	1.27	1.06	1.04	0.93	1.11	0.95	1.00	1.06
	AD-20069	0.79	0.72	0.96	1.16	0.35	0.39	0.46	0.86	0.17	0.21	0.58	0.89
	AD-20092	0.68	0.93	1.11	1.15	0.36	0.58	0.85	0.91	0.27	0.63	1.08	0.96
	AD-20099	0.51	0.72	0.95	1.07	0.18	0.37	0.58	0.88	0.08	0.22	0.56	0.86
	AD-20101	0.72	0.76	1.34	1.53	0.33	0.40	0.78	1.00	0.18	0.39	0.98	0.95
	AD-20111	1.25	1.15	1.30	1.32	0.89	1.10	0.87	1.00	0.93	0.95	0.98	0.95
	AD-20113	0.56	0.73	1.02	1.03	0.22	0.44	0.75	0.80	0.12	0.35	0.88	0.82
	AD-20116	0.82	1.23	1.64	1.88	0.41	0.70	0.92	0.98	0.17	0.51	0.75	0.73
	AD-20193	1.22	0.84	1.31	1.67	0.46	0.53	0.66	0.80	0.16	0.30	0.48	0.74
	AD-12115	0.60	0.65	1.03	1.00	0.19	0.26	0.62	0.93	0.08	0.26	0.63	0.61
	PLK	0.47	0.80	0.65	1.67	0.12	0.54	0.74	1.00	0.06	0.64	0.88	0.78
	AD-19200	0.62	0.85	0.72	1.55	0.64	0.78	0.92	0.81	0.64	0.83	0.81	0.87
	AD-1955	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

MEL-202	AD-20039	1.21	0.98	1.02	0.93	0.72	0.56	0.79	1.00	0.78	0.66	0.75	0.88
	AD-20045	0.95	0.90	0.95	0.85	0.47	0.38	0.61	0.92	0.57	0.36	0.47	0.80
	AD-20051	0.70	0.78	0.80	0.38	0.62	0.36	0.77	0.82	0.82	0.42	0.62	0.80
	AD-20052	0.98	1.02	1.06	0.93	0.46	0.42	0.67	1.02	0.33	0.55	0.95	1.03
	AD-20057	0.61	0.91	0.86	0.85	0.30	0.27	0.47	0.86	0.31	0.34	0.53	0.88
	AD-20063	0.91	1.00	1.02	0.37	0.74	0.81	1.01	0.91	1.54	1.32	1.08	1.04
	AD-20069	0.77	1.03	0.91	1.00	0.28	0.44	0.43	0.64	0.34	0.37	0.49	0.79
	AD-20092	0.87	0.88	0.95	0.87	0.25	0.44	0.70	0.80	0.26	0.73	0.97	1.11
	AD-20099	0.80	0.69	0.75	0.41	0.24	0.36	0.53	0.68	0.13	0.26	0.66	1.16
	AD-20101	0.72	0.92	0.80	0.87	0.16	0.48	0.57	0.73	0.17	0.51	1.21	0.92
	AD-20111	1.18	0.90	0.75	0.84	0.67	0.83	0.74	0.80	1.30	1.37	1.25	1.03
	AD-20113	0.63	0.55	0.74	0.36	0.18	0.31	0.66	0.68	0.15	0.37	1.00	1.05
	AD-20116	0.42	0.59	0.62	0.93	0.41	0.51	0.73	0.90	0.29	0.38	0.71	0.77
	AD-20193	0.39	0.53	0.53	0.94	0.36	0.49	0.70	0.76	0.29	0.32	0.59	0.80
	AD-12115	0.22	0.22	0.30	0.50	0.09	0.12	0.42	0.78	0.03	0.04	0.08	0.68
	PLK	0.23	0.27	0.37	0.63	0.10	0.16	0.56	0.65	0.03	0.07	0.97	1.19
	AD-19200	0.37	0.52	0.49	0.56	0.29	0.75	0.76	0.74	0.44	1.15	1.04	0.85
	AD-1955	1	1	1	1	1	1	1	1	1	1	1	1

MEL-285	AD-20039	0.58	1.37	1.23	1.23	1.07	1.25	1.13	1.09	0.82	1.06	0.96	0.93
	AD-20045	1.16	1.31	1.15	1.05	1.10	1.10	1.12	1.24	0.84	0.85	0.97	0.90
	AD-20051	1.14	1.20	0.97	0.98	1.27	1.16	1.06	1.03	0.84	0.99	0.89	0.97
	AD-20052	0.63	1.40	1.26	1.04	1.03	1.40	1.42	1.21	0.92	1.02	1.13	0.98
	AD-20057	1.14	1.17	1.20	1.04	1.00	1.04	1.26	1.35	0.98	0.98	1.03	1.00

	Day 3				Day 5				Day 7			
AD-20063	1.10	1.14	0.88	0.89	1.33	1.14	0.94	0.95	1.16	1.05	0.87	0.91
AD-20069	0.46	1.09	1.08	0.96	1.17	1.20	1.03	1.04	1.29	1.17	1.01	0.99
AD-20092	1.02	1.14	1.15	0.96	1.02	1.11	1.03	1.05	1.06	1.11	1.04	1.02
AD-20099	0.89	1.10	0.95	0.95	0.48	0.92	0.96	1.00	0.54	0.91	0.89	1.05
AD-20101	0.70	1.16	1.12	1.04	0.47	1.12	1.41	1.42	0.66	1.01	1.22	1.03
AD-20111	1.12	1.05	1.13	1.01	1.21	1.49	1.30	1.29	1.04	1.18	1.04	1.03
AD-20113	0.81	0.97	1.02	1.03	0.41	0.85	0.81	0.97	0.31	0.76	0.85	1.01
AD-20116	0.50	0.86	1.07	1.01	1.03	0.98	1.03	1.01	0.99	0.91	1.01	0.94
AD-20193	0.91	0.88	1.03	0.94	0.58	0.86	1.25	1.24	0.72	0.80	1.09	1.03
AD-12115	0.34	0.35	0.81	0.43	0.10	0.12	0.54	1.02	0.07	0.12	0.82	1.00
PLK	0.23	0.65	0.46	0.97	0.31	0.54	1.40	1.31	0.18	0.72	1.38	1.17
AD-19200	0.53	0.81	0.68	0.94	0.53	0.77	1.22	1.32	0.46	0.97	1.22	1.15
AD-1955	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Example 7. *In vivo* efficacy studies

The dsRNAs are screened for *in vivo* inhibition of the target gene in mice. Mice are injected with varying amounts of the dsRNA. Target gene protein levels are assayed using, e.g., mouse plasma and an ELISA with a target gene specific antibody. Target gene mRNA levels are assayed using, e.g., mouse liver and branched DNA assays. The lead candidates are dsRNA that reduce levels of the target gene protein and/or mRNA in a dose-dependent manner.

Regimen for treatment of mice with dsRNA

A single-dose IV bolus efficacy study is designed for each dsRNA to be tested: dose level, dosing days, formulation, and number of animals. Mice are intravenously (i.v.) administered target gene specific dsRNA, control dsRNA) or PBS systemically and/or subcutaneously in a range of concentrations, e.g., 1.0 mg/kg, 3.0 mg/kg, or 6.0 mg/kg.

Mice are observed for forty-hours then anesthetized with 200 μl of ketamine, and are exsanguinated by severing the right caudal artery. Whole blood is isolated and placed into EDTA plasma separator tubes and centrifuged at 3000 rpm for 10 minutes. Plasma is isolated and stored at 80°C until assaying. Liver tissue is collected, flash-frozen and stored at -80°C until processing.

Efficacy of treatment is evaluated by methods including (i) measurement of protein in plasma at prebleed and at 48 hours post-dose, (ii) measurement of mRNA in liver at 48 hours post-dose, and (iii) efficacy in modulation of target gene specific phenotype, e.g., anti-tumor activity.

Assay of target gene protein in mouse plasma

Target plasma levels are assayed by ELISA utilizing the commercially available anti GNAQ antibodies, for example G alpha q (K-17) or G alpha q (E-17) (Santa Cruz Biotechnology Inc. Santa Cruz, CA, USA, cat# SC-26791 and cat # SC-393), according to manufacturer's guidelines.

