COMPOUNDS INCLUDING A MUTANT KRAS SEQUENCE AND A LIPID AND USES THEREOF

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on _____ is named _____ and is _____ bytes in size.

BACKGROUND OF THE INVENTION

RAS proteins are essential components of cell signaling pathways. Oncogenic activation of RAS proteins by mutation is frequently detected in several types of cancer, including pancreatic cancer. There continues to be a need for further and more effective cancer treatments.

SUMMARY

The disclosure provides compounds that can be used in therapeutic methods.

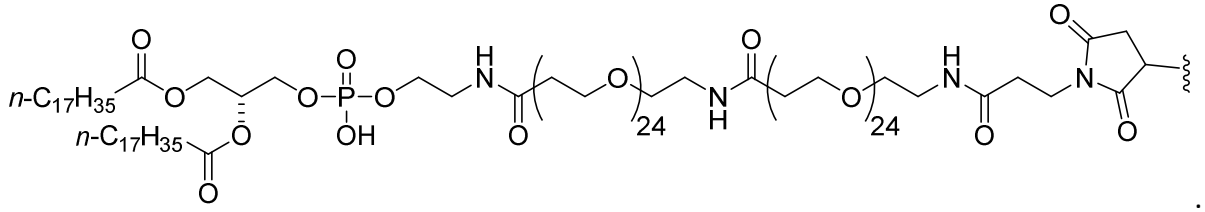
Accordingly, in one example, the disclosure features a compound including a mutant KRAS sequence and a lipid, where the mutant KRAS sequence is conjugated to the lipid by a linker, and (i) the linker includes one or more polyethylene glycol blocks, (ii) the lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), and (iii) the mutant KRAS sequence comprises or consists of an amino acid sequence selected from the group consisting of YKLVVVGADGVGKSALTI (SEQ ID NO:23), YKLVVVGAVGVGKSALTI (SEQ ID NO:24), YKLVVVGARGVGKSALTI (SEQ ID NO:25), YKLVVVGAAGVGKSALTI (SEQ ID NO:26), YKLVVVGASGVGKSALTI (SEQ ID NO:27), YKLVVVGACGVGKSALTI (SEQ ID NO:28), YKLVVVGATGVGKSALTI (SEQ ID NO:29), and YKLVVVGAGDVGKSALTI (SEQ ID NO:30).

In another example, the disclosure features a compound including a mutant KRAS sequence and a lipid, where the mutant KRAS sequence is conjugated to the lipid by a linker, and (i) the linker includes one or more polyethylene glycol blocks, (ii) the lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), and (iii) the mutant KRAS sequence comprises or consists of an amino acid sequence selected from the group consisting of CYKLVVVGADGVGKSALTI (SEQ ID NO:1), CYKLVVVGAVGVGKSALTI (SEQ ID NO:2), CYKLVVVGARGVGKSALTI (SEQ ID NO:3), CYKLVVVGAAGVGKSALTI (SEQ ID NO:4), CYKLVVVGASGVGKSALTI (SEQ ID NO:5), CYKLVVVGACGVGKSALTI (SEQ ID NO:6), CYKLVVVGATGVGKSALTI (SEQ ID NO:22), and CYKLVVVGAGDVGKSALTI (SEQ ID NO:7). In one aspect, the present disclosure refers to a compound comprising a mutant KRAS sequence and a lipid, wherein the mutant KRAS sequence is conjugated to the lipid by a linker, and (i) the linker comprises one or more polyethylene glycol blocks, (ii) the lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), and (iii) the mutant KRAS sequence consists of an amino acid sequence selected from the group consisting of CYKLVVVGADGVGKSALTI (SEQ ID NO:1), CYKLVVVGAVGVGKSALTI (SEQ ID NO:2), CYKLVVVGARGVGKSALTI (SEQ ID NO:3), CYKLVVVGAAGVGKSALTI (SEQ ID NO:4), CYKLVVVGASGVGKSALTI (SEQ ID NO:5), CYKLVVVGACGVGKSALTI (SEQ ID NO:6), CYKLVVVGATGVGKSALTI (SEQ ID NO:22), and CYKLVVVGAGDVGKSALTI (SEQ ID NO:7).

