

CLAIMS:

1. A bifunctional shRNAs capable of reducing an expression of a K-ras gene, wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene and wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.
2. The bifunctional shRNAs of claim 1, wherein the bifunctional shRNA comprises at least one sequence defined by SEQ ID NOS: 1 to 22 or 53 to 56.
3. The bifunctional shRNAs of claim 1, wherein the bifunctional shRNA comprises at least one sequence defined by SEQ ID NOS: 31 to 52.
4. The bifunctional shRNAs of claim 1, wherein at least one target site sequence is within a human K-ras gene cDNA sequence (SEQ ID NOS: 27 to 30).
5. The bifunctional shRNAs of claim 1, wherein the K-ras is a mutated K-ras.
6. An expression vector comprising:
a promoter; and
a nucleic acid insert operably linked to the promoter, wherein the insert encodes one or more shRNA capable of inhibiting an expression of at least one target gene that is a K-ras gene via RNA interference;
wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.
7. The expression vector of claim 6, wherein the ~~target gene sequence~~ bifunctional shRNA comprises SEQ ID NO: 1 to 5.
8. The expression vector of claim 6, wherein a sequence arrangement for the shRNA comprises a 5' stem arm-19 nucleotide target, which is K-ras-TA-15 nucleotide loop-19

nucleotide target complementary sequence-3' stem arm-Spacer-5' stem arm-19 nucleotide target variant-TA-15 nucleotide loop-19 nucleotide target complementary sequence-3' stem arm.

9. The expression vector of claim 6, wherein the nucleic acid insert comprises at least one sequence selected from SEQ ID NO: 6 to 27 or 53 to 56.

10. The expression vector of claim 6, wherein the vector comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 copies of bifunctional shRNAs inserts capable of reducing an expression of one or more mutated K-ras genes.

11. The expression vector of claim 6, wherein at least one shRNA has a target site sequence that is within a mutated K-ras gene cDNA sequence.

12. A therapeutic delivery system comprising:

a therapeutic agent carrier; and

an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter, the nucleic acid insert encoding one or more short hairpin RNA (shRNA) capable inhibiting an expression of a target gene sequence that is K-ras gene via RNA interference;

wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.

13. The delivery system of claim 12, wherein the therapeutic agent carrier is a compacted DNA nanoparticle, or a compacted DNA nanoparticle with one or more polycations.

14. The delivery system of claim 13, wherein the one or more polycations is a 10 kDa polyethylene glycol (PEG)-substituted cysteine-lysine 3-mer peptide (CK₃₀PEG10k).

15. The delivery system of claim 13, wherein the compacted DNA nanoparticles are further encapsulated in at least one of a liposome, a reversibly masked liposome, or a bilamellar invaginated vesicle (BIV).

16. The delivery system of claim 12, wherein the target gene sequence comprises SEQ ID NOS: 27 to 30.

17. The delivery system of claim 12, wherein the nucleic acid insert comprises at least one of the sequences selected from SEQ ID NOS: 31 to 52.

18. The delivery system of claim 12, wherein the nucleic acid insert is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

19. Use of a composition to reduce the expression of ~~A method to deliver one or more shRNAs to a target tissue expressing a~~ mutant K-ras gene comprising the steps of:

~~preparing~~ an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter that encodes the one or more shRNA, wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras as compared to wild-type K-ras;

~~combining the expression vector with~~ in a therapeutic agent carrier, wherein the therapeutic agent carrier comprises a liposome; ~~and~~

~~administering a therapeutically effective amount of the expression vector and therapeutic agent carrier complex to a patient in need thereof.~~

20. The use method of claim 19, wherein the therapeutic agent carrier is a compacted DNA nanoparticle.

21. The use method of claim 20, wherein the DNA nanoparticle is compacted with one or more polycations, wherein the one or more polycations comprise a 10 kDA polyethylene glycol (PEG)-substituted cysteine-lysine 3-mer peptide (CK₃₀PEG10k) or a 30-mer lysine condensing peptide.

22. The use method of claim 20, wherein the compacted DNA nanoparticles are further encapsulated in a liposome, wherein the liposome is a bilamellar invaginated vesicle (BIV) that comprises one or more receptor targeting moieties.