Assay of target gene mRNA levels in mouse liver

Target gene mRNA levels are assayed utilizing the Branched DNA assays Quantigene 2.0 (Panomics cat #: QS0011). Briefly, mouse liver samples are ground and tissue lysates are prepared. Liver lysis Mixture (a mixture of 1 volume of lysis mixture, 2 volume of nuclease-free water and 10ul of Proteinase-K/ml for a final concentration of 20mg/ml.) is incubated at 65 °C for 35 minutes. 20μl of Working Probe Set (target probe for detection of target gene and GAPDH probe for endogenous control) and 80ul of tissue-lysate are then added into the Capture Plate. Capture Plates are incubated at 55 °C ±1 °C (aprx. 16-20hrs). The next day, the Capture Plate are washed 3 times with 1X Wash Buffer (nuclease-free water, Buffer Component 1 and Wash Buffer Component 2), then dried by centrifuging for 1 minute at 240g. 100ul of pre-Amplifer Working Reagent is added into the Capture Plate, which is sealed with aluminum foil and incubated for 1 hour at 55°C ±1°C. Following 1 hour incubation, the wash step is repeated, then 100μl of Amplifier Working Reagent is added. After 1 hour, the wash and dry steps are repeated, and 100μl of Label Probe is added. Capture plates are incubated 50 °C ±1 °C for 1 hour. The plate is then washed with 1X Wash Buffer, dried and 100μl Substrate is added into the Capture Plate. Capture Plates are read using the SpectraMax Luminometer following a 5 to 15 minute incubation. bDNA data are analyzed by subtracting the average background from each triplicate sample, averaging the triplicate GAPDH (control probe) and target gene probe (experimental probe) then taking the ratio: (experimental probe-background)/(control probe-background).

GNAQ materials and methods

The GNAQ specific dsRNA are formulated in lipid particles (SNALP) as describe herein and administered systemically or subcutaneously to mice with GNAQ-mutant human uveal melanoma cell tumors implanted in the liver to assess in vivo target knockdown and antitumor activity. The dsRNA duplexes with positive results are selected for further studies to develop a Phase I/II trial in patients with GNAQ-mutant uveal melanoma metastatic to liver.

Example 8. *Inhibition of GNAQ in humans*

A human subject is treated with a dsRNA targeted to a GNAQ gene to inhibit expression of the GNAQ gene to treat a condition.

A subject in need of treatment is selected or identified. The subject can have uveal melanoma, cutaneous melanoma, Blue nevi, Nevi of Ota, a neuroendocrine tumor, or a small lung tumor.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an anti- GNAQ siRNA is administered to the subject. The dsRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated, e.g., by measuring tumor growth. This measurement can be accompanied by a measurement of GNAQ expression in said subject, and/or the products of the successful siRNA-targeting of GNAQ mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

After treatment, the subject's tumor growth rate is lowered relative to the rate existing prior to the treatment, or relative to the rate measured in a similarly afflicted but untreated subject.

Example 9. ***GNAQ mRNA sequences***

Human GNAQ mRNA NM_002072.2 (SEQ ID NO:1761)

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  1 agggggtgcc ggcggggctg cagcggaggc actttggaag aatgactctg gagtccatca
 61 tggcgtgctg cctgagcgag gaggccaagg aagcccggcg gatcaacgac gagatcgagc
121 ggcagctccg cagggacaag cgggacgccc gccgggagct caagctgctg ctgctcggga
181 caggagagag tggcaagagt acgtttatca agcagatgag aatcatccat gggtcaggat
241 actctgatga agataaaagg ggcttcacca agctggtgta tcagaacatc ttcacggcca
301 tgcaggccat gatcagagcc atggacacac tcaagatccc atacaagtat gagcacaata
361 aggctcatgc acaattagtt cgagaagttg atgtggagaa ggtgtctgct tttgagaatc
421 catatgtaga tgcaataaag agtttatgga atgatcctgg aatccaggaa tgctatgata
481 gacgacgaga atatcaatta tctgactcta ccaaatacta tcttaatgac ttggaccgcg
541 tagctgaccc tgcctacctg cctacgcaac aagatgtgct tagagttcga gtccccacca
601 cagggatcat cgaatacccc tttgacttac aaagtgtcat tttcagaatg gtcgatgtag
661 ggggccaaag gtcagagaga agaaaatgga tacactgctt tgaaaatgtc acctctatca
721 tgtttctagt agcgcttagt gaatatgatc aagttctcgt ggagtcagac aatgagaacc
781 gaatggagga aagcaaggct ctctttagaa caattatcac ataccccctgg ttccagaact
841 cctcggttat tctgttctta aacaagaaag atcttctaga ggagaaaatc atgtattccc
901 atctagtcga ctacttccca gaatatgatg gaccccagag agatgcccag gcagcccgag
961 aattcattct gaagatgttc gtggacctga acccagacag tgacaaaatt atctactccc
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1021 acttcacgtg cgccacagac accgagaata tccgctttgt ctttgctgcc gtcaaggaca
1081 ccatcctcca gttgaacctg aaggagtaca atctggtcta attgtgcctc ctagacaccc
1141 gccctgccct tccctggtgg gctattgaag atacacaaga gggactgtat ttctgtggaa
1201 aacaatttgc ataatactaa tttattgccg tcctggactc tgtgtgagcg tgtccacaga
1261 gtttgtagta aatattatga ttttatttaa actattcaga ggaaaaacag aggatgctga
1321 agtacagtcc cagcacattt cctctctatc tttttttag gcaaaacctt gtgactcagt
1381 gtattttaaa ttctcagtca tgcactcaca aagataagac ttgtttcttt ctgtctctct
1441 ctctttttct tttctatgga gcaaaacaaa gctgatttcc cttttttctt cccccgctaa
1501 ttcatacctc cctcctgatg tttttcccag gttacaatgg cctttatcct agttccattc
1561 ttggtcaagt ttttctctca aatgatacag tcaggacaca tcgttcgatt taagccatca
1621 tcagcttaat ttaagtttgt agtttttgct gaaggattat atgtattaat acttacggtt
1681 ttaaatgtgt tgctttggat acacacatag tttcttttt aatagaatat actgtcttgt
1741 ctcactttgg actgggacag tggatgccca tctaaaagtt aagtgtcatt tcttttagat
1801 gtttaccttc agccatagct tgattgctca gagaaatatg cagaaggcag gatcaaagac
1861 acacaggagt cctttctttt gaaatgccac gtgccattgt ctttcctccc ttctttgctt
1921 ctttttctta ccctctcttt caattgcaga tgccaaaaaa gatgccaaca gacactacat
1981 taccctaatg gctgctaccc agaacctttt tataggttgt tcttaatttt tttgttgttg
2041 ttgttcaagc ttttcctttc tttttttct tagtgtttgg gccacgattt taaaatgact
2101 tttattatgg gtatgtgttg ccaaagctgg ctttttgtca aataaaatga atacgaactt
2161 aaaaaataaa aaaaaaaaaa aaaaaaaa

Rat GNAQ mRNA NM_031036 (SEQ ID NO:1762)

1 atgactctgg agtccatcat ggcgtgctgc ctgagcgagg aggccaagga agcccggagg
61 atcaacgacg agatcgagcg gcagctgcgc agggacaagc gcgacgcccg ccgggagctc
121 aagctgctgc tgctggggac aggggagagt ggcaagagta ccttcattaa gcagatgagg
181 atcatccacg ggtcggggta ctctgatgaa gacaagaggg gctttaccaa actggtgtat
241 cagaacatct ttacagccat gcaggccatg gtcagagcta tggacactct caagatccca
301 tacaagtatg aacacaataa ggctcatgca caattggttc gagaggttga tgtggagaag
361 gtgtctgctt ttgagaatcc atatgtagac gcaataaaga gcttgtggaa tgatcctgga
421 atccaggaat gctacgatag acggcgagaa tatcagctat ctgactctac caaatactat
481 ctgaacgact tggaccgtgt ggctgaccct tcctatctgc ctacacaaca agatgtgctt
541 agagttcgag tccccaccac agggatcatt gagtacccct tcgacttaca gagtgtcatc
601 ttcagaatgg tcgatgtagg aggccaaagg tcagagagaa gaaaatggat acactgcttt
661 gaaaacgtca cctcgatcat gtttctggta gcgcttagcg aatacgatca agttcttgtg
721 gagtcagaca atgagaaccg aatggaggag agcaaagcac tctttagaac cattatcaca
781 tatccctggt tccagaactc ctctgttatt ctgttcttaa acaagaaaga tcttctagag

841 gagaaaatta tgtattccca cctagtcgac tacttcccag aatatgatgg accccagaga

901 gatgcccagg cagcacgaga attcatcctg aagatgttcg tggacctgaa ccccgacagt

961 gacaaaatca tctactcgca cttcacgtgt gccacagaca cggagaacat ccgcttcgtg

1021 tttgctgctg tcaaggacac catcctgcag ctgaacctga aggagtacaa tctggtctaa

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of a G-alpha q subunit (GNAQ) of a heterotrimeric G gene, wherein said dsRNA comprises a sense strand and an antisense strand each being at least 15 nucleotides in length, and wherein the antisense strand comprises a sequence of at least 15 contiguous nucleotides of SEQ ID NO: 74, SEQ ID NO:75, SEQ ID NO:80, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:96, SEQ ID NO:100, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:112, SEQ ID NO:888, SEQ ID NO:890, SEQ ID NO:893, SEQ ID NO:894 or SEQ ID NO:896 and wherein the sense strand comprises a sequence hybridizing to and forming a duplex with said antisense strand, and optionally wherein said sense strand and/or antisense strand comprises one or more modified nucleotide residues.