In one example, the mutant KRAS sequence, at its N-terminus, is conjugated to the linker through a cysteine-maleimide linkage.

In another example the linker includes 48 repeat units of polyethylene glycol.

In an additional example, the mutant KRAS sequence, at its N-terminus, is conjugated to the following structure:



In one example, the disclosure features a composition including one or more compounds of the disclosure and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a composition comprising one or more compounds of the disclosure, and a pharmaceutically acceptable carrier.

In one example, the composition includes (1) a compound including the amino acid sequence YKLVVVGADGVGKSALTI (SEQ ID NO:23), (2) a compound including the amino acid sequence YKLVVVGAVGVGKSALTI (SEQ ID NO:24), (3) a compound including the amino acid sequence YKLVVVGARGVGKSALTI (SEQ ID NO:25), (4) a compound including the amino acid sequence YKLVVVGAAGVGKSALTI (SEQ ID NO:26), (5) a compound including the amino acid sequence YKLVVVGASGVGKSALTI (SEQ ID NO:27), (6) a compound including the amino acid sequence YKLVVVGACGVGKSALTI (SEQ ID NO:28) or a compound including the amino acid sequence YKLVVVGATGVGKSALTI (SEQ ID NO:29), and (7) a compound including the amino acid sequence YKLVVVGAGDVGKSALTI (SEQ ID NO:30).

In one example, the composition includes (1) a compound including the amino acid sequence YKLVVVGADGVGKSALTI (SEQ ID NO:23), (2) a compound including the amino acid sequence YKLVVVGAVGVGKSALTI (SEQ ID NO:24), (3) a compound including the amino acid sequence YKLVVVGARGVGKSALTI (SEQ ID NO:25), (4) a compound including the amino acid sequence YKLVVVGAAGVGKSALTI (SEQ ID NO:26), (5) a compound including the amino acid sequence YKLVVVGASGVGKSALTI (SEQ ID NO:27), (6) a compound including the amino acid sequence YKLVVVGACGVGKSALTI (SEQ ID NO:28), and (7) a compound including the amino acid sequence YKLVVVGAGDVGKSALTI (SEQ ID NO:30).

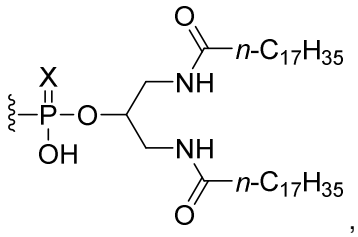
In another example, the composition includes (1) a compound including the amino acid sequence CYKLVVVGADGVGKSALTI (SEQ ID NO:1), (2) a compound including the amino acid sequence CYKLVVVGAVGVGKSALTI (SEQ ID NO:2), (3) a compound including the amino acid sequence CYKLVVVGARGVGKSALTI (SEQ ID NO:3), (4) a compound including the amino acid sequence CYKLVVVGAAGVGKSALTI (SEQ ID NO:4), (5) a compound including the amino acid sequence CYKLVVVGASGVGKSALTI (SEQ ID NO:5), (6) a compound including the amino acid sequence CYKLVVVGACGVGKSALTI (SEQ ID NO:6) or a compound including the amino acid sequence CYKLVVVGATGVGKSALTI (SEQ ID NO:22), and (7) a compound including the amino acid sequence CYKLVVVGAGDVGKSALTI (SEQ ID NO:7).