23. The use method of claim 22, wherein the one or more receptor targeting moieties comprise small molecule bivalent beta-turn mimics.

24. The use method of claim 19, wherein the nucleic acid insert comprises a sequence selected from SEQ ID NOS: 1 to 22 or 53 to 56.

25. ~~A method to inhibit~~ The use of a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras ~~an expression of a K-ras gene in one or more target cells comprising the steps of:~~

~~selecting the one or more target cells; and~~

~~transfecting the target cell with a vector that expresses one or more short hairpin RNA (shRNAs) capable of inhibiting an expression of a K-ras gene in the one or more target cells via RNA interference, wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.~~

26. — ~~The method of claim 25, wherein the shRNA incorporates siRNA (cleavage-dependent) and miRNA (cleavage-independent) motifs.~~

27. Use of a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of the target gene, wherein the bifunctional shRNA selectively knocks-down mutant K-ras to suppress tumor cell growth in vitro to trigger apoptosis, arrested cell proliferation, or reduced invasiveness of a tumor cell. ~~A method of suppressing a tumor cell growth in a human subject comprising the steps of:~~

~~identifying the human subject in need for suppression of the tumor cell growth; and~~

~~administering an expression vector in a therapeutic agent carrier complex to the human subject in an amount sufficient to suppress the tumor cell growth, wherein the expression vector expresses one or more shRNA capable inhibiting an expression of a target gene that is a mutated K-ras in the one or more target cells via RNA interference;~~

~~wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of the target gene,~~

~~wherein the inhibition results in an apoptosis, an arrested proliferation, or a reduced invasiveness of the tumor cells.~~

28. The use method of claim 27, wherein the therapeutic agent carrier comprises a bilamellar invaginated vesicle (BIV).
29. The use method of claim 27, wherein the therapeutic agent carrier comprises one or more receptor targeting moieties that comprise small molecule bivalent beta-turn mimics.
30. The use method of claim 27, wherein administering is selected from the group consisting of intratumoral, subcutaneous, intravenous, intraperitoneal, intramuscular, and intravenous injection.
31. The use method of claim 27, wherein administering comprises injecting with a DNA:lipoplex.
32. The use method of claim 27, wherein the tumor cell growth expresses a mutated K-ras.
33. The use method of claim 27, wherein the tumor cell growth is human pancreatic ductal adenocarcinoma.
34. The use method of claim 27, wherein the tumor cell growth is selected from the group consisting of lung cancer, colon cancer, melanoma, insulinoma, mesothelioma, ovarian cancer, and pancreatic cancer.
35. The use method of claim 27, wherein the bishRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.
36. The use method of claim 27, further comprising a combination therapy with a second anti-neoplastic agent.
37. The use method of claim 27, further comprising a combination therapy with cetuximab or vemurafenib.
38. An expression vector comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 bifunctional shRNAs inserts capable of reducing an expression of a mutant K-ras gene; wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene, wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of mutant K-ras, wherein the bifunctional shRNA preferentially knocks-down mutant K-ras as compared to wild-type K-ras.

39. The expression vector of claim 38, wherein the bifunctional shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

40. The expression vector of claim 38, wherein the bifunctional shRNA comprise triplet inserts that target specific K-ras mutations selected from at least one of G12D-G12V-G12R, G12C-G12D-G12R, G12D-G12V-G12R, or G12C-G12D-G12R.

41. The expression vector of claim 38, wherein the bifunctional shRNA comprise triplet inserts that target specific K-ras mutations selected from SEQ ID NO.: 2, 18, 20, 21, or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.

42. A cell comprising an expression unit that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 bifunctional shRNAs inserts capable of reducing an expression of a mutant K-ras gene; wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene, wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of mutant K-ras and not wild-type K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.

43. The cell of claim 42, wherein the bifunctional shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

44. The cell of claim 42, wherein the bifunctional shRNA has less activity against ~~increases the expression of~~ wild-type K-ras.