2. The dsRNA according to claim 1, wherein each strand is between 15 and 30 nucleotides in length.

3. The dsRNA according to claim 1 or claim 2, wherein the antisense strand comprises at least 15 contiguous nucleotides of SEQ ID NO: 92 and wherein the antisense strand is complementary to at least the first 11 nucleotides of SEQ ID NO: 19, and optionally wherein the sense strand and or antisense strand comprises one or more modified nucleotide residues.

4. The dsRNA according to claim 1 or claim 2, wherein:

a) the antisense strand comprises SEQ ID NO: 74 and the sense strand comprises SEQ ID NO: 1; or

b) the antisense strand comprises SEQ ID NO: 75 and the sense strand comprises SEQ ID NO: 2; or

c) the antisense strand comprises SEQ ID NO: 80 and the sense strand comprises SEQ ID NO: 7; or

d) the antisense strand comprises SEQ ID NO: 91 and the sense strand comprises SEQ ID NO: 18; or

e) the antisense strand comprises SEQ ID NO: 92 and the sense strand comprises SEQ ID NO: 19; or

f) the antisense strand comprises SEQ ID NO: 96 and the sense strand comprises SEQ ID NO: 23; or

g) the antisense strand comprises SEQ ID NO: 100 and the sense strand comprises SEQ ID NO: 27; or

h) the antisense strand comprises SEQ ID NO: 103 and the sense strand comprises SEQ ID NO: 30; or

i) the antisense strand comprises SEQ ID NO: 104 and the sense strand comprises SEQ ID NO: 31; or

j) the antisense strand comprises SEQ ID NO: 112 and the sense strand comprises SEQ ID NO: 39; or

k) the antisense strand comprises SEQ ID NO: 888 and the sense strand comprises SEQ ID NO: 887; or

l) the antisense strand comprises SEQ ID NO: 890 and the sense strand comprises SEQ ID NO: 879; or

m) the antisense strand comprises SEQ ID NO: 893 and the sense strand comprises SEQ ID NO: 882; or

n) the antisense strand comprises SEQ ID NO: 894 and the sense strand comprises SEQ ID NO: 883; or

o) the antisense strand comprises SEQ ID NO: 896 and the sense strand comprises SEQ ID NO: 885,

and optionally wherein said sense strand and/or antisense strand comprises one or more modified nucleotide residues.

5. The dsRNA according to claim 1 or claim 2, wherein:

a) the antisense strand consists of SEQ ID NO: 74 and the sense strand consists of SEQ ID NO: 1; or

b) the antisense strand consists of SEQ ID NO: 75 and the sense strand consists of SEQ ID NO: 2; or

c) the antisense strand consists of SEQ ID NO: 80 and the sense strand consists of SEQ ID NO: 7; or

d) the antisense strand consists of SEQ ID NO: 91 and the sense strand consists of SEQ ID NO: 18; or

e) the antisense strand consists of SEQ ID NO: 92 and the sense strand consists of SEQ ID NO: 19; or

f) the antisense strand consists of SEQ ID NO: 96 and the sense strand consists of SEQ ID NO: 23; or

g) the antisense strand consists of SEQ ID NO: 100 and the sense strand consists of SEQ ID NO: 27; or

h) the antisense strand consists of SEQ ID NO: 103 and the sense strand consists of SEQ ID NO: 30; or

i) the antisense strand consists of SEQ ID NO: 104 and the sense strand consists of SEQ ID NO: 31; or

j) the antisense strand consists of SEQ ID NO: 112 and the sense strand consists of SEQ ID NO: 39; or

k) the antisense strand consists of SEQ ID NO: 888 and the sense strand consists of SEQ ID NO: 887; or

l) the antisense strand consists of SEQ ID NO: 890 and the sense strand consists of SEQ ID NO: 879; or

m) the antisense strand consists of SEQ ID NO: 893 and the sense strand consists of SEQ ID NO: 882; or

n) the antisense strand consists of SEQ ID NO: 894 and the sense strand consists of SEQ ID NO: 883; or

o) the antisense strand consists of SEQ ID NO: 896 and the sense strand consists of SEQ ID NO: 885,

and optionally wherein said sense strand and/or antisense strand comprises one or more modified nucleotide residues.

6. The dsRNA according to any one of claims 1 to 5, wherein each strand of the dsRNA comprises a 3' overhang consisting of dTdT.

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7. The dsRNA according to any one of claims 1 to 6, wherein the sense strand and/or the antisense strand comprises one or more modified nucleotide residues.

8. The dsRNA according to claim 7, wherein a modified nucleotide is selected from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

9. The dsRNA according to claim 7, wherein a modified nucleotide is selected from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

10. The dsRNA according to claim 7, wherein the sense strand and/or the antisense strand comprises at least one 2'-O-methyl modified nucleotide and at least one 2'-deoxythymidine-3'-phosphate nucleotide comprising a 5'-phosphorothioate group.

11. The dsRNA according claim 7, wherein all pyrimidine residues of the sense strand are 2'-O-methyl modified pyrimidines and wherein each pyrimidine residue of the antisense strand adjacent to an A residue is a 2'-O-methyl modified pyrimidines and wherein each strand comprises dTdT at a 3' end thereof.

12. The dsRNA according to claim 7, wherein all pyrimidine residues of the sense strand are 2'-O-methyl modified pyrimidines and wherein each pyrimidine residue of the antisense strand adjacent to A residue is a 2'-O-methyl modified pyrimidines and each strand comprises dTsdT at a 3' end thereof.

13. The dsRNA according to claim 12, wherein

a) the antisense strand comprises SEQ ID NO: 476 (5'-AUuAGuAUuAUGcAAAUUGdTdT -3') and the sense strand comprises SEQ ID NO: 475(5'- cAAuuuGcAuAAuAcuAAudTdT -3'); or

b) the antisense strand comprises SEQ ID NO: 442 (5'-ACGGcAAuAAAUuAGuAUUdTdT -3') and the sense strand comprises SEQ ID NO: 441 (5'- AAuAcuAAuuuAuuGccGudTdT -3'); or

c) the antisense strand comprises SEQ ID NO: 484 (5’-GACGGcAAuAAAUuAGuAUdTdT -3’) and the sense strand comprises SEQ ID NO: 483 (5’- AuAcuAAuuuAuuGccGucdTdT -3’); or

d) the antisense strand comprises SEQ ID NO: 944 (5’-GACGGcAAuAAAUuAGuAdTdT -3’) and the sense strand comprises SEQ ID NO: 943 (5’- uAcuAAuuuAuuGccGuccdTdT -3’); or

e) the antisense strand comprises SEQ ID NO: 456 (5’-AGGACGGcAAuAAAUuAGUdTdT -3’) and the sense strand comprises SEQ ID NO: 455 (5’- AcuAAuuuAuuGccGuccudTdT -3’); or

f) the antisense strand comprises SEQ ID NO: 440 (5’-cAGGACGGcAAuAAAUuAGdTdT -3’) and the sense strand comprises SEQ ID NO: 439 (5’- cuAAuuuAuuGccGuccuGdTdT -3’); or

g) the antisense strand comprises SEQ ID NO: 956 (5’-CcAGGACGGcAAuAAAUuAdTdT -3’) and the sense strand comprises SEQ ID NO: 955 (5’- uAAuuuAuuGccGuccuGGdTdT -3’); or

h) the antisense strand comprises SEQ ID NO: 498 (5’-UCcAGGACGGcAAuAAAUUdTdT -3’) and the sense strand comprises SEQ ID NO: 497 (5’- AAuuuAuuGccGuccuGGAdTdT -3’); or

i) the antisense strand comprises SEQ ID NO: 474 (5’-GUCcAGGACGGcAAuAAAUdTdT -3’) and the sense strand comprises SEQ ID NO: 473 (5’- AuuuAuuGccGuccuGGAcdTdT -3’); or