In another example, the composition includes (1) a compound including the amino acid sequence CYKLVVVGADGVGKSALTI (SEQ ID NO:1), (2) a compound including the amino acid sequence CYKLVVVGAVGVGKSALTI (SEQ ID NO:2), (3) a compound including the amino acid sequence CYKLVVVGARGVGKSALTI (SEQ ID NO:3), (4) a compound including the amino acid sequence CYKLVVVGAAGVGKSALTI (SEQ ID NO:4), (5) a

compound including the amino acid sequence CYKLVVVGASGVGKSALTI (SEQ ID NO:5), (6) a compound including the amino acid sequence CYKLVVVGACGVGKSALTI (SEQ ID NO:6), and (7) a compound including the amino acid sequence CYKLVVVGAGDVGKSALTI (SEQ ID NO:7).

In some examples, 700 µg of each compound is included in the composition.

In an additional example, the composition further includes a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



,

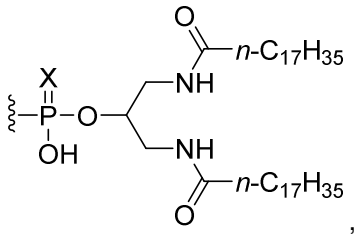
or a salt thereof, where X is O or S. In one example, the nucleotide sequence is bonded to the lipid. In another example, all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8) are phosphorothioates.

In one aspect, the present disclosure refers to use of the composition of the disclosure in the manufacture of a medicament for treating a cancer in a human patient. In one example, the disclosure features a method of treating a cancer in a human patient, the method including administering the composition of the disclosure to the patient.

In one example, the use or method further includes administering an adjuvant, such as an adjuvant including a CpG nucleotide sequence (e.g., 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3'; SEQ ID NO:8).

In some examples, 0.1 mg, 0.5 mg, or 2.5 mg of the adjuvant is administered.

In one example, the use or method further includes administering to the patient a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



,

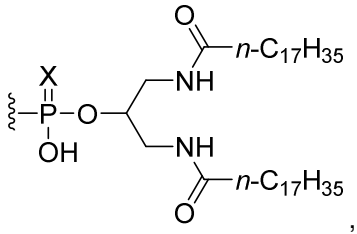
or a salt thereof, where X is O or S. In one example, the nucleotide sequence is bonded to the lipid. In one example, 0.1 mg, 0.5 mg, or 2.5 mg of the compound is administered.

In another example, all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8) are phosphorothioates.

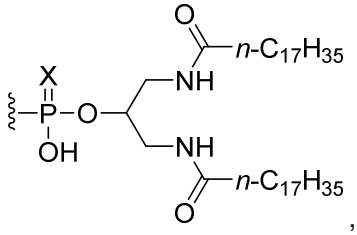
In one example, the cancer is a pancreatic cancer, a lung cancer, or a colorectal cancer.

In another example, the disclosure features a kit including (i) a compound of the disclosure, or a composition of the disclosure, and (ii) a compound consisting of the nucleotide sequence

5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



, or a salt thereof, where X is O or S. In one aspect, the present disclosure refers to a kit comprising (i) a compound of the disclosure, and (ii) a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



, or a salt thereof, wherein X is O or S. In one example, the nucleotide sequence is bonded to the lipid. In another example, all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8) are phosphorothioates.

Definitions

A "carbonyl," as used herein, refers to a group –C(O)–.

A "carboxylate," as used herein, refers to a group –C(O)–OH or a salt thereof.

As used herein, "conjugated" refers to a covalent bonding of a mutant KRAS sequence to a linker and, optionally, further covalent bonding of the linker to a lipid. In various examples, the covalent bonds connecting the linker to the mutant KRAS sequence or to the lipid may be a covalent bond between a sulfur atom and a sp^3-hybridized carbon atom in succinimide, between a nitrogen atom and a carbon atom of a carbonyl, or between oxygen atom and the phosphorus atom of a thiophosphoryl or phosphoryl. In various examples, each of the KRAS sequence and the lipid may contain a group for bonding to a complementary group in the linker. For example, the KRAS polypeptide may include a sulfur atom (e.g., the sulfur atom in a cysteine residue) bonded to a complementary group, e.g., succinimide, in the linker. The bond from the sulfur atom in the KRAS polypeptide to the succinimide in the linker may be formed by a reaction of a thiol (e.g., a thiol in a cysteine residue) in the KRAS polypeptide and a maleimide in the linker. Alternatively, the KRAS polypeptide may include a nitrogen atom or a carbonyl group bonded to a carbonyl group or a nitrogen atom, respectively, in the linker. The bond between the carbonyl group and the nitrogen atom may be formed by a reaction between an amine or carboxylate in the KRAS polypeptide and a carboxylate or an amine, respectively, in the linker.