45. A method of evaluating a candidate drug believed to be useful in treating cells or tissues in vitro that comprise at least one mutated K-ras that is effectively silenced, the method comprising:

(a) measuring one or more of the following:

- a. the level of expression of at least a wild-type K-ras and one or more mutated K-ras genes in the cells or tissues in vitro;
- b. the level of expression of a candidate gene or a group of candidate genes in an cellular environment with the lowered expression of one or more mutated K-ras genes in the cancer cells or tissues in vitro;

- c. the effect of a candidate drug on the phenotype of such cells comprised of lowered expression of one or more mutated K-ras genes in the cancer cells or tissues in vitro;
- (b) administering a candidate drug to a first subset of said cells or tissues, and a placebo to a second subset of said cells or tissues in vitro;
- (c) repeating step (a) after the administration of the candidate drug or the placebo; and
- (d) determining if the candidate drug is effective in producing determined phenotype in an cellular environment with reduced expression of mutated K-ras as compared to K-ras mutant expressing cellular environment that is statistically significant as compared to any reduction occurring in the second subset of cells or tissues, wherein a statistically significant reduction indicates that the candidate drug is useful in treating disease without significant K-ras mutation background, wherein the candidate agent is a bifunctional shRNA selectively knocks-down mutant K-ras.

46. The method of claim 44, wherein the cells or tissue further express one or more detectable genes have modified to comprise a wild-type K-ras and one or more mutated K-ras, wherein the level of expression of the detectable label correlates with the effect of the candidate substance on the wild-type K-ras and one or more mutated K-ras.

47. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNAs capable of reducing an expression of a mutant K-ras gene, wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene and wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of mutant K-ras.

~~48. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNAs selected from SEQ ID NOS: 1 to 22 or 53 to 56. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNA that comprises one or more inserts that target specific K-ras mutations selected from SEQ ID NO.: 2, 18, 20, 21, or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.~~

49. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNA that comprises one or more inserts that target specific K-ras mutations selected from SEQ ID NO.: 2, 18, 20, 21, or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.

What is claimed is:

1. A bifunctional shRNAs capable of reducing an expression of a K-ras gene, wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene and wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a
5 cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.
2. The bifunctional shRNAs of claim 1, wherein the bifunctional shRNA comprises at least one sequence defined by SEQ ID NOS: 1 to 22 or 53 to 56.
3. The bifunctional shRNAs of claim 1, wherein the bifunctional shRNA comprises at least
10 one sequence defined by SEQ ID NOS: 31 to 52.
4. The bifunctional shRNAs of claim 1, wherein at least one target site sequence is within a human K-ras gene cDNA sequence (SEQ ID NOS: 27 to 30).
5. The bifunctional shRNAs of claim 1, wherein the K-ras is a mutated K-ras.
6. An expression vector comprising:
15 a promoter; and
a nucleic acid insert operably linked to the promoter, wherein the insert encodes one or more shRNA capable of inhibiting an expression of at least one target gene that is a K-ras gene via RNA interference;
wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a
20 cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.
7. The expression vector of claim 6, wherein the target gene sequence comprises SEQ ID NO: 1 to 5.
8. The expression vector of claim 6, wherein a sequence arrangement for the shRNA
25 comprises a 5' stem arm-19 nucleotide target, which is K-ras-TA-15 nucleotide loop-19 nucleotide target complementary sequence-3' stem arm-Spacer-5' stem arm-19 nucleotide target variant-TA-15 nucleotide loop-19 nucleotide target complementary sequence-3' stem arm.
9. The expression vector of claim 6, wherein the nucleic acid insert comprises at least one sequence selected from SEQ ID NO: 6 to 27 or 53 to 56.

10. The expression vector of claim 6, wherein the vector comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 copies of bifunctional shRNAs inserts capable of reducing an expression of one or more mutated K-ras genes.

11. The expression vector of claim 6, wherein at least one shRNA has a target site sequence
5 that is within a mutated K-ras gene cDNA sequence.

12. A therapeutic delivery system comprising:

a therapeutic agent carrier; and

an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter, the nucleic acid insert encoding one or more short hairpin RNA (shRNA) capable
10 inhibiting an expression of a target gene sequence that is K-ras gene via RNA interference;

wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.