j) the antisense strand comprises SEQ ID NO: 960 (5’-AGAGUCcAGGACGGcAAuAdTdT -3’) and the sense strand comprises SEQ ID NO: 959 (5’- uAuuGccGuccuGGAcucudTdT -3’); or

k) the antisense strand comprises SEQ ID NO: 492 (5’-ACGCUcAcAcAGAGUCcAGdTdT -3’) and the sense strand comprises SEQ ID NO: 491 (5’- cuGGAcucuGuGuGAGcGudTdT -3’); or

l) the antisense strand comprises SEQ ID NO: 452 (5’-GGAcACGCUcAcAcAGAGUdTdT -3’) and the sense strand comprises SEQ ID NO: 451 (5’- AcucuGuGuGAGcGuGuccdTdT -3’); or

m) the antisense strand comprises SEQ ID NO: 500 (5'- CUGUGGAcACGCUcAcAcAdTdT -3') and the sense strand comprises SEQ ID NO: 499 (5'- uGuGuGAGcGuGuccAcAGdTdT -3'); or

n) the antisense strand comprises SEQ ID NO: 516 (5'- AUUuACuAcAAACUCUGUGdTdT -3') and the sense strand comprises SEQ ID NO: 515 (5'- cAcAGAGuuuGuAGuAAAudTdT -3'); or

o) the antisense strand comprises SEQ ID NO: 954 (5'- AAAUcAuAAuAUUuACuACdTdT -3') and the sense strand comprises SEQ ID NO: 953 (5'- GuAGuAAAuAuuAuGAuuudTdT -3'),

wherein 2'-O-methyladenosine is indicated by a lower case letter "a", 2'-O-methylcytidine is indicated by a lower case letter "c", 2'-O-methylguanosine is indicated by a lower case letter "g", 2'-O-methyluridine is indicated by a lower case letter "u", and 2'-deoxythymidine is indicated by "dT".

14. The dsRNA according to claim 12, wherein

a) the antisense strand consists of SEQ ID NO: 476 (5'- AUuAGuAUuAUGcAAAUUGdTdT -3') and the sense strand consists of SEQ ID NO: 475(5'- cAAuuuGcAuAAuAcuAAudTdT -3'); or

b) the antisense strand consists of SEQ ID NO: 442 (5'- ACGGcAAuAAAUuAGuAUUdTdT -3') and the sense strand consists of SEQ ID NO: 441 (5'- AAuAcuAAuuuAuuGccGudTdT -3'); or

c) the antisense strand consists of SEQ ID NO: 484 (5'- GACGGcAAuAAAUuAGuAUdTdT -3') and the sense strand consists of SEQ ID NO: 483 (5'- AuAcuAAuuuAuuGccGucdTdT -3'); or

d) the antisense strand consists of SEQ ID NO: 944 (5'- GACGGcAAuAAAUuAGuAdTdT -3') and the sense strand consists of SEQ ID NO: 943 (5'- uAcuAAuuuAuuGccGuccdTdT -3'); or

e) the antisense strand consists of SEQ ID NO: 456 (5'- AGGACGGcAAuAAAUuAGUdTdT -3') and the sense strand consists of SEQ ID NO: 455 (5'- AcuAAuuuAuuGccGuccudTdT -3'); or

f) the antisense strand consists of SEQ ID NO: 440 (5'- cAGGACGGcAAuAAAUuAGdTdT -3') and the sense strand consists of SEQ ID NO: 439 (5'- cuAAuuuAuuGccGuccuGdTdT -3'); or

g) the antisense strand consists of SEQ ID NO: 956 (5'- CcAGGACGGcAAuAAAUuAdTdT -3') and the sense strand consists of SEQ ID NO: 955 (5'- uAAuuuAuuGccGuccuGGdTdT -3'); or

h) the antisense strand consists of SEQ ID NO: 498 (5'- UCcAGGACGGcAAuAAAUUdTdT -3') and the sense strand consists of SEQ ID NO: 497 (5'- AAuuuAuuGccGuccuGGAdTdT -3'); or

i) the antisense strand consists of SEQ ID NO: 474 (5'- GUCcAGGACGGcAAuAAAUdTdT -3') and the sense strand consists of SEQ ID NO: 473 (5'- AuuuAuuGccGuccuGGAcdTdT -3'); or

j) the antisense strand consists of SEQ ID NO: 960 (5'- AGAGUCcAGGACGGcAAuAdTdT -3') and the sense strand consists of SEQ ID NO: 959 (5'- uAuuGccGuccuGGAcucudTdT -3'); or

k) the antisense strand consists of SEQ ID NO: 492 (5'- ACGCUcAcAcAGAGUCcAGdTdT -3') and the sense strand consists of SEQ ID NO: 491 (5'- cuGGAcucuGuGuGAGcGudTdT -3'); or

l) the antisense strand consists of SEQ ID NO: 452 (5'- GGAcACGCUcAcAcAGAGUdTdT -3') and the sense strand consists of SEQ ID NO: 451 (5'- AcucuGuGuGAGcGuGuccdTdT -3'); or

m) the antisense strand consists of SEQ ID NO: 500 (5'- CUGUGGAcACGCUcAcAcAdTdT -3') and the sense strand consists of SEQ ID NO: 499 (5'- uGuGuGAGcGuGuccAcAGdTdT -3'); or

n) the antisense strand consists of SEQ ID NO: 516 (5'- AUUuACuAcAAACUCUGUGdTdT -3') and the sense strand consists of SEQ ID NO: 515 (5'- cAcAGAGuuuGuAGuAAAudTdT -3'); or

o) the antisense strand consists of SEQ ID NO: 954 (5'- AAAUcAuAAuAUUuACuACdTdT -3') and the sense strand consists of SEQ ID NO: 953 (5'- GuAGuAAAuAuuAuGAuuudTdT -3'),

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wherein 2'-O-methyladenosine is indicated by a lower case letter “a”, 2'-O-methylcytidine is indicated by a lower case letter “c”, 2'-O-methylguanosine is indicated by a lower case letter “g”, 2'-O-methyluridine is indicated by a lower case letter “u”, and 2'-deoxythymidine is indicated by “dT”.

15. The dsRNA according to any one of claims 1 to 14, further comprising a ligand.

16. The dsRNA according to claim 15, wherein the ligand is conjugated to a 3'-end of the sense strand of the dsRNA.

17. A composition for inhibiting expression of a GNAQ gene comprising the dsRNA according to any one of claims 1 to 16 and a pharmaceutical formulation.

18. The composition according to claim 17, wherein the pharmaceutical formulation is a lipid formulation.

19. The composition according to claim 17 or claim 18, wherein the pharmaceutical formulation is a LNP formulation, a LNP01 formulation, a MC3 comprising formulation, or a LNP11 formulation.

20. The composition according to any one of claims 17 to 19, wherein the pharmaceutical formulation is a (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3).

21. The composition according to any one of claims 17 to 19, wherein the pharmaceutical formulation is a LNP11 formulation.

22. A vector comprising a nucleotide sequence that encodes at least one strand of the dsRNA according to any one of claims 1 to 14.

23. An isolated cell comprising the dsRNA according to any one of claims 1 to 16 or the composition according to any one of claims 17 to 21 or the vector of claim 22.

24. The dsRNA according to any one of claims 1 to 16 or the composition according to any one of claims 17 to 21 or the vector according to claim 22 when used to moderate expression of a G-alpha q subunit a (GNAQ) of a heterotrimeric G gene.

25. A method of inhibiting GNAQ expression in a cell, the method comprising:

(a) introducing the dsRNA according to any one of claims 1 to 16 or the composition according to any one of claims 17 to 21 or the vector according to claim 22 into a cell; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a GNAQ gene, thereby inhibiting expression of the GNAQ gene in the cell.

26. A method of treatment of a disorder mediated by GNAQ expression comprising administering a therapeutically effective amount of the dsRNA according to any one of claims 1 to 16 or the composition according to any one of claims 17 to 21 or the vector according to claim 22 to a subject in need thereof.

27. The method according to claim 26, wherein the disorder is uveal melanoma, cutaneous melanoma, Blue nevi, Nevi of Ota, a small lung tumor, or a neuroendocrine tumor.

28. Use of the dsRNA according to any one of claims 1 to 16 in the preparation of a medicament for treatment of a disorder mediated by GNAQ, wherein said disorder is selected from the group consisting of uveal melanoma, cutaneous melanoma, Blue nevi, Nevi of Ota, a small lung tumor and a neuroendocrine tumor.