A "lipid" is any of a group of organic compounds that are greasy to the touch, insoluble in water, and soluble in organic solvents, and in general includes fatty acids and their derivatives. One example of a lipid used in the disclosure is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE).

A "linker," as used herein, refers to a monovalent or divalent group, in which one valency is covalently bonded to one biologically functional group, and the other valency is covalently bonded to another biologically functional group. In one example, a linker connects a KRAS sequence to a lipid, as noted above. Optionally, such a linker can include one or more polyethylene glycol blocks. In another example, a linker connects a nucleotide sequence of, e.g., a CpG oligonucleotide, to a lipid (e.g., -P(X)(OH)-O-CH(CH_2NHCO-(CH_2)$_{16}$-CH_3)$_2$, or a salt thereof, where X is O or S, as described herein). Such linkers can optionally include one or more nucleotides, for example, a dinucleotide (e.g., GG).

A "pharmaceutically acceptable carrier," as used herein, refers to a vehicle capable of suspending or dissolving an active compound, and having the properties of being nontoxic and non-inflammatory in a

subject (e.g., a human patient) to whom it is administered. Moreover, a pharmaceutically acceptable carrier may include a pharmaceutically acceptable additive, such as a preservative, antioxidant, fragrance, emulsifier, dye, or excipient known or used in the field of drug formulation and that does not significantly interfere with the therapeutic effectiveness of the biological activity of the active agent, and that is non-toxic to the subject.

A "phosphoryl," as used herein, refers to a group $–P(O)(OR^A)(OR^B)$, where R^A is H, and R^B is a valency.

A "polyethylene glycol," as used herein, refers to a block $–(OCH_2CH_2)_n–$, where n is a number of repeat units and is an integer from 2 to 50 (e.g., 24 or 48).

A "thiol," as used herein, refers to a group –SH.

A "thiophosphoryl," as used herein, refers to a group $–P(S)(OR^A)(OR^B)$, where R^A is H, and R^B is a valency.

The terms "treat," "treatment," and "treating" refer to therapeutic approaches in which the goal is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a condition associated with a disease or disorder, e.g., cancer. These terms include reducing or alleviating at least one adverse effect or symptom of a condition, disease, or disorder. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced, or if a desired response (e.g., a specific immune response) is induced. Alternatively, treatment is "effective" if the progression of a disease is reduced or halted.

The disclosure provides several advantages. For example, in including DSPE moieties, certain compounds of the disclosure bind to endogenous albumin in subjects to whom they are administered, which enhances delivery of the compounds to the lymph nodes of the subjects. This facilitates the induction of a therapeutic immune response against the KRAS sequences of the compounds, leading to effective cancer treatment.

Other features and advantages of the disclosure will be apparent from the following detailed description, the drawings, and the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows that amphiphile-KRas (aKRas), in combination with amphiphile-CpG (aCpG), activated splenocytes, while soluble KRas, in combination with aCpG, and the untreated and soluble KRas and polyIC (pIC) control groups, did not.

Figure 2 shows that both aKRas G12R and G12V, in combination with aCpG, activated splenocytes, while neither soluble KRas G12R nor G12V in combination with aCpG, and the untreated and soluble KRas and pIC control groups, did not.

Figure 3 shows a KRas-specific $CD8^+$ T-cell response for mice immunized with the combination of aKRas G12D and aCpG, but not for soluble KRas G12D in combination with CpG or soluble wild-type KRas in combination with CpG.