13. The delivery system of claim 12, wherein the therapeutic agent carrier is a compacted
15 DNA nanoparticle, or a compacted DNA nanoparticle with one or more polycations.

14. The delivery system of claim 13, wherein the one or more polycations is a 10 kDA polyethylene glycol (PEG)-substituted cysteine-lysine 3-mer peptide (CK₃₀PEG10k).

15. The delivery system of claim 13, wherein the compacted DNA nanoparticles are further encapsulated in at least one of a liposome, a reversibly masked liposome, or a bilamellar
20 invaginated vesicle (BIV).

16. The delivery system of claim 12, wherein the target gene sequence comprises SEQ ID NOS: 27 to 30.

17. The delivery system of claim 12, wherein the nucleic acid insert comprises at least one of the sequences selected from SEQ ID NOS: 31 to 52.

25 18. The delivery system of claim 12, wherein the nucleic acid insert is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

19. A method to deliver one or more shRNAs to a target tissue expressing a K-ras gene comprising the steps of:

30 preparing an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter that encodes the one or more shRNA, wherein the one or more shRNA

comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras;

combining the expression vector with a therapeutic agent carrier, wherein the therapeutic agent carrier comprises a liposome; and

5 administering a therapeutically effective amount of the expression vector and therapeutic agent carrier complex to a patient in need thereof.

20. The method of claim 19, wherein the therapeutic agent carrier is a compacted DNA nanoparticle.

10 21. The method of claim 20, wherein the DNA nanoparticle is compacted with one or more polycations, wherein the one or more polycations comprise a 10 kDA polyethylene glycol (PEG)-substituted cysteine-lysine 3-mer peptide (CK₃₀PEG10k) or a 30-mer lysine condensing peptide.

15 22. The method of claim 20, wherein the compacted DNA nanoparticles are further encapsulated in a liposome, wherein the liposome is a bilamellar invaginated vesicle (BIV) that comprises one or more receptor targeting moieties.

23. The method of claim 22, wherein the one or more receptor targeting moieties comprise small molecule bivalent beta-turn mimics.

24. The method of claim 19, wherein the nucleic acid insert comprises a sequence selected from SEQ ID NOS: 1 to 22 or 53 to 56.

20 25. A method to inhibit an expression of a K-ras gene in one or more target cells comprising the steps of:

selecting the one or more target cells; and

25 transfecting the target cell with a vector that expresses one or more short hairpin RNA (shRNAs) capable of inhibiting an expression of a K-ras gene in the one or more target cells via RNA interference, wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.

26. The method of claim 25, wherein the shRNA incorporates siRNA (cleavage-dependent) and miRNA (cleavage-independent) motifs.

30 27. A method of suppressing a tumor cell growth in a human subject comprising the steps of:

identifying the human subject in need for suppression of the tumor cell growth; and

administering an expression vector in a therapeutic agent carrier complex to the human subject in an amount sufficient to suppress the tumor cell growth, wherein the expression vector expresses one or more shRNA capable inhibiting an expression of a target gene that is a mutated

5 K-ras in the one or more target cells via RNA interference;

wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of the target gene;

10 wherein the inhibition results in an apoptosis, an arrested proliferation, or a reduced invasiveness of the tumor cells.

28. The method of claim 27, wherein the therapeutic agent carrier comprises a bilamellar invaginated vesicle (BIV).

29. The method of claim 27, wherein the therapeutic agent carrier comprises one or more receptor targeting moieties that comprise small molecule bivalent beta-turn mimics.

15 30. The method of claim 27, wherein administering is selected from the group consisting of intratumoral, subcutaneous, intravenous, intraperitoneal, intramuscular, and intravenous injection.

31. The method of claim 27, wherein administering comprises injecting with a DNA:lipoplex.

20 32. The method of claim 27, wherein the tumor cell growth expresses a mutated K-ras.

33. The method of claim 27, wherein the tumor cell growth is human pancreatic ductal adenocarcinoma.

25 34. The method of claim 27, wherein the tumor cell growth is selected from the group consisting of lung cancer, colon cancer, melanoma, insulinoma, mesothelioma, ovarian cancer, and pancreatic cancer.