29. The dsRNA according to any one of claims 1 to 16 or 24 or the composition according to any one of claims 17 to 21 or 24 or the vector according to claim 22 or 24 or the isolated cell according to claim 23 or the method according to any one of claims 25 to 27 or the use according to claim 28 substantially as described with reference to the accompanying drawings and/or examples.

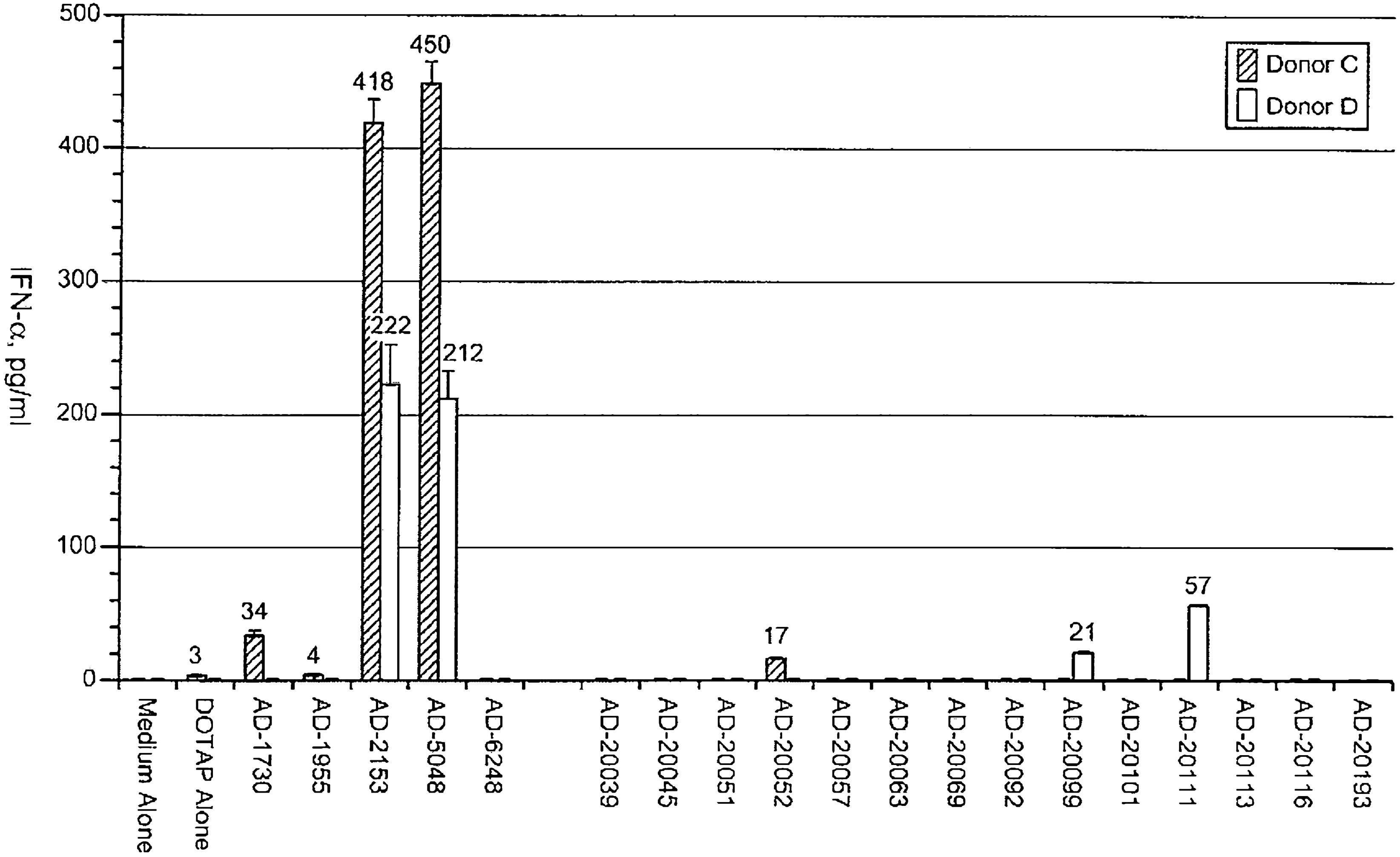
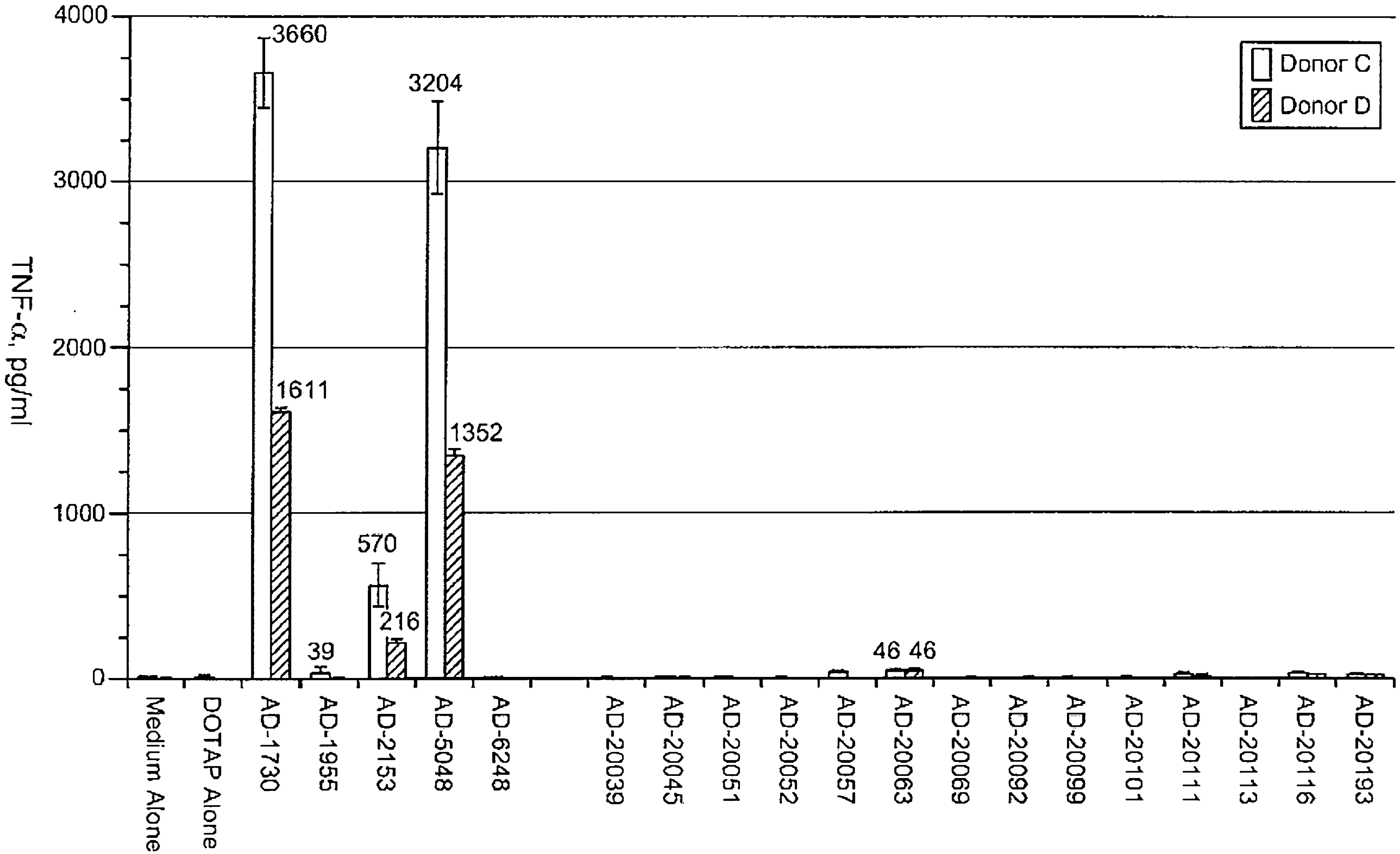
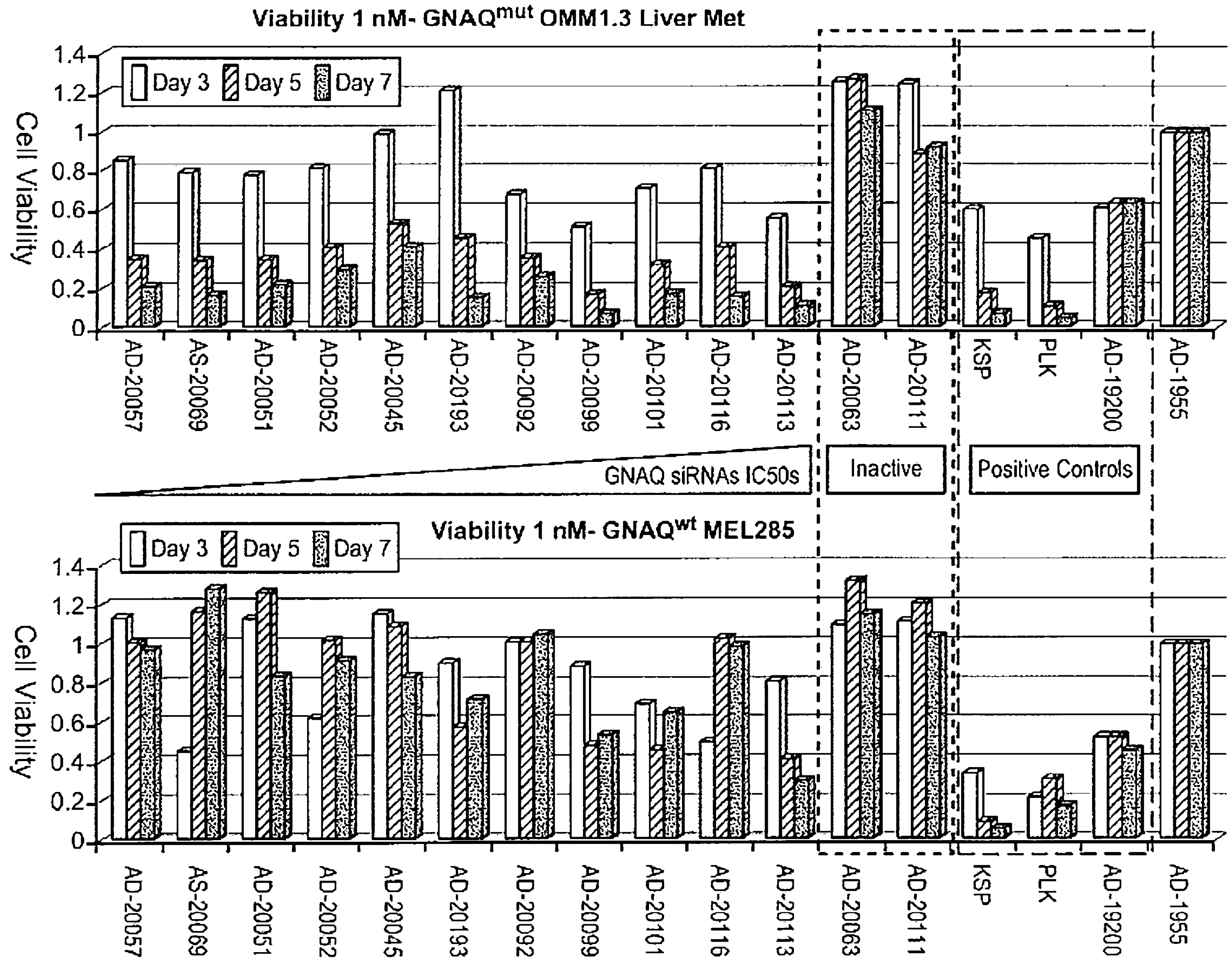