Figure 4 shows a KRas-specific $CD8^+$ T-cell response (9mers) and $CD8^+$ or $CD4^+$T-cell response (18mer) for mice immunized with the combination of aKRas G12D and aCpG, but not for soluble KRas G12D in combination with CpG or the untreated control.

CLAIMS

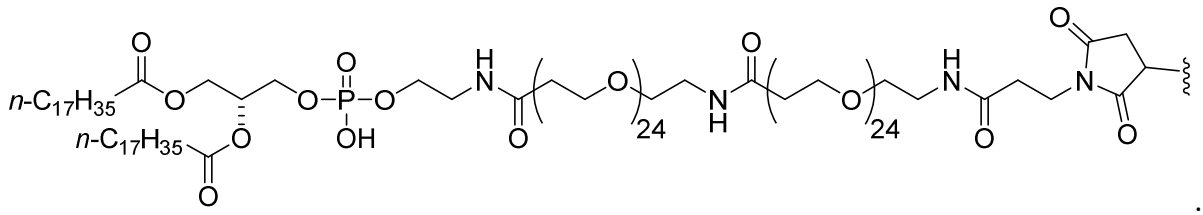
1. A compound comprising a mutant KRAS sequence and a lipid, wherein the mutant KRAS sequence is conjugated to the lipid by a linker, and (i) the linker comprises one or more polyethylene glycol blocks, (ii) the lipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE), and (iii) the mutant KRAS sequence consists of an amino acid sequence selected from the group consisting of CYKLVVVGADGVGKSALTI (SEQ ID NO:1), CYKLVVVGAVGVGKSALTI (SEQ ID NO:2), CYKLVVVGARGVGKSALTI (SEQ ID NO:3), CYKLVVVGAAGVGKSALTI (SEQ ID NO:4), CYKLVVVGASGVGKSALTI (SEQ ID NO:5), CYKLVVVGACGVGKSALTI (SEQ ID NO:6), CYKLVVVGATGVGKSALTI (SEQ ID NO:22), and CYKLVVVGAGDVGKSALTI (SEQ ID NO:7).

2. The compound of claim 1, wherein the mutant KRAS sequence consists of an amino acid sequence selected from the group consisting of YKLVVVGADGVGKSALTI (SEQ ID NO:23), YKLVVVGAVGVGKSALTI (SEQ ID NO:24), YKLVVVGARGVGKSALTI (SEQ ID NO:25), YKLVVVGAAGVGKSALTI (SEQ ID NO:26), YKLVVVGASGVGKSALTI (SEQ ID NO:27), YKLVVVGACGVGKSALTI (SEQ ID NO:28), YKLVVVGATGVGKSALTI (SEQ ID NO:29), and YKLVVVGAGDVGKSALTI (SEQ ID NO:30).

3. The compound of claim 1 or 2, wherein the mutant KRAS sequence, at its N-terminus, is conjugated to the linker through a cysteine-maleimide linkage.

4. The compound of claim 1 or 2, wherein linker comprises 48 repeat units of polyethylene glycol.

5. The compound of claim 1 or 2, wherein the mutant KRAS sequence, at its N-terminus, is conjugated to the following structure:



.

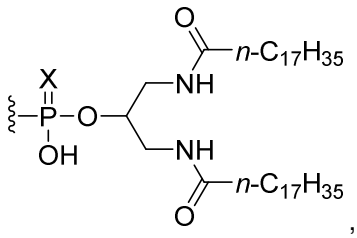
6. A composition comprising one or more compounds of claim 1 or 2, and a pharmaceutically acceptable carrier.

7. The composition of claim 6, wherein the composition comprises (1) a compound comprising the amino acid sequence YKLVVVGADGVGKSALTI (SEQ ID NO:23), (2) a compound comprising the amino acid sequence YKLVVVGAVGVGKSALTI (SEQ ID NO:24), (3) a compound comprising the amino acid sequence YKLVVVGARGVGKSALTI (SEQ ID NO:25), (4) a compound comprising the amino acid sequence YKLVVVGAAGVGKSALTI (SEQ ID NO:26), (5) a compound comprising the amino acid sequence

YKLVVVGASGVGKSALTI (SEQ ID NO:27), (6) a compound comprising the amino acid sequence YKLVVVGACGVGKSALTI (SEQ ID NO:28) or a compound comprising the amino acid sequence YKLVVVGATGVGKSALTI (SEQ ID NO:29), and (7) a compound comprising the amino acid sequence YKLVVVGAGDVGKSALTI (SEQ ID NO:30).