35. The method of claim 27, wherein the shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

36. The method of claim 27, further comprising a combination therapy with a second anti-neoplastic agent.

37. The method of claim 27, further comprising a combination therapy with cetuximab or vemurafenib.

38. An expression vector comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 bifunctional shRNAs inserts capable of reducing an expression of a mutant K-ras gene; wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene, wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.

39. The expression vector of claim 38, wherein the bifunctional shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

40. The expression vector of claim 38, wherein the bifunctional shRNA comprise triplet inserts that target specific K-ras mutations selected from at least one of G12D-G12V-G12R, G12C-G12D-G12R, G12D-G12V-G12R, or G12C-G12D-G12R.

41. The expression vector of claim 38, wherein the bifunctional shRNA comprise triplet inserts that target specific K-ras mutations selected from SEQ ID NO.: 2, 18, 20, 21, or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.

42. A cell comprising an expression unit that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 bifunctional shRNAs inserts capable of reducing an expression of a mutant K-ras gene; wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene, wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.

43. The cell of claim 42, wherein the bifunctional shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

44. The cell of claim 42, wherein the bifunctional shRNA increases the expression of wild-type K-ras.

45. A method of evaluating a candidate drug believed to be useful in treating cells or tissues that comprise at least one mutated K-ras that is effectively silenced, the method comprising:

(a) measuring one or more of the following:

a. the level of expression of at least a wild-type K-ras and one or more mutated K-ras genes in the cells or tissues;

b. the level of expression of a candidate gene or a group of candidate genes in an cellular environment with the lowered expression of one or more mutated K-ras genes in the cancer cells or tissues;

5 c. the effect of a candidate drug on the phenotype of such cells comprised of lowered expression of one or more mutated K-ras genes in the cancer cells or tissues;

(b) administering a candidate drug to a first subset of said cells or tissues, and a placebo to a second subset of said cells or tissues;

(c) repeating step (a) after the administration of the candidate drug or the placebo; and

10 (d) determining if the candidate drug is effective in producing determined phenotype in an cellular environment with reduced expression of mutated K-ras as compared to K-ras mutant expressing cellular environment that is statistically significant as compared to any reduction occurring in the second subset of cells or tissues, wherein a statistically significant reduction indicates that the candidate drug is useful in
15 treating disease without significant K-ras mutation background.

46. The method of claim 44, wherein the cells or tissue further express one or more detectable genes have modified to comprise a wild-type K-ras and one or more mutated K-ras, wherein the level of expression of the detectable label correlates with the effect of the candidate substance on the wild-type K-ras and one or more mutated K-ras.

20 47. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNAs capable of reducing an expression of a K-ras gene, wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene and wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras.
25

48. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNAs selected from SEQ ID NOS: 1 to 22 or 53 to 56. The method of claim 44, wherein the cells or tissue have been modified to express a bifunctional shRNA that comprises one or more inserts that target specific K-ras mutations selected from SEQ ID NO.: 2, 18, 20, 21,
30 or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.

What is claimed is:

1. A bifunctional shRNA capable of reducing an expression of a K-ras gene, wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene and wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.
2. The bifunctional shRNAs of claim 1, wherein the bifunctional shRNA comprises at least one sequence defined by SEQ ID NOS: 1 to 22 or 53 to 56.
3. The bifunctional shRNAs of claim 1, wherein the bifunctional shRNA comprises at least one sequence defined by SEQ ID NOS: 31 to 52.
4. The bifunctional shRNAs of claim 1, wherein at least one target site sequence is within a human K-ras gene cDNA sequence (SEQ ID NOS: 27 to 30).
5. The bifunctional shRNAs of claim 1, wherein the K-ras is a mutated K-ras.
6. An expression vector comprising:
a promoter; and
a nucleic acid insert operably linked to the promoter, wherein the insert encodes one or more shRNA capable of inhibiting an expression of at least one target gene that is a K-ras gene via RNA interference;
wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.
7. The expression vector of claim 6, wherein the bifunctional shRNA comprises SEQ ID NO: 1 to 5.
8. The expression vector of claim 6, wherein a sequence arrangement for the shRNA comprises a 5' stem arm-19 nucleotide target, which is K-ras-TA-15 nucleotide loop- 19 nucleotide target

complementary sequence-3 'stem arm-Spacer-5' stem arm-19 nucleotide target variant-TA-15 nucleotide loop- 19 nucleotide target complementary sequence-3'stem arm.