Donor C
Donor D
IFN-α, pg/ml
500
400
300
200
100
0
418
450
222
212
34
3
4
17
21
57
Medium Alone
DOTAP Alone
AD-1730
AD-1955
AD-2153
AD-5048
AD-6248
AD-20039
AD-20045
AD-20051
AD-20052
AD-20057
AD-20063
AD-20069
AD-20092
AD-20099
AD-20101
AD-20111
AD-20113
AD-20116
AD-20193

FIG. 1



4000
3000
2000
1000
0
TNF-α, pg/ml
Donor C
Donor D
3660
1611
39
570
216
3204
1352
46 46
Medium Alone
DOTAP Alone
AD-1730
AD-1955
AD-2153
AD-5048
AD-6248
AD-20039
AD-20045
AD-20051
AD-20052
AD-20057
AD-20063
AD-20069
AD-20092
AD-20099
AD-20101
AD-20111
AD-20113
AD-20116
AD-20193

FIG. 2



Viability 1 nM- GNAQ^mut OMM1.3 Liver Met
Day 3
Day 5
Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955
GNAQ siRNAs IC50s
Inactive
Positive Controls
Viability 1 nM- GNAQ^wt MEL285

FIG. 3

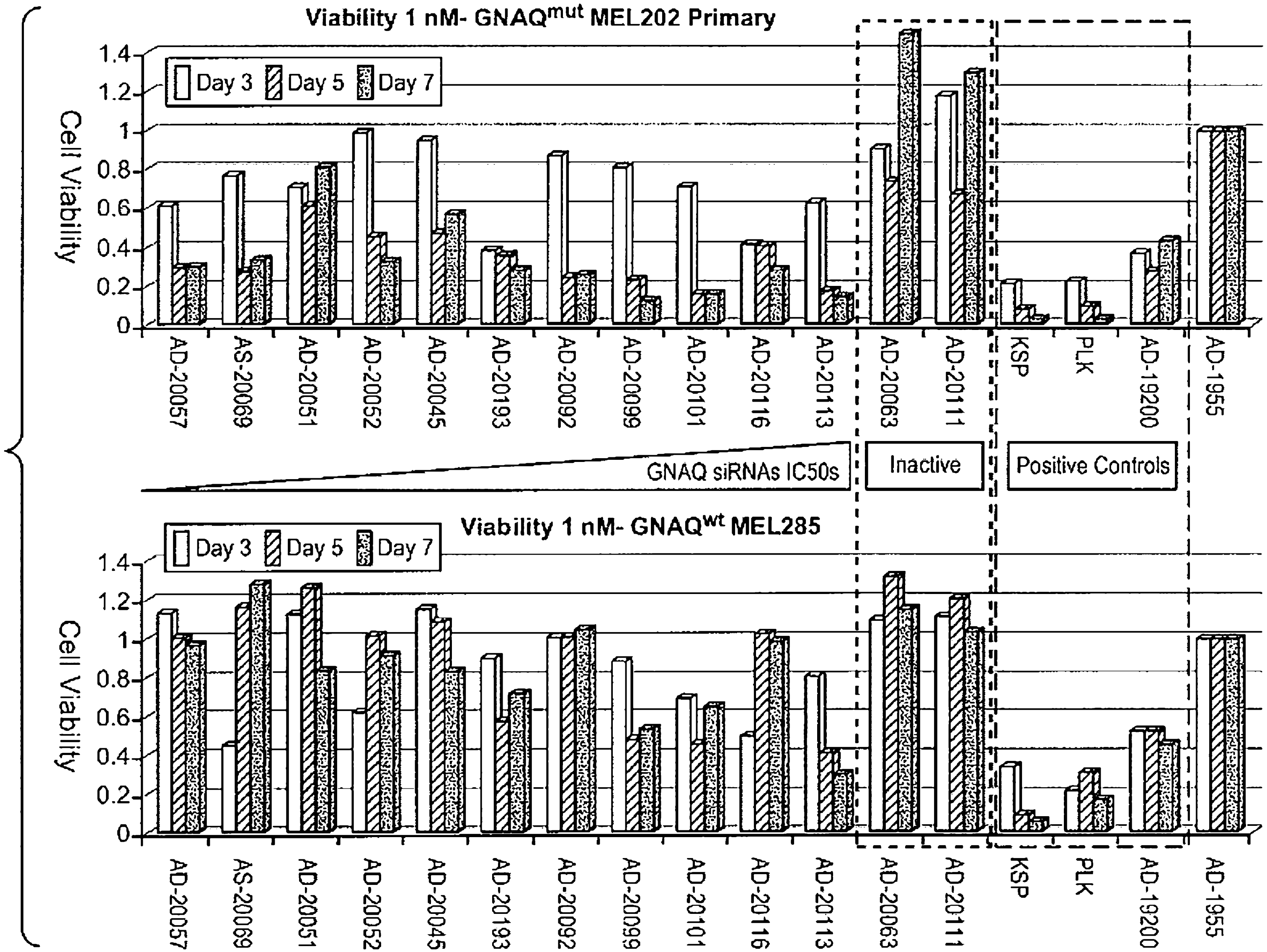
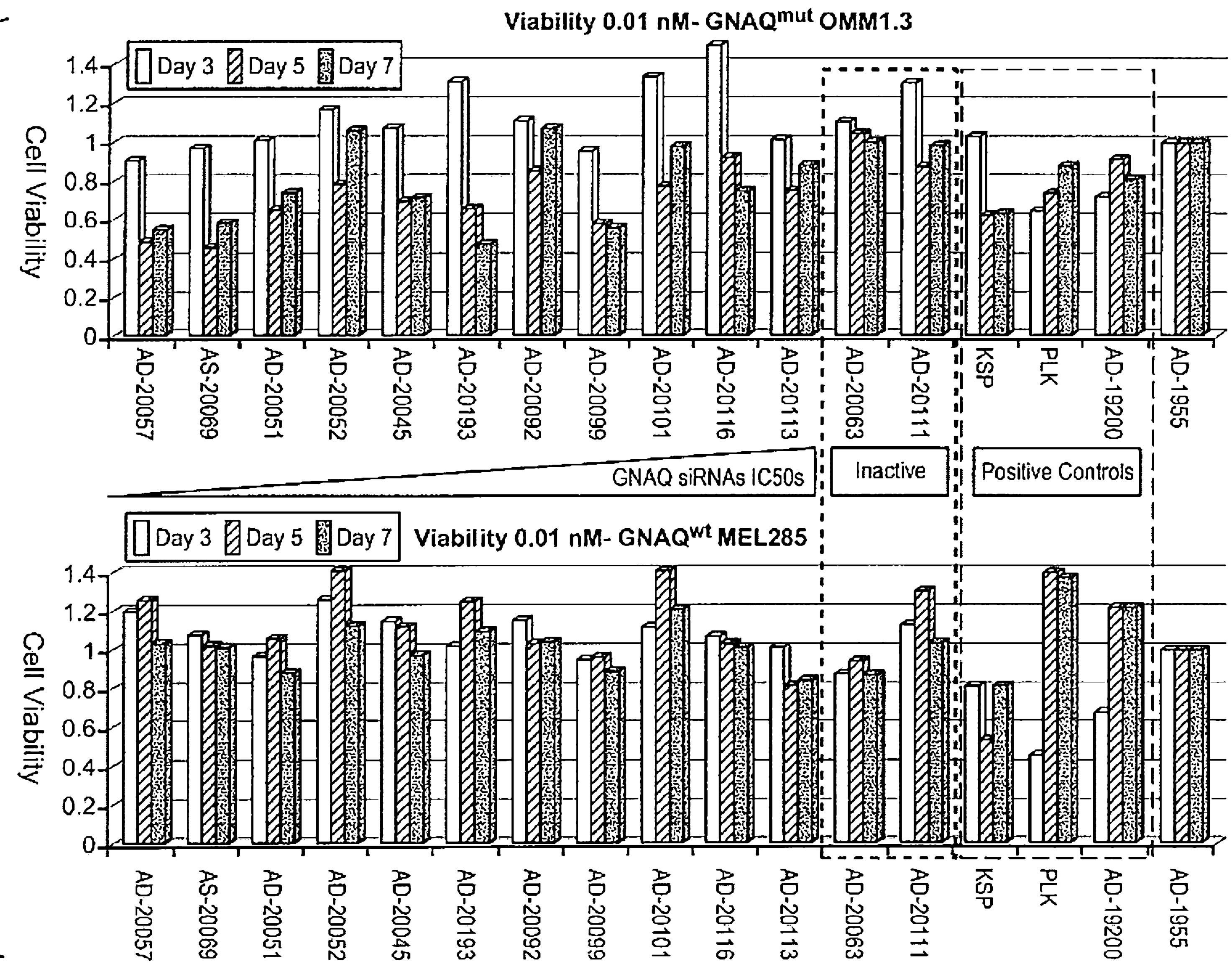
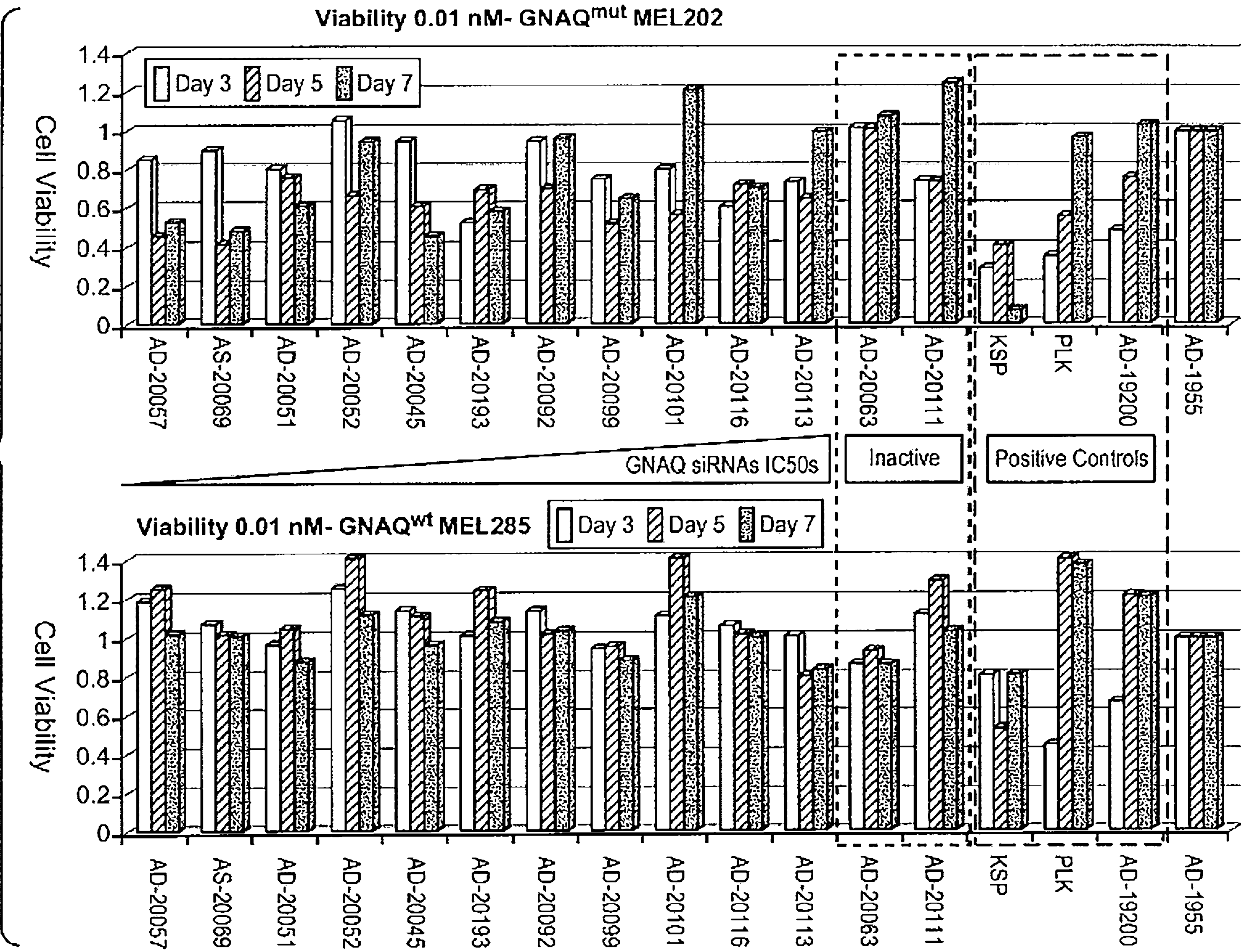

Viability 1 nM- GNAQ^mut MEL202 Primary
Day 3 Day 5 Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955
GNAQ siRNAs IC50s
Inactive
Positive Controls
Viability 1 nM- GNAQ^wt MEL285
Day 3 Day 5 Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955

FIG. 4



Viability 0.01 nM- GNAQ^{mut} OMM1.3
Day 3
Day 5
Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955
GNAQ siRNAs IC50s
Inactive
Positive Controls
Viability 0.01 nM- GNAQ^{wt} MEL285
Day 3
Day 5
Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955

FIG. 5



Viability 0.01 nM- GNAQ^mut MEL202
Day 3
Day 5
Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955
GNAQ siRNAs IC50s
Inactive
Positive Controls
Viability 0.01 nM- GNAQ^wt MEL285
Day 3
Day 5
Day 7
Cell Viability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AD-20057
AS-20069
AD-20051
AD-20052
AD-20045
AD-20193
AD-20092
AD-20099
AD-20101
AD-20116
AD-20113
AD-20063
AD-20111
KSP
PLK
AD-19200
AD-1955