8. The composition of claim 6, wherein the composition comprises (1) a compound comprising the amino acid sequence CYKLVVVGADGVGKSALTI (SEQ ID NO:1), (2) a compound comprising the amino acid sequence CYKLVVVGAVGVGKSALTI (SEQ ID NO:2), (3) a compound comprising the amino acid sequence CYKLVVVGARGVGKSALTI (SEQ ID NO:3), (4) a compound comprising the amino acid sequence CYKLVVVGAAGVGKSALTI (SEQ ID NO:4), (5) a compound comprising the amino acid sequence CYKLVVVGASGVGKSALTI (SEQ ID NO:5), (6) a compound comprising the amino acid sequence CYKLVVVGACGVGKSALTI (SEQ ID NO:6) or a compound comprising the amino acid sequence CYKLVVVGATGVGKSALTI (SEQ ID NO:22), and (7) a compound comprising the amino acid sequence CYKLVVVGAGDVGKSALTI (SEQ ID NO:7).

9. The composition of claim 6, wherein the composition further comprises a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



,

or a salt thereof,
wherein X is O or S.

10. The composition of claim 6, wherein the composition comprises 700 µg of each compound.

11. The composition of claim 9, wherein the nucleotide sequence is bonded to the lipid.

12. Use of the composition of claim 6 in the manufacture of a medicament for treating a cancer in a human patient.

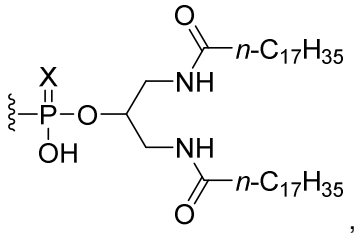
13. The use of claim 12, wherein the medicament is to be administered with an adjuvant.

14. The use of claim 13, wherein the adjuvant comprises a CpG nucleotide sequence.

15. The use of claim 14, wherein the CpG nucleotide sequence comprises 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO: 8).

16. The use of claim 12, wherein 0.1 mg, 0.5 mg, or 2.5 mg of the adjuvant is to be administered.

17. The use of claim 12, wherein the medicament is to be administered to the patient with a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:

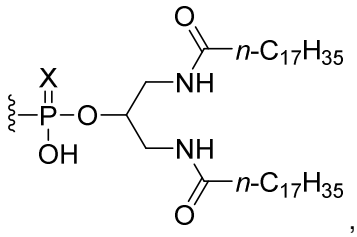


,

or a salt thereof,
wherein X is O or S.

18. The use of claim 17, wherein the nucleotide sequence is bonded to the lipid.

19. The use of claim 17, wherein 0.1 mg, 0.5 mg, or 2.5 mg of the compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



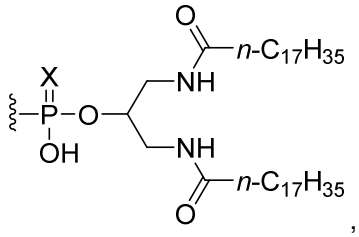
,

or a salt thereof,
wherein X is O or S,
is to be administered.

20. The use of claim 12, wherein the cancer is a pancreatic cancer, a lung cancer, or a colorectal cancer.

21. The use of claim 12, wherein all internucleoside groups connecting the nucleosides in 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8) are phosphorothioates.

22. A kit comprising (i) a compound of claim 1 or 2, and (ii) a compound consisting of the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:8), which, at its 5' end, is bonded or linked by a linker to the following lipid:



,

or a salt thereof,

wherein X is O or S.