9. The expression vector of claim 6, wherein the nucleic acid insert comprises at least one sequence selected from SEQ ID NO: 6 to 27 or 53 to 56.

10. The expression vector of claim 6, wherein the vector comprises 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 copies of bifunctional shRNAs inserts capable of reducing an expression of one or more mutated K-ras genes.

11. The expression vector of claim 6, wherein at least one shRNA has a target site sequence that is within a mutated K-ras gene cDNA sequence.

12. A therapeutic delivery system comprising:

a therapeutic agent carrier; and

an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter, the nucleic acid insert encoding one or more short hairpin RNA (shRNA) capable inhibiting an expression of a target gene sequence that is K-ras gene via RNA interference; wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.

13. The delivery system of claim 12, wherein the therapeutic agent carrier is a compacted DNA nanoparticle, or a compacted DNA nanoparticle with one or more polycations.

14. The delivery system of claim 13, wherein the one or more polycations is a 10 kDA polyethylene glycol (PEG)-substituted cysteine-lysine 3-mer peptide (CK30PEG10k).

15. The delivery system of claim 13, wherein the compacted DNA nanoparticles are further encapsulated in at least one of a liposome, a reversibly masked liposome, or a bilamellar invaginated vesicle (BIV).

16. The delivery system of claim 12, wherein the target gene sequence comprises SEQ ID NOS: 27 to 30.

17. The delivery system of claim 12, wherein the nucleic acid insert comprises at least one of the sequences selected from SEQ ID NOS: 31 to 52.

18. The delivery system of claim 12, wherein the nucleic acid insert is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

19. Use of a composition to reduce the expression of a mutant K-ras gene comprising an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter that encodes the one or more shRNA, wherein the one or more shRNA comprise a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras as compared to wild-type K-ras in a therapeutic agent carrier, wherein the therapeutic agent carrier comprises a liposome.

20. The use of claim 19, wherein the therapeutic agent carrier is a compacted DNA nanoparticle.

21. The use of claim 20, wherein the DNA nanoparticle is compacted with one or more polycations, wherein the one or more polycations comprise a 10 kDA polyethylene glycol (PEG)-substituted cysteine-lysine 3-mer peptide (CK30PEG10k) or a 30-mer lysine condensing peptide.

22. The use of claim 20, wherein the compacted DNA nanoparticles are further encapsulated in a liposome, wherein the liposome is a bilamellar invaginated vesicle (BIV) that comprises one or more receptor targeting moieties.

23. The use of claim 22, wherein the one or more receptor targeting moieties comprise small molecule bivalent beta-turn mimics.

24. The use of claim 19, wherein the nucleic acid insert comprises a sequence selected from SEQ ID NOS: 1 to 22 or 53 to 56.

25. The use of a bifunctional RNA molecule that activates a cleavage-dependent and cleavage-independent RNA-induced silencing complex for reducing the expression level of K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.

26. Use of a bifunctional RNA molecule that activates a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of the target

gene, wherein the bifunctional shRNA selectively knocks-down mutant K-ras to suppress tumor cell growth in vitro to trigger apoptosis, arrested cell proliferation, or reduced invasiveness of a tumor cell.

27. The use of claim 26, wherein the therapeutic agent carrier comprises a bilamellar invaginated vesicle (BIV).

28. The use of claim 26, wherein the therapeutic agent carrier comprises one or more receptor targeting moieties that comprise small molecule bivalent beta-turn mimics.

29. The use of claim 26, wherein administering is selected from the group consisting of intratumoral, subcutaneous, intravenous, intraperitoneal, intramuscular, and intravenous injection.

30. The use of claim 26, wherein administering comprises injecting with a DNA:lipoplex.

31. The use of claim 26, wherein the tumor cell growth expresses a mutated K-ras.

32. The use of claim 26, wherein the tumor cell growth is human pancreatic ductal adenocarcinoma.

33. The use of claim 26, wherein the tumor cell growth is selected from the group consisting of lung cancer, colon cancer, melanoma, insulinoma, mesothelioma, ovarian cancer, and pancreatic cancer.