FIG. 6

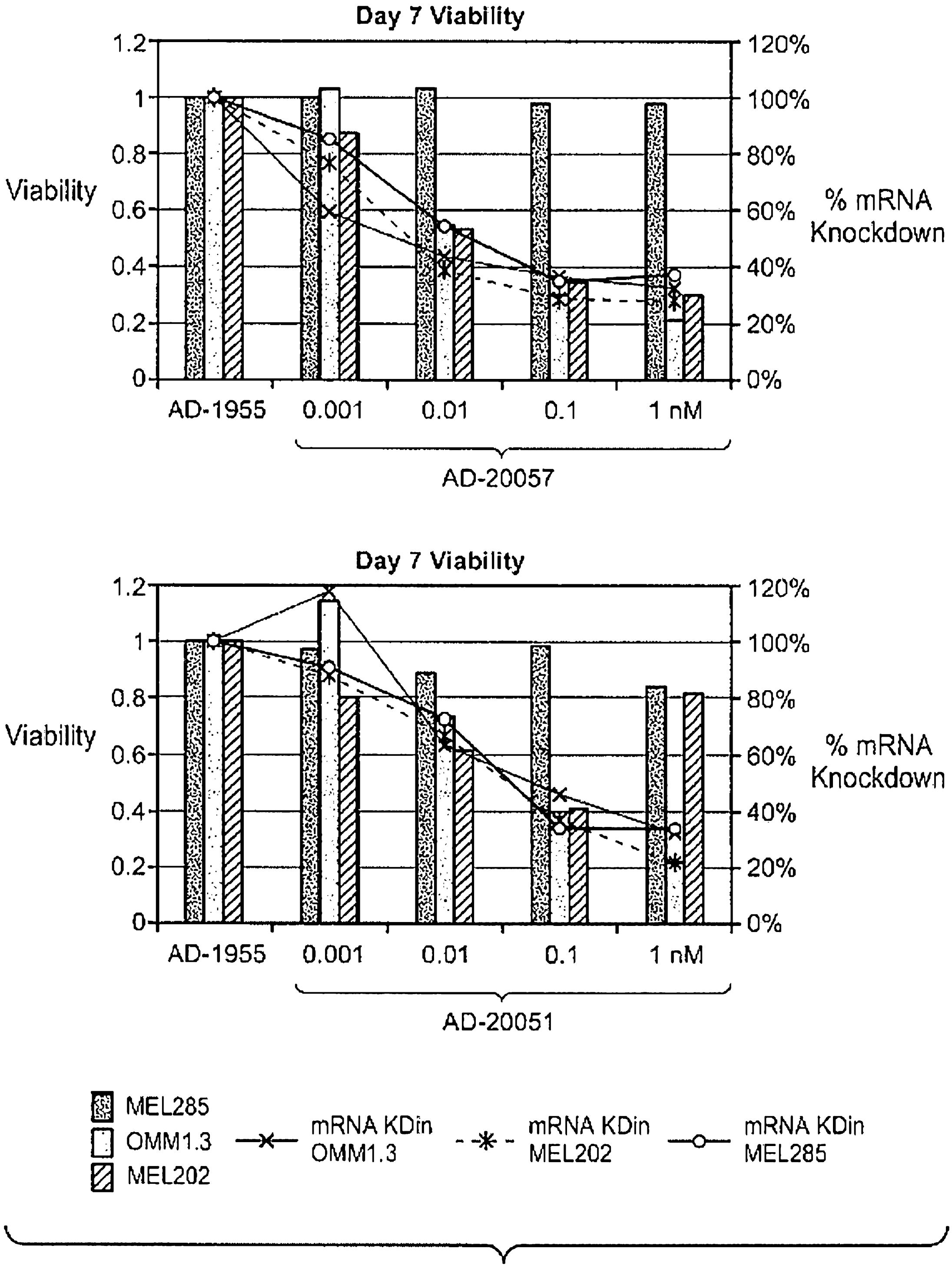

Day 7 Viability
1.2
1
0.8
0.6
0.4
0.2
0
Viability
120%
100%
80%
60%
40%
20%
0%
% mRNA Knockdown
AD-1955
0.001
0.01
0.1
1 nM
AD-20057
Day 7 Viability
1.2
1
0.8
0.6
0.4
0.2
0
Viability
120%
100%
80%
60%
40%
20%
0%
% mRNA Knockdown
AD-1955
0.001
0.01
0.1
1 nM
AD-20051
MEL285
OMM1.3
MEL202
mRNA KDin OMM1.3
mRNA KDin MEL202
mRNA KDin MEL285

FIG. 7

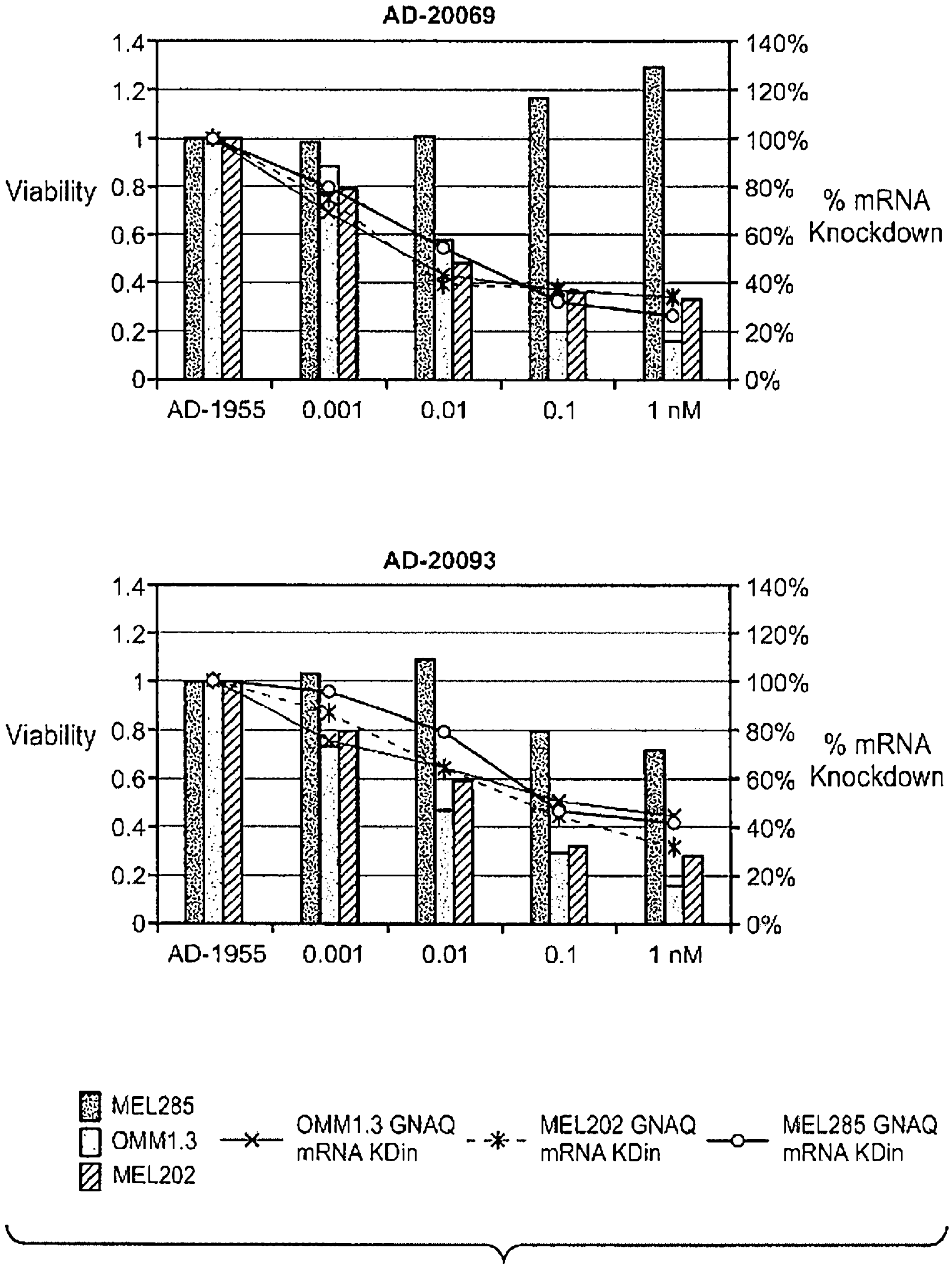

AD-20069
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Viability
140%
120%
100%
80%
60%
40%
20%
0%
% mRNA Knockdown
AD-1955
0.001
0.01
0.1
1 nM
AD-20093
Viability
% mRNA Knockdown
MEL285
OMM1.3
MEL202
OMM1.3 GNAQ mRNA KDin
MEL202 GNAQ mRNA KDin
MEL285 GNAQ mRNA KDin

FIG. 8

EDITORIAL NOTE
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The specification totals more than 1000 pages.

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