34. The use of claim 26, wherein the bishRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

35. The use of claim 26, further comprising a combination therapy with a second anti-neoplastic agent.

36. The use of claim 26, further comprising a combination therapy with cetuximab or vemurafenib.

37. An expression vector comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 bifunctional shRNAs inserts capable of reducing an expression of a mutant K-ras gene; wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene, wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing

the expression level of mutant K-ras, wherein the bifunctional shRNA preferentially knocks-down mutant K-ras as compared to wild-type K-ras.

38. The expression vector of claim 37, wherein the bifunctional shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

39. The expression vector of claim 37, wherein the bifunctional shRNA comprise triplet inserts that target specific K-ras mutations selected from at least one of G12D-G12V-G12R, G12C-G12D-G12R, G12D-G12V-G12R, or G12C-G12D-G12R.

40. The expression vector of claim 37, wherein the bifunctional shRNA comprise triplet inserts that target specific K-ras mutations selected from SEQ ID NO.: 2, 18, 20, 21, or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.

41. A cell comprising an expression unit that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 50, 75, or 100 bifunctional shRNAs inserts capable of reducing an expression of a mutant K-ras gene; wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene, wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of mutant K-ras and not wild-type K-ras, wherein the bifunctional shRNA selectively knocks-down mutant K-ras.

42. The cell of claim 41, wherein the bifunctional shRNA is selected from SEQ ID NOS: 1 to 22 or 53 to 56.

43. The cell of claim 41, wherein the bifunctional shRNA has less activity against wild-type K-ras.

44. A method of evaluating a candidate drug believed to be useful in treating cells or tissues *in vitro* that comprise at least one mutated K-ras that is effectively silenced, the method comprising:

(a) measuring one or more of the following:

a. the level of expression of at least a wild-type K-ras and one or more mutated K-ras genes in the cells or tissues *in vitro*;

b. the level of expression of a candidate gene or a group of candidate genes in an cellular environment with the lowered expression of one or more mutated K- ras genes in the cancer cells or tissues *in vitro*;

c. the effect of a candidate drug on the phenotype of such cells comprised of lowered expression of one or more mutated K-ras genes in the cancer cells or tissues *in vitro*;

(b) administering a candidate drug to a first subset of said cells or tissues, and a placebo to a second subset of said cells or tissues *in vitro*;

(c) repeating step (a) after the administration of the candidate drug or the placebo; and

(d) determining if the candidate drug is effective in producing determined phenotype in an cellular environment with reduced expression of mutated K-ras as compared to K- ras mutant expressing cellular environment that is statistically significant as compared to any reduction occurring in the second subset of cells or tissues, wherein a statistically significant reduction indicates that the candidate drug is useful in treating disease without significant K-ras mutation background, wherein the candidate agent is a bifunctional shRNA selectively knocks-down mutant K-ras.

45. The method of claim 43, wherein the cells or tissue further express one or more detectable genes have modified to comprise a wild-type K-ras and one or more mutated K-ras, wherein the level of expression of the detectable label correlates with the effect of the candidate substance on the wild-type K-ras and one or more mutated K-ras.

46. The method of claim 43, wherein the cells or tissue have been modified to express a bifunctional shRNAs capable of reducing an expression of a mutant K-ras gene, wherein at least one target site sequence of the bifunctional RNA molecule is located within the K-ras gene and wherein the bifunctional RNA molecule is capable of activating a cleavage-dependent and a cleavage-independent RNA-induced silencing complex for reducing the expression level of mutant K-ras.

47. The method of claim 43, wherein the cells or tissue have been modified to express a bifunctional shRNAs selected from SEQ ID NOS: 1 to 22 or 53 to 56.

48. The method of claim 43, wherein the cells or tissue have been modified to express a bifunctional shRNA that comprises one or more inserts that target specific K-ras mutations

selected from SEQ ID NO.: 2, 18, 20, 21, or a combination of SEQ ID NOS: 2, 18 and 20; or a combination of SEQ ID NOS: 21, 2 and 